Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(2), 3258-3268 | ISSN:2251-6727

Management of Cerebral Palsy in LPS Induced Rat Model

Chandra Sekhar Thota [1*], P Muralidharan [2]

[1*]Department of Pharmacology, C.L Baid Metha College of Pharmacy, Tamil Nadu-600097 (Chennai) India.

[2]Department of Pharmacology, Chettinad school of pharmaceutical sciences, Tamil Nadu-603103 (Chennai) India.

(Received: 07 January 2024 Revised: 12 February 2024 Accepted: 06 March 2024)

KEYWORDS
Curcumin Longa, Cerebral Palsy, Neuroinflammation, Oxidative stress, TNF-α, and NF-κB.

ABSTRACT:

Introduction: Cerebral palsy (CP) is a complex neurological disorder linked to motor and cognitive impairments, often stemming from perinatal brain injury. Inflammatory processes play a role in CP, with animal models exposed to lipopolysaccharide (LPS) mimicking key features of the condition. *Curcuma longa L* (Turmeric), is known for its potent anti-inflammatory and antioxidant properties, is investigated for its potential neuroprotective effects in this study.

Objective: This study investigates about pro-inflammatory cytokines, oxidative stress markers, and neuroinflammatory pathways.

Material and Methods: The study administered LPS through the intracerebroventricular route in adult Wistar rats, with oral treatment of *Curcuma longa L's* hydroalcoholic extract (HAECL) in various doses. The investigation focused on pro-inflammatory cytokines, oxidative stress markers, and neuroinflammatory pathways. Behavioral tests and histological evaluations, including assessments of TNF-α, NF-κB, COX-2, IL-6, and neuronal viability, were conducted.

Results: The findings revealed that HAECL treatment significantly enhanced motor functions and reduced cognitive impairments, indicating improved learning and memory performance. Brain analysis showed decreased neuroinflammation and neuronal damage, suggesting neuroprotective properties. Oxidative stress markers were also attenuated, highlighting antioxidant effects.

Discussion: *Curcuma longa L* demonstrated notable neuroprotective effects, attributed to its anti-inflammatory and antioxidant properties. It mitigated motor deficits, preserved neuronal integrity, and reduced oxidative stress. The results position curcumin as a potential therapeutic agent for managing cerebral palsy-associated symptoms, warranting further investigation for clinical applications.

1. Introduction

Cerebral palsy (CP), initially documented by William Little in the 1840s, is a prevalent developmental disability characterized by diverse degrees of involvement, ranging from mild with minimal disability to severe, often accompanied by additional co-morbid conditions. Alongside autism and mental retardation, CP ranks among the three most common lifelong developmental disabilities, imposing significant challenges on affected individuals and their families **[1]**. CP encompasses a spectrum of conditions impacting movement and posture control **[2]**. Described as an "umbrella term," it encompasses non-progressive motor impairment syndromes stemming from brain lesions or anomalies during early development **[3]**. The condition arises due to damage to the brain areas regulating movement, resulting in abnormal muscle control **[4]**. Notably, CP symptoms vary in severity, but the condition itself does not worsen with age.

Paradoxically, the incidence and severity of CP have risen with a decline in infant mortality rates. Premature babies face a significantly higher incidence of CP compared to term or normal births. Understanding the complexities of CP is crucial for healthcare professionals

Journal of Chemical Health Risks

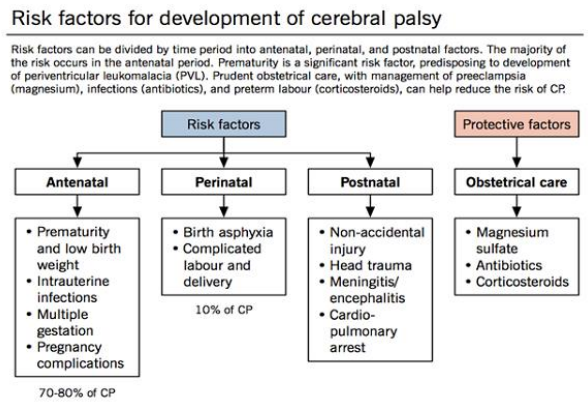
www.jchr.org

JCHR (2024) 14(2), 3258-3268 | ISSN:2251-6727

grappling with diagnostic and therapeutic challenges associated with this impactful developmental disability.

Etiology of CP

The etiology of cerebral palsy (CP) is notably diverse and multifaceted, encompassing congenital, genetic, inflammatory, infectious, anoxic, traumatic, and metabolic factors. The injuries to the developing brain can occur during prenatal, natal, or postnatal phases. Predominantly, 75% to 80% of CP cases result from prenatal injuries, whereas less than 10% are linked to significant birth trauma or asphyxia. Prematurity and low birth weight emerge as pivotal risk factors, with the likelihood of CP escalating as gestational age and birth weight decrease.



Notably, cerebral palsy is observed in 10% to 18% of infants weighing between 500 and 999 grams at birth. Although CP is more prevalent in very premature or full-term infants, term infants, constituting the majority of all births, comprise about half of the cases of cerebral palsy **[5]**. Understanding the intricate array of factors contributing to CP is crucial for comprehending its diverse origins, with prematurity and low birth weight emerging as prominent risk indicators. This nuanced understanding is essential for healthcare professionals and researchers aiming to address the complex etiological landscape of cerebral palsy.

2. Experimental Design

Material and Methods:

Lipopolysaccharide

LPS is employed globally in experimental models, both in vitro and in vivo, to study neuroinflammation and amyloidosis **[6]**. The induction of systemic inflammation by LPS finds application in various neurodegenerative diseases; Alzheimer's disease, Parkinson disease, amyotrophic lateral sclerosis, and multiple sclerosis **[7]**.

LPS constitutes a segment of the outer membrane in gram-negative bacteria, serving as a robust endotoxin. Its resilience to degradation by mammalian enzymes establishes a sustained inflammatory stimulus, eliciting the release of proinflammatory cytokines. These cytokines, in turn, activate both the neuroimmune and neuroendocrine systems, inducing responses akin to those triggered by behavioral stress **[8]**. LPS forms a complex with CD14 on microglia membranes, subsequently interacting with toll-like receptor (TLR)-4. TLR-4 activation initiates signal transduction cascades in microglia, triggering the rapid transcription and release of various proinflammatory cytokines, including interleukin (IL)-1, IL-6, IL-12, IL-17A, IL-18, p40, inducible nitric oxide synthase (iNOS), and tumor necrosis factor-α (TNF-α) **[9]**. Additionally, chemokines like CCL2, CCL5, and CXCL8, complement system proteins such as C3, C3a, and C5a receptors, and anti-inflammatory cytokines like IL-10 and transforming growth factor-β (TGF-β) are released. Studies indicate increased expression levels of TNF-α, IL-1β, and IL-6 in the hippocampus three days post LPS administration compared to control groups **[10]**.

Animals

Adult Wistar rats of both sex weighing approximately 220-250 g were obtained from the animal house of C.L Baid Metha College of Pharmacy. The animals were housed individually in cases under standard conditions (room temperature: 24–26°C; humidity: 60–65%; 12/12-h natural light/dark cycle). The animals had free access to food and water. The rats were acclimatized for at least 6 days prior to the study. The experimental protocols and animal handling procedures were approved by the Ethics Committee of the Integrated Research and Testing Laboratory, C.L Baid Metha College of Pharmacy (IAEC approval number 04/321/PO/Re/S/01/CPCSEA/2019).

Extraction procedure

The plant material was collected from the agriculture land in Andhra Pradesh, and roots were separated to air dried. The dried material is authenticated at Madras Christian College, Chennai, Tamil Nadu – 600059. The Hydroalcoholic Extraction (HAE) process is chosen for

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(2), 3258-3268 | ISSN:2251-6727

isolating the components of the study material. In this method, finely dried and coarsely powdered product is immersed in a mixture of water and ethanol at a ratio of 4:6. This extraction process is conducted over a period of 24 to 48 hours, with intermittent stirring. The resulting mixture is then filtered and left to air-dry, yielding the final product **[11]**. The percentage yield of turmeric was 14.32% W/W.

LPS administration:

All animals were trained on In-vivo parameters for 2 weeks (1-14 days) continuously prior to initiation of the LPS demonstration. Initially, animals undergo pre-surgical preparation, and the following procedure is employed to induce CP in the study. A precise incision is made from the base of the neck to the midpoint between the eyes of the rat. The skull is cleansed using sterile cotton swabs dipped in hydrogen peroxide to optimize the bregma **[12]**. The syringe/needle, with the beveled portion facing posteriorly, is carefully positioned until it touches the bregma. The needle is then moved laterally to the right by 1.0 mm and anteriorly by 0.3 mm. Subsequently, the needle is slowly driven through the skull until it is flush with the top, and a brief pause of 2-3 minutes allows for sealing around the needle. The endotoxin (LPS) is administered at a controlled rate of 1 µL per second **[13]**. A cotton swab is pressed against the skull at the needle's base during LPS injection. Once the needle is withdrawn, the cotton swab is rolled over the needle hole and held for 1 minute to prevent any LPS leakage. The skin is sutured, antibiotic ointment is applied, and the mouse is placed on a heated recovery pad. Daily monitoring is conducted for one-week (15-21 days) post-surgery to assess recovery and any potential complications **[14]**.

Study design

The rats were divided into five groups as shown in the Table 1 below: the Group 1 serves as control for the study receiving oral administration of 0.9 % of saline solution of turmeric extract, the Group 2 serves as negative control for the study receiving LPS at a fixed dose (0.25 mg/kg) **[15]** via intracerebroventricular (ICV) route, the Group 3, Group 4 and Group 5 serving as treatment groups for the study receiving a fixed dose of LPS via ICV route + oral hydroalcoholic extract of *Curcuma longa L* (HAECL) at 200, 400 and 600 mg/kg, respectively.

Groups	Treatment	No. of Wistar rat's animals
Group 1	Control (0.9% Saline Solution of Turmeric extract)	6
Group 2	Negative Control (LPS 0.25mg/kg)	6
Group 3	Low dose (LPS + 200 mg/kg of HAECL)	6
Group 4	Mid dose (LPS + 400 mg/kg of HAECL)	6
Group 5	High dose (LPS + 600 mg/kg of HAECL)	6
Total		30 M/F
**Note: No standard is used in the study, as we do not have any standard drug to treat cerebral palsy.		

Table 1

After recovery, the treatment group (Group 3, Group 4 and Group 5) animals were subjected to receive a daily dose of oral HAECL from day 22 to day 28 at 200, 400 and 600 mg/kg, respectively. On day 29, the rat's blood was collected from sinus orbitals to determine the neuronal markers. All animals were sacrificed and dissected the brain, homogenized at 4°C using a homogenizer **[16]**. The homogenates were used for the biochemical assays.

3. Experimental Procedures

In-vivo observations
Elevated plus-maze

The *Elevated Plus Maze* (EPM) test is used to assess anxiety-related behavior in rodent models of CNS disorders. The EPM apparatus consists of a "+"-shaped maze elevated above the floor with two oppositely positioned closed arms, two oppositely positioned open arms, and a center area. As subjects freely explore the maze, their behavior is recorded by means of a video camera mounted above the maze and analyzed using a video tracking system. The preference for being in open arms over closed arms (expressed as either as a percentage of entries and/or a percentage of time spent in the open arms) is calculated to measure anxiety-like behavior. This test can be used to phenotype strains of transgenic mice and to screen for putative anxiolytic compounds **[17]**.

Forced swim test

Morris water maze test: The Morris Water Maze (MWM) is designed to test spatial memory and long-term memory by observing and recording escape latency, thigmotaxis duration, distance moved, and velocity during the time spend in the MWM water tank. Tempera paint is added into the water until it becomes opaque. A hidden platform, 1/10 the length of the diameter of the water body, is placed about 1cm below

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(2), 3258-3268 | ISSN:2251-6727

the water surface. Three fourths of the water tank is surrounded by privacy blinds with 3 visual cues **[17]**.

Muscle spascity test

Rotarod test: Rotor-Rod is a test used to assess sensorimotor coordination and motor learning in rodent models of CNS disorders. The subjects are placed on a rotating rod with either constant rotation or a steady acceleration; the latency to fall is recorded, where the subjects fall safely 9" below the rotating rod. During training, subjects learn to balance on a stationary rod, then on a rod constantly rotating at 10 rpm. At least two weeks of training are needed to ensure that all subjects have learned the task to the same degree. In the fixed rotation protocol, the animals are placed on a rod which accelerates to and then constantly rotates at 10 rpm. Subjects receive three sessions of testing per week, three trials per session; the average of the three trials is presented daily. In the accelerating protocol, the animals are placed on a rod that accelerates quickly from 0-5 rpm and then gradually from 5-20 rpm. Testing consists of three sessions per week, two trials per session; the average of the two trials is presented daily. A trial is complete when the animal falls or the time period ends; overall testing can run as long as three weeks. This test is used to phenotype strains of transgenic mice and evaluate novel chemical entities for their effect on motor performance **[18]**.

Memory impairment

Novel Object Recognition (NOR) task is used to evaluate cognition, particularly recognition memory, in rodent models of CNS disorders. This test is based on the spontaneous tendency of rodents to spend more time exploring a novel object than a familiar one. The choice to explore the novel object reflects the use of learning and recognition memory. The Object-Location Memory task assesses cognition, specifically spatial memory and discrimination, in rodent models of CNS disorders. This test is based on the spontaneous tendency of rodents to spend more time exploring a novel object than a familiar object and also to recognize when an object has been relocated. Testing occurs in an open field arena, to which the animals are first habituated. The next day, four objects of similar material but different shapes are introduced to the arena. They are spaced roughly equidistant from each other with space in the middle for introducing the subject. In the first trial, the animal is allowed to explore the arena with the four objects. In the second trial shortly thereafter, the animal again encounters the four objects, except that two of them have switched positions. The trials are recorded using a camera mounted above the arena and scored for the amount of time spent sniffing the objects; the object-location discrimination index is calculated. The Object-Location Memory task is useful for assessing cognitive deficits in transgenic strains of mice and for evaluating novel chemical entities for their effect on cognition **[19]**.

Y-maze (Spontaneous Alternation Test)

Y-Maze Spontaneous Alternation is a behavioral test for measuring the willingness of rodents to explore new environments. Rodents typically prefer to investigate a new arm of the maze rather than returning to one that was previously visited. Many parts of the brain--including the hippocampus, septum, basal forebrain, and prefrontal cortex--are involved in this task. Testing occurs in a Y-shaped maze with three white, opaque plastic arms at a 120° angle from each other. After introduction to the center of the maze, the animal is allowed to freely explore the three arms. Over the course of multiple arm entries, the subject should show a tendency to enter a less recently visited arm. The number of arm entries and the number of triads are recorded in order to calculate the percentage of alternation. An entry occurs when all four limbs are within the arm. This test is used to quantify cognitive deficits in transgenic strains of mice and evaluate novel chemical entities for their effects on cognition **[20]**.

In-Vitro observations

Neuronal viability

The MTT assay was employed to evaluate cell viability, building on a prior study. In summary, 3000 BV2 cells were seeded per well in 96-well plates. After attachment, cells were provided with fresh media containing either lipopolysaccharide (LPS) or Curcumin at the specified concentration. Following 24 hours of drug treatment, cells were exposed to 5 mg/ml MTT for 4 hours. Subsequently, the culture media were replaced with 200 µl of DMSO, and the optical density at the wavelength of 540 nm was measured using a microplate reader **[21]**.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(2), 3258-3268 | ISSN:2251-6727

Nitric oxide (NO) Oxidative Stress analysis

To determine NO content, rat brain tissue was treated with 9 volumes of PBS. The mixture underwent three cycles of freeze-thaw, followed by homogenization using a homogenizer. The supernatant obtained after centrifugation at 10,005 g for 10 minutes was utilized for assessing NO content. In the case of BV2 cell cultures, the supernatant from cells cultured for 2 days was collected. Measurement of NO content in both tissue and cell supernatants was conducted using the NO Assay Kit **[21]**.

NF-κ B, and TNFα

The quantification of NF-κ B and TNFα expressions in the hippocampus region was performed through Enzyme-Linked Immunosorbent Assays (ELISA) following the manufacturer's guidelines. Briefly, samples and standards were carefully dispensed into pre-coated wells containing specific antibodies for NF-κ B and TNFα. Subsequently, the wells underwent washing, and enzyme-linked antibodies specific to NF-κ B and TNFα were introduced, followed by an incubation period. After three meticulous washes, any residual wash buffer was eliminated through aspiration. The plate was inverted over clean paper towels to remove unbound antibody-enzyme reagent. Finally, a substrate solution comprising equal volumes of Reagents A and B was added to the wells, and the resulting solution was read at 450 nm using a microplate reader **[22, 23]**.

Statistical analysis

Statistical validation of the data was performed using GraphPad Prism version 9, employing appropriate statistical comparison tests. The values presented in the tabular column denote the Mean ± SD (Standard Deviation), with a sample size of n = 6, encompassing six rats in each experimental group **[24]**.

4. Results

Effect of HAECL on Elevated plus-maze

The assessment of rats working memory involved the use of elevated plus maze. Notably, Group II animals exhibited a substantial reduction in retention latency in comparison to the other groups, with p-values indicating statistical significance at levels of $p<0.001$ and $p<0.0001$.

The investigation into the impact of a plant extract on the rat memory formation process utilized the elevated plus-maze. Notably, the normal control group (Group I) exhibited a reduced Retention Latency (RL), indicating enhanced retention memory. In contrast, Group II animals, which only received ICV LPS, showed no improvement in RL, suggesting potential damage to the hippocampus and other temporal lobe brain structures. However, groups treated with the hydroalcoholic extract of *Curcuma longa L* (HAECL) demonstrated a significant ($p<0.001$ and $p<0.0001$) dose-dependent improvement in RL. This indicates the extract's ability to prevent damage in hippocampal regions. The findings underscore the memory-enhancing properties of *Curcuma longa L*, as depicted in Figure-1.

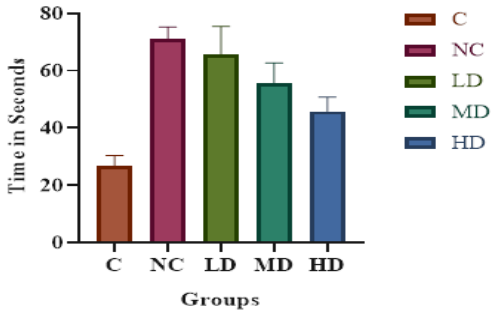


Figure-1. Effect of *HAECL* on Elevated Plus Maze (RL)

Effect of HAECL on Forced swim test

Morris water maze (MWM) is a behavioural model for assessing spatial and related forms of learning and memory. The Group II animals showed increase in escape latency period on comparison with Group I animals ($p<0.0001$). Treatment with HAECL at three dose levels (200, 400 and 600mg/kg) decreased the latency time significantly ($p<0.0001$) Group III, IV and Group V respectively) on comparison with Group II animals.

The MWM task involves animals locating a concealed platform to escape from swimming in a water pool. To accomplish this, the animal creates a "spatial orientation map" in the brain using visual cues from extra maze features in the testing environment. During training, learning is evaluated by the time taken for the animal to reach the platform and escape the water (escape latency)

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(2), 3258-3268 | ISSN:2251-6727

and by the percentage of time or path length spent in the quadrant containing the platform (target quadrant). Spatial memory, indicative of hippocampus-dependent cognition, is a key outcome.

In this study, the negative control group exhibited a reduced escape latency, possibly indicating neuronal degeneration in the hippocampus due to LPS infusion. Conversely, the results revealed a dose-dependent improvement in spatial memory for HAECL-treated groups (III, IV, and V). This improvement could stem from enhanced neuronal impulse transmission due to the extract treatment or the extract's potential to alleviate neuroinflammatory processes. Detailed findings are illustrated in Figure-2.

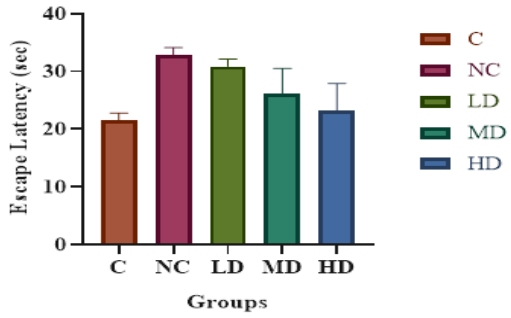


Figure-2. Effect of *HAECL* on Morris Water Maze

Effect of HAECL on Muscle spascity test

To assess the sensorimotor function involving motor control and coordination in rats using rotarod model. The Group II (LPS infused) animals showed significant ($p<0.0001$) reduced residence time of rats in muscle coordination and control on compared with control (Group I) and HAECL treated (Group III, IV and V) animals.

To explore the impact of Curcumin on LPS-induced motor coordination impairment, we conducted the Rota-rod test, assessing the animals' motor abilities. Our findings revealed that rats treated solely with LPS experienced a notable reduction in their residence time on the Rota-rod. Significantly, co-treatment with HAECL and LPS demonstrated a dose-dependent restoration of the rats' residence duration on the Rota-rod. Particularly it was the observation that rats administered a higher dose of HAECL (600 mg/kg) exhibited significantly longer residence times on the rotarod compared to those treated with LPS alone, the findings are illustrated in Figure-3. These results strongly suggest that HAECL treatment mitigates the disruptive effects of LPS on balance and motor coordination.

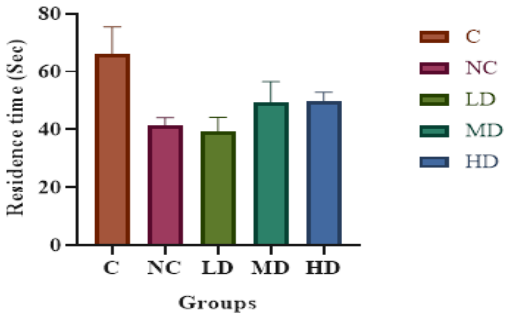


Figure-3. Effect of *HAECL* on Muscle Spascity Test

Effect of HAECL on NOR test

The assessment of rat's recognition memory involved conducting a Novel Object Recognition (NOR) task. A notable disparity in object-directed activity was observed between Group II (LPS-infused) rats and both the control group (Group I) and rats treated with extracts (Group III, IV, and V).

The NOR task was employed to analyze habituation behavior and the recognition and remembrance capabilities of rats. Recognition of novelty, a key indicator of cognitive skills in animals, is primarily mediated by the brain's perirhinal cortex. In the classical NOR task, where animals explore both a known and a novel object, the frequency and duration of exploration for the unique object are expected to exceed those for the familiar one. The preference for the novel object signifies the retention of the known object's appearance in the animal's memory.

The NOR task's utility extends from assessing short-term memory to evaluating intermediate or even long-term memory in rodents by adjusting the retention interval. Recognition memory formation is primarily governed by the perirhinal cortex and hippocampus. Any damage to these brain areas can compromise performance in the NOR task. In this study, rats infused with LPS failed to recognize and explore the presented novel object compared to the control group, potentially due to LPS-ICV infusion causing damage to brain structures like the hippocampus and cortex. Extracts treatment mitigated this damage, resulting in HAECL-treated groups demonstrating commendable improvement in novel

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(2), 3258-3268 | ISSN:2251-6727

object recognition in a dose-dependent manner. There was a significant ($p<0.0001$) difference in object-directed activity produced by Group II (LPS infused) rats when compared with Group I rats and extracts treated rats (Group III, IV and V). Results were illustrated in Figure-4.

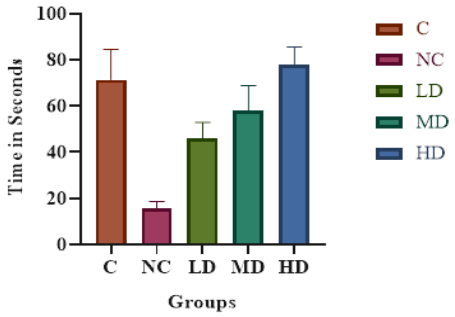


Figure-4. Effect of *HAECL* on Memory Impairment model (NOR)

Effect of HAECL on Spatial Memory (Y-Maze)

The Y-maze test serves as a valuable tool for assessing spatial memory impairments, particularly evident in the alterations observed in the number of visits among different rat groups (Groups I, III, IV, and V) when compared to Group II, which received LPS infusion.

Significantly, rats infused with LPS (Group II) exhibited a noteworthy decrease in the number of entries into the arms ($p<0.0001$). Conversely, groups treated with various doses of HAECL (Groups III, IV, and V) demonstrated a dose-dependent enhancement in spatial memory. Notably, the higher dose of HAECL at 600 mg/kg displayed a remarkable and statistically significant ($p<0.0001$) increase in the number of entries compared to the LPS-induced group. This increase in entries associated with the higher HAECL dose suggests a potential reversal of memory impairment induced by LPS infusion. Results were illustrated in Figure-5.

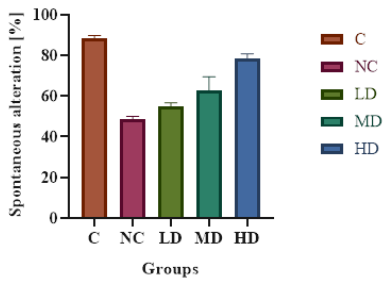


Figure-5. Effect of *HAECL* on Short-term Spatial Memory (Y-Maze)

Effect of HAECL on Neuronal viability

The cell viability-boosting capacity of the plant extract (HAECL) against LPS-induced cell cytotoxicity in SH–SY5Y neuroblastoma cell lines validated its neuroprotective effects. The MTT assay was employed to determine cell viability.

LPS induces the formation of reactive oxygen species, altering mitochondrial membrane permeability, leading to the release of cytochrome c into the cytoplasm, thereby initiating the caspase cascade and apoptosis. Co-administration of the extract (HAECL) at progressively doubled concentrations (ranging from 6.25µg/ml to 100 µg/ml) resulted in a significant ($p<0.001$ and $p<0.0001$) enhancement in cell viability percentage compared to the LPS group. Plant extract exhibited these effects in a dose-dependent manner. The HAECL extract showed intense result with 95.32 % cell viability at a concentration of 100 µg/ml, Cell viability enhancing capability of HAECL is given in Figure-6.

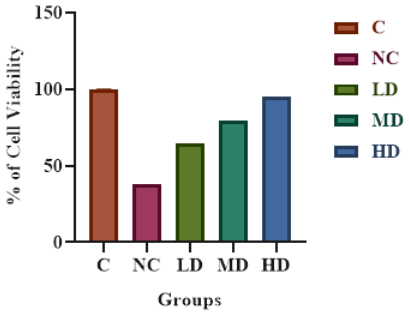


Figure-6. Effect of *HAECL* on Cell viability

Effect of HAECL on Nitric Oxide

The LPS-induced oxidative stress was assessed by measuring the NO content in cerebral tissues. LPS treatment elevated oxidative stress in BV2 cells, as evidenced by increased NO levels ($p<0.0001$) when compared with control group (Group I). Notably, HAECL demonstrated a remarkable suppression of both NO and ROS contents ($p<0.0001$), indicating that the inflammatory response and oxidative stress induced by LPS were moderately mitigated by HAECL treatment in a dose-dependent manner (Group, III, IV and V), the findings are illustrated in Figure-7.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(2), 3258-3268 | ISSN:2251-6727

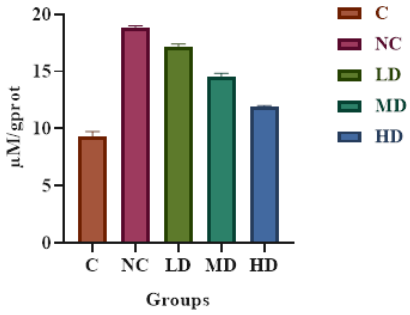


Figure-7. Effect of *HAECL* on NO Content

Effect of HAECL on NF-κ B, and TNFα

A single ICV LPS dose administered to rats significantly elevated NF-κ B levels compared to Group I animals. Notably, animals treated with HAECL exhibited a significant reduction in NF-κ B expression compared to the LPS group. Results were illustrated in Figure-8.

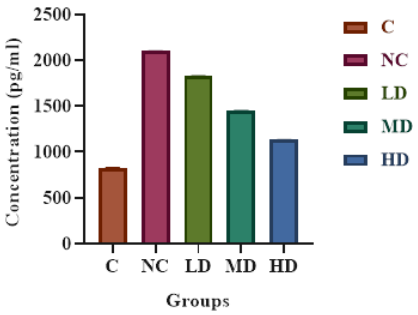


Figure-8. Effect of *HAECL* on Nf-kB levels

TNF-α, a proinflammatory cytokine produced by monocytes in the periphery and microglia, neurons, and astrocytes in the CNS, plays a pivotal role in inflammation. Functioning as a key regulator of acute phase inflammation, TNF-α initiates cascades in inflammatory cytokine signaling. The brain TNF-α levels in Group II animals showed a significant increase ($p<0.0001$) compared to Group I animals. However, treatment with HAECL at doses of 200, 400, and 600 mg/kg (Group III, Group IV, and Group V) resulted in a substantial and significant ($p<0.0001$) decrease in TNF-α levels compared to Group II animals. Results were illustrated in Figure-9.

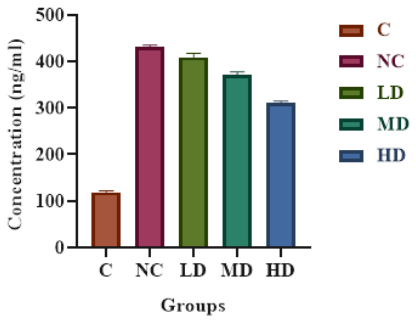


Figure-9. Effect of *HAECL* on TNF-α levels

5. Discussion

Cerebral Palsy (CP) stands as a prominent neurological disorder marked by impairments in motor and developmental skills. Common manifestations encompass issues with posture, gait, muscle tone, and movement coordination **[25]**. Presently, there is no universally accepted standard treatment for CP; however, the impact can be mitigated, and complications prevented through physiotherapy interventions, incorporating measures to address muscular spasms, spasticity, anticonvulsants, and muscle coordination and relaxant strategies. The long-term use of such treatments, however, may raise concerns about potential drug toxicity **[26, 27]**. The acceptance of drugs derived from medicinal plants is steadily growing due to their cost-effectiveness and a reduced profile of toxicity. This trend reflects an increasing recognition of the potential benefits offered by medicinal plant-based medications in addressing the challenges associated with CP, providing an alternative avenue for managing the condition.

The current study highlights the neuroprotective potential of the hydroalcoholic extract of *Curcuma longa L* in a rat model of lipopolysaccharide (LPS)-induced cerebral palsy. LPS, a major component of Gram-negative bacteria membranes, plays a crucial role in maintaining structural integrity and creating a permeability barrier for bacterial cells. Infusion of LPS into the lateral cerebral ventricles in rats has been observed to induce physiological and neurological disorders resembling cerebral palsy **[28]**. To model this condition, we chose LPS as inducing agent, as it mimics some aspects of cerebral in rats.

The effects of the plant extract on rat memory formation were explored using the elevated plus maze, with

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(2), 3258-3268 | ISSN:2251-6727

retention latency (RL) on the second day as a parameter. While Group II animals showed no improvement in RL, the hydroalcoholic extract of *Curcuma longa L* (HAECL) treated groups demonstrated a significant dose-dependent improvement, suggesting a memory-enhancing property of Curcumin.

Additionally, habituation behavior and recognition memory were analyzed through the Novel Object Recognition (NOR) task. The recognition of novelty, indicative of cognitive skills, is primarily mediated by the brain's perirhinal cortex. In the NOR task, where animals explore both a known and a novel object, HAECL-treated groups exhibited a significant improvement in recognizing and exploring the novel object, contrasting with the LPS-infused Group II rats that failed to do so. This suggests that HAECL treatment prevented damage to brain structures, such as the hippocampus and cortex, induced by LPS-ICV infusion. Overall, the study indicates the potential of *Curcuma longa L* in ameliorating memory deficits and cognitive impairments associated with cerebral palsy.

The Y-maze test serves as a valuable tool for evaluating an animal's aptitude in navigating novel environments, commonly employed to gauge short-term spatial working memory and learning. In contrast to the control group, the LPS-infused group (Group II) exhibited a reduced percentage of spontaneous alternation. Notably, groups treated with *Curcuma longa L* (HAECL) showcased a substantial, dose-dependent enhancement, indicating a pronounced memory-improving characteristic associated with Curcumin.

Beyond neuroinflammation, oxidative stress emerges as another contributory factor in the pathogenesis of cerebral palsy (CP). This phenomenon results in compromised cell viability and contributes to the degeneration of dopamine neurons associated with CP **[29]**. In alignment with earlier research, our findings corroborate the upregulation of oxidative stress markers, including elevated levels of nitric oxide (NO) and reactive oxygen species (ROS), along with increased inducible nitric oxide synthase (iNOS) mRNA expression **[30]** in HAECL treated rat BV2 cells, showed suppressed the over production of these oxidative stress markers **[31]**.

The phytochemical analysis of *Curcuma longa L* revealed the predominance of phenolic compounds and terpenoids, encompassing diarylheptanoids (commonly known as curcuminoids), diarylheptanoids, monoterpenes, sesquiterpenes, diterpenes, triterpenoids, alkaloids, sterols, and more **[32]**. In the current study, the hydroalcoholic extract of *Curcuma longa L* (HAECL) demonstrated substantial neuroprotection against LPS-induced cerebral palsy in rats, potentially attributed to the diverse array of phytoconstituents present in the extract.

6. Conclusion

The current study's results indicate that the hydroalcoholic root extract of *Curcuma longa L* possesses neuroprotective properties coupled with significant antioxidant effects. These outcomes primarily arise from the modulation of glutamate levels in the rat brain with LPS-induced cerebral palsy. Initial phytochemical analysis and HR LCMS (High-Resolution Liquid Chromatography–Mass Spectrometer) examination of the ethanolic root extract of Curcuma longa revealed the presence of diverse terpenoids and diarylheptanoids, potentially accounting for its neuroprotective and memory-enhancing effects. However, further investigation into Curcuma longa is warranted to isolate potential phytoconstituents and assess their specific mechanisms underlying neuropharmacological effects.

7. Acknowledgment

This publication is a part of the Ph.D. thesis of The Tamil Nadu DR.M.G. R Medical University, Chennai, Tamil Nadu, India. The authors are very much thankful to the Department of Pharmacology, C.L. Baid Metha College of Pharmacy, Chennai, India, for research facilities and other logistic supports.

Plant authentication approval: The fresh root of *Curcuma longa L* was collected from agricultural land in Kadapa, Andhra Pradesh, India, and authentified by Botanist Prof. Senthilkumar Umapathy, Ph.D., Assistant Professor in Botany, Madras Christian College, Tambaram, Chennai. India.

References

1. Chitra Sankar, NandiniMundkur, Cerebral palsy-definition, classification, etiology and early diagnosis. Indian J Pediatr. 2005 Oct;72(10):865-8. DOI: 10.1007/BF02731117.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(2), 3258-3268 | ISSN:2251-6727

2. Cerebral Palsy guide. https://www.cerebralpalsyguide.com/cerebral-palsy/ (accessed 2020-01-19).
3. V.Soumya, K.V. Phani Madhavi*, B. Devi Madhavi, A study on maternal and perinatal risk factors of cerebral palsy among children attending a cerebral palsy clinic in Visakhapatnam. Int J Community Med Public Health. 2018 Jan;5(1):317-321. DOI:10.18203/2394-6040.ijcmph20175805
4. National Institute of Neurological disorders and stroke. https://www.ninds.nih.gov/health-information/disorders/cerebral-palsy# (accessed on 2020-01-19)
5. Centre for disease control and prevention. https://www.cdc.gov/ncbddd/cp/causes.html (accessed on 2020-01-19).
6. Judith Miklossy, Chronic Inflammation and Amyloidogenesis in Alzheimer's Disease – Role of Spirochetes. Journal of Alzheimer's Disease 13 (2008) 381–391. https://www.researchgate.net/publication/267626158_Chronic_Inflammation_and_Amyloidogenesis_in_Alzheimer%27s_Disease_-_Role_of_Spirochetes
7. Greg M. Cole, Bruce Teter, Sally A. Frautschy, Adv Exp Med Biol. 2007; 595: 197–212. DOI: 10.1007/978-0-387-46401-5_8
8. Urmila Maitra, Hui Deng, Trevor Glaros, Bianca Baker, Daniel G S Capelluto, Zihai Li, Liwu Li. Molecular mechanisms responsible for the selective and low-grade induction of proinflammatory mediators in murine macrophages by lipopolysaccharide J Immunol. 2012 July 15; 189(2): 1014–1023. DOI: 10.4049/jimmunol.1200857
9. Jie Sun, Susu Zhang, Xiang Zhang, Xiaobao Zhang, Hongquan Dong, Yanning Qian. IL-17A is implicated in lipopolysaccharide-induced neuroinflammation and cognitive impairment in aged rats via microglial activation. Journal of Neuroinflammation (2015) 12:165. DOI: 10.1186/s12974-015-0394-5
10. Welser-Alves, Milner, Microglia are the major source of TNF-α and TGF-β in postnatal glial cultures; regulation by cytokines, lipopolysaccharide, and vitronectin. Neurochem Int. 2013 July; 63(1). DOI: 10.1016/j.neuint.2013.04.007
11. Tábata T. Garmus, Sarah F. M. Kopf, Julia T. Paula, Ana C. Aguiar, Gustavo H. B. Duarte, Marcos N. Eberlin, Fernando A. Cabral, Brazilian Journal of Chemical Engineering, Vol. 36, No. 02, pp. 1041 - 1051, April - June, 2019. DOI: 10.1590/0104-6632.20190362s20180159
12. Heidi M Houdek, Jordan Larson, John A Watt, Thad A Rosenberger. Bacterial lipopolysaccharide induces a dose-dependent activation of neuroglia and loss of basal forebrain cholinergic cells in the rat brain. Inflamm Cell Signal. 2014;1(1): DOI:10.14800/ics.47
13. Sarah L. DeVos, Timothy M. Miller, Direct intraventricular delivery of drugs to the rodent central nervous system J. Vis. Exp. (75), e50326. DOI: 10.3791/50326
14. Anroop B. Nair and Shery Jacob, A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm. March 2016-May 2016; 7(2): 27–31. DOI: 10.4103/0976-0105.177703
15. R Zakaria, W Mh Wan Yaacob, Z Othman, I Long, A H Ahmad, B Al-Rahbi, Lipopolysaccharide-induced memory impairment in rats: a model of Alzheimer's disease. Physiol Res. 2017 Sep 22;66(4):553-565. DOI: 10.33549/physiolres.933480
16. Stanford behavioral and functional neuroscience laboratory (SBFNL). https://med.stanford.edu/content/dam/sm/sbfnl/documents/news/SBFNL-booklet-SfN-2018-Final-PDF.pdf (accessed on 08-Jun-2022).
17. Khulud Abdullah Bahaidrah, Noor Ahmed Alzahrani,Rahaf Saeed Aldhahri, Rasha Abdulrashed Mansouri, Badrah Saeed Alghamdi, Effects of Different Lipopolysaccharide Doses on Short- and Long-Term Spatial Memory and Hippocampus Morphology in an Experimental Alzheimer's Disease Model. Clin. Transl. Neurosci. 2022, 6(3), 20, DOI: https://doi.org/10.3390/ctn6030020
18. Wenhui Cao, Yan Dong, Weina Zhao, Xin Lu, Li Sun. Mulberrin attenuates 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine (MPTP)-induced Parkinson's disease by promoting Wnt/β-catenin signaling pathway. Journal of Chemical Neuroanatomy 98 (2019) 63–70. DOI: https://doi.org/10.1016/j.jchemneu.2019.04.003
19. Nida Jamali-Raeufy, Fahimeh Alizadeh, Zhila Mehrabi, Soraya Mehrabi, Mina Goudarzi, Acetyl-L-carnitine confers neuroprotection against lipopolysaccharide (LPS) -induced

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(2), 3258-3268 | ISSN:2251-6727

neuroinflammation by targeting TLR4/NFκB, autophagy, inflammation and oxidative stress. Metab Brain Dis 36, 1391–1401 (2021), DOI: https://doi.org/10.1007/s11011-021-00715-6

20. Stanford behavioral and functional neuroscience laboratory (SBFNL). https://med.stanford.edu/sbfnl/services/bm/lm/y-maze.html (accessed on 08-Jun-2022).
21. Yangyang Yu, Qian Shen, Yihong Lai, Sun Y Park, Xingmei Ou, Dongxu Lin, Meiling Jin, Weizhen Zhang, Anti-inflammatory Effects of Curcumin in Microglial Cells. Front Pharmacol. 2018 Apr 20:9:386. DOI: 10.3389/fphar.2018.00386
22. Leyla Acar, Nazan Atalan, E. Hande Karagedik, Arzu Ergen, Tumour Necrosis Factor-alpha and Nuclear Factor-kappa B Gene Variants in Sepsis. Balkan Med J. 2018 Feb; 35(1): 30–35. DOI: 10.4274/balkanmedj.2017.0246
23. K Hensley, N Hall, R Subramaniam, P Cole, M Harris, M Aksenov, M Aksenova, S P Gabbita, J F Wu, J M Carney, et al, Brain regional correspondence between Alzheimer's disease histopathology and biomarkers of protein oxidation. Journal of neurochemistry.1995;65(5):2146-2156. DOI: 10.1046/j.1471-4159.1995.65052146.x
24. Zulfiqar Ali, S Bala Bhaskar, Basic statistical tools in research and data analysis. Indian J Anaesth. 2016 Sep; 60(9): 662–669. DOI: 10.4103/0019-5049.190623
25. Mayo Clinical. Cerebral Palsy (disease and conditions) https://www.mayoclinic.org/diseases-conditions/cerebral-palsy/symptoms-causes/syc-20353999 (accessed on 2020-01-19).
26. Cerebral Palsy: Wikipedia https://en.wikipedia.org/wiki/Cerebral_palsy
27. Christian R. H. Raetz, C. Whitfield, Lipopolysaccharide Endotoxins. Annu Rev Biochem. 2002; 71: 635–700. DOI: 10.1146/annurev.biochem.71.110601.135414
28. Aisha Farhana, Yusuf S. Khan, Biochemistry, Lipopolysaccharide. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. https://www.ncbi.nlm.nih.gov/books/NBK554414/
29. Adriana Fernanda K Vizuete, Fernanda Fróes, Marina Seady, Caroline Zanotto, Larissa Daniele Bobermin, Ana Cristina Roginski, Moacir Wajner, André Quincozes-Santos, Carlos Alberto Gonçalves, Early effects of LPS-induced neuroinflammation on the rat hippocampal glycolytic pathway. J Neuroinflammation. 2022 Oct 11;19(1):255. DOI: 10.1186/s12974-022-02612-w.
30. G W Kreutzberg, Microglia: a sensor for pathological events in the CNS. Trends Neurosci.1996 Aug;19(8):312-8. DOI: 10.1016/0166-2236(96)10049-7.
31. Peter Jenner, Oxidative stress in Parkinson's disease. Ann Neurol 2003:53 Suppl 3:S26-36. DOI: 10.1002/ana.10483.
32. Shiyou Li, Wei Yuan, Guangrui Deng, Ping Wang, Peiying Yang, Bharat B. Aggarwal, Chemical Composition and Product Quality Control of Turmeric (*Curcuma longa L*). Pharmaceutical Crops, 2011, 2, 28-54. https://scholarworks.sfasu.edu/agriculture_facultypubs/1