International Journal of Medical Toxicology & Legal Medicine **Volume 27, No. 5, 2024**
e-ISSN: 0974-4614
p-ISSN: 0972-0448
https://doi.org/10.47059/ijmtlm/V27I5/065

Evaluation of Withania somniferaL.on oxidative stress and neuroinflammatory activities in rat's brain by LPS induced cerebral palsy model

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Received: 13.09.2024 Revised: 10.10.2024 Accepted: 09.11.2024

ABSTRACT
This study explores the potential neuroprotective effects of Withaniasomnifera L. (Ashwagandha), which is recognized for its anti-inflammatory and antioxidant benefits. Adult Wistar rats were subjected to lipopolysaccharide (LPS) exposure and treated orally with the hydroalcoholic extract of Withaniasomnifera (HAEWS). The results indicated significant enhancements in motor functions and a reduction in cognitive impairments, pointing to improved learning and memory capabilities. Additionally, the study revealed decreased neuroinflammation and neuronal damage, highlighting the strong neuroprotective properties of Withaniasomnifera. This positions Withaniasomnifera as a promising therapeutic option for addressing cerebral palsy.

Keywords: Withaniasomnifera L., Cerebral Palsy, Neuro-inflammation, Oxidative stress, TNF-α, NF-κB.

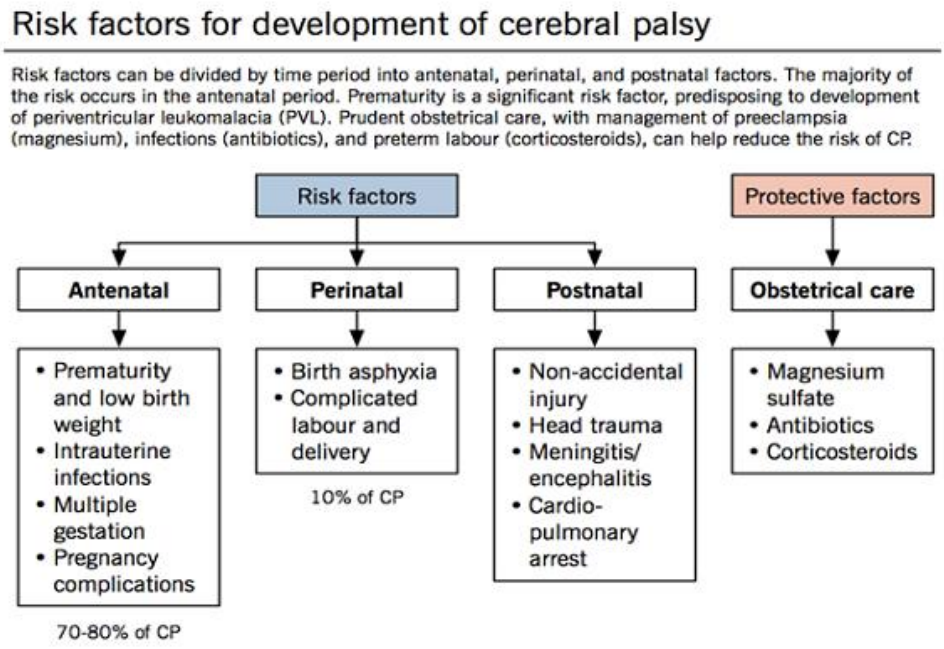
1. INTRODUCTION

Cerebral palsy (CP), originally delineated by William Little in the 1840s, stands as a prevalent developmental disability marked by a spectrum of involvement, spanning from mild with minimal disability to severe, often accompanied by additional co-morbid conditions. In the realm of lifelong developmental disabilities, CP, alongside autism and mental retardation, holds a prominent position, presenting substantial challenges for individuals affected and their families [1]. This encompassing term describes a variety of conditions affecting movement and posture control [2] and is classified as an "umbrella term" for non-progressive motor impairment syndromes resulting from brain lesions or anomalies during early development [3]. The condition emerges from damage to brain areas regulating movement, leading to abnormal muscle control [4]. Importantly, while CP symptoms exhibit variability in severity, the condition itself does not deteriorate with age.

On a global scale, CP impacts 2 to 5 per 1000 live births, and despite advancements in neonatal and obstetric care, its incidence has not diminished. Paradoxically, the incidence and severity of CP have increased alongside a decline in infant mortality rates. Premature infants, in particular, face a significantly higher risk of CP compared to term or normal births. A profound understanding of the intricacies of CP is imperative for healthcare professionals grappling with the diagnostic and therapeutic complexities associated with this impactful developmental disability.

1.1 Etiology of CP[1,5]

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.



Scheme-1

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. 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.

1.2 Types of CP [6]

Cerebral Palsy manifests in various types, each presenting distinct characteristics:

1. **Spastic Cerebral Palsy (70-80%):**
   In this prevalent type, muscles exhibit stiffness, complicating movement. Spastic hemiplegia may affect one side of the body, with the arm typically more severely impacted than the leg. The most severe form is spastic quadriplegia, impacting all four limbs, the trunk, and often involving muscles controlling the mouth and tongue. It is commonly associated with mental retardation and additional challenges.
2. **Athetoid or Dyskinetic Cerebral Palsy (10-20%):**
   Athetoid CP affects the entire body, marked by fluctuations in muscle tone ranging from tight to loose. Uncontrolled movements, either slow and writhing or rapid and jerky, are characteristic. Children may struggle with body control, affecting tasks like sitting and walking. Issues with facial muscles and tongue may lead to difficulties in sucking, swallowing, and speech.
3. **Ataxic Cerebral Palsy (5-10%):**
   Ataxic CP influences balance and coordination, resulting in an unsteady gait with wide-set feet. Precise coordination tasks, such as writing, pose challenges for individuals with this type.
4. **Mixed Cerebral Palsy:**
   Combining features of different types, children with mixed CP may exhibit mild spasticity, dystonia, and/or athetoid movements. Ataxia may coexist with other motor dysfunctions, with Spastic Ataxic Diplegia being a common mixed type often linked to hydrocephalus.

Withania somnifera, popularly known as Ashwagandha, stands as an ancient medicinal herb deeply ingrained in traditional Ayurvedic practices, lauded for its multifaceted benefits, including anti-inflammatory, antioxidant, and immunomodulatory properties. This venerable herb has become a focal point of extensive scientific scrutiny [7]. The root of Withania somnifera harbors a treasure trove of over 35 chemical constituents, featuring prominent biologically active compounds such as alkaloids (isopellertierine, anferine), steroidal lactones (withanolides, withaferins), saponins with an additional acyl group (sitoindoside VII and VIII), and withanolides with glucose at carbon 27 (sitonidoside XI and X). Notably, the roots are rich in iron as well [8, 9].

Beyond its chemical complexity, Ashwagandha has been employed traditionally for a range of purposes, serving as an immune system booster, an anti-inflammatory agent, and a stress alleviator. Its versatile applications extend to the promotion of vitality and enhancement of cognitive functions [10]. This dual identitydeeply rooted

in ancient practices and subject to contemporary scientific investigation underscores the enduring significance of Withania somnifera in the pursuit of holistic well-being.

2. Experimenital Design

2.1 Material and Methods

2.1.1 Lipopolysaccharide

Lipopolysaccharide (LPS) is commonly used in experimental models worldwide, both in vitro and in vivo, to investigate neuroinflammation and amyloidosis [11]. This approach of inducing systemic inflammation with LPS has applications in various neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and multiple sclerosis [12].

LPS is a component of the outer membrane in gram-negative bacteria, acting as a potent endotoxin. Its resistance to degradation by mammalian enzymes results in a sustained inflammatory stimulus, leading to the release of proinflammatory cytokines. These cytokines activate both the neuroimmune and neuroendocrine systems, triggering responses similar to those induced by behavioral stress [13]. LPS forms a complex with CD14 on microglia membranes and interacts with toll-like receptor (TLR)-4. TLR-4 activation initiates signal transduction cascades in microglia, prompting 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) [11, 13, 14]. Additionally, the release includes 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). Studies indicate elevated expression levels of TNF-α, IL-1β, and IL-6 in the hippocampus three days after LPS administration compared to control groups [15].

2.1.2 Animals

Adult Wistar rats of both sexes, weighing approximately 220-250 g, were procured from the animal house of C.L Baid Metha College of Pharmacy. Each rat was housed individually under standard conditions, maintaining a room temperature of 24–26°C, humidity levels at 60–65%, and a natural light/dark cycle of 12/12 hours. They were provided with unrestricted access to food and water throughout the study period. A minimum acclimatization period of 6 days was observed for the rats before the commencement of 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).

2.2 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 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 [16, 17].The percentage yield of Ashwagandha was 17.94% W/W.

2.3 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. 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. 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 [18, 19, 20].

2.4 Study design

The rats were divided into five groups as shown in the Table 1below: the Group 1 serves as control for the study receiving oral administration of 0.9 % of saline solution ofashwagandha extract, the Group 2 serves as negative

control for the study receiving LPS at a fixed dose (0.25 mg/kg) [21]via intracerebroventricular (ICV) route, the Group 3, Group 4 and Group 5 serving as treatment groups for the studyreceiving a fixed dose of LPS via ICV route + oral hydroalcoholic extract of Withania somnifera L. (HAEWS) at 200, 400 and 600 mg/kg, respectively.

Table 1

Groups	Treatment	No. of Wistar rat's animals
Group 1	Control (0.9% Saline Solution of Ashwagandha extract)	6
Group 2	Negative Control (LPS 0.25mg/kg)	6
Group 3	Low dose (LPS + 200 mg/kg of HAEWS)	6
Group 4	Mid dose (LPS + 400 mg/kg of HAEWS)	6
Group 5	High dose (LPS + 600 mg/kg of HAEWS)	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.		

After recovery, the treatment group(Group 3, Group 4, and Group 5) animals were subjected to receive a daily dose of oral HAEWSfrom day 22 to day 28at 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 [22]. The homogenates were used for the biochemical assays.

3. Experimental Procedures

3.1 In-vivo observations

3.1.1 Elevated plus-maze [23]

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.

3.1.2 Forced swim test [23]

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

3.1.3 Muscle spascity test [24]

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.

3.1.4 Memory impairment [25]

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.

3.1.5 Y-maze (Spontaneous Alternation Test) [25]

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.

3.2 In-Vitro observations

3.2.1 Neuronal viability [24]

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 lipopolysaccharide (LPS) 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.

3.2.2 Nitric oxide (NO) Oxidative Stress analysis[24]

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.

3.2.3 NF-κ B, and TNFα [25]

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.

4. 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.

5. RESULTS AND DISCUSSION

5.1 Effect of HAEWS 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 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, when compared with Group I animals.However, groups treated with the hydroalcoholic extract of Withania somnifera L. (HAEWS) 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 Withania somnifera L., as depicted in Figure-1.

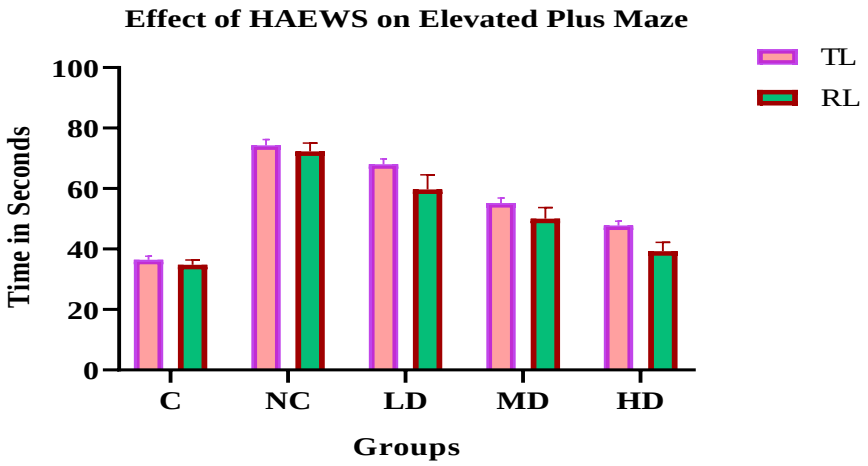


Figure-1

5.2 Effect of HAEWS on Forced swim test

Morris water maze (MWM) serves as a behavioral model to evaluate spatial and associated forms of learning and memory. In comparison with Group I animals, Group II animals exhibited an increase in the escape latency period ($p<0.0001$). Treatment with HAEWS at the lower dose (200 mg/kg) did not result in a significant change in latency time. However, both the median dose (400 mg/kg) and high dose levels (600 mg/kg) demonstrated a significant reduction in latency time ($p<0.0001$) when compared 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 extramaze 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) 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 HAEWS-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.

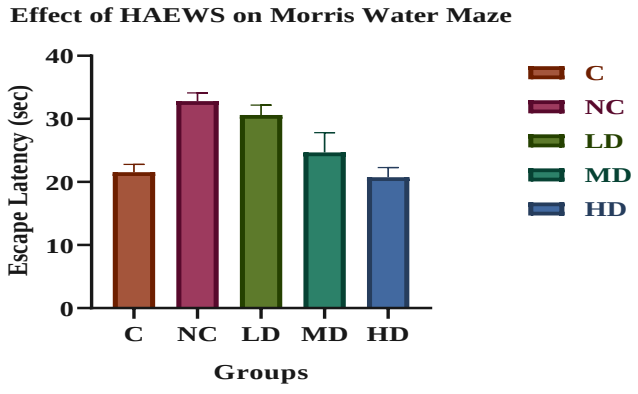


Figure-2

5.3 Effect of HAEWS 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 HAEWS treated (Group III, IV and V) animals.

To explore the impact of ashwagandha 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 HAEWS 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 HAEWS (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 HAEWS treatment mitigates the disruptive effects of LPS on balance and motor coordination.

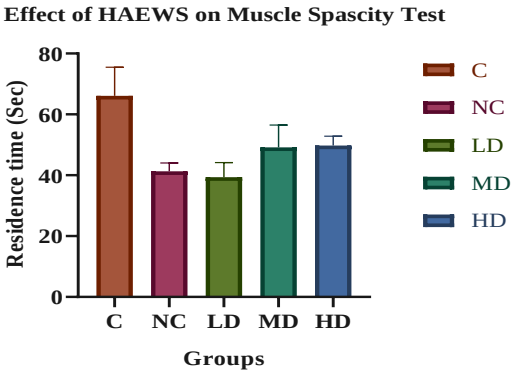


Figure-3

5.4 Effect of HAEWS 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 HAEWS-treated groups demonstrating commendable improvement in novel 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.

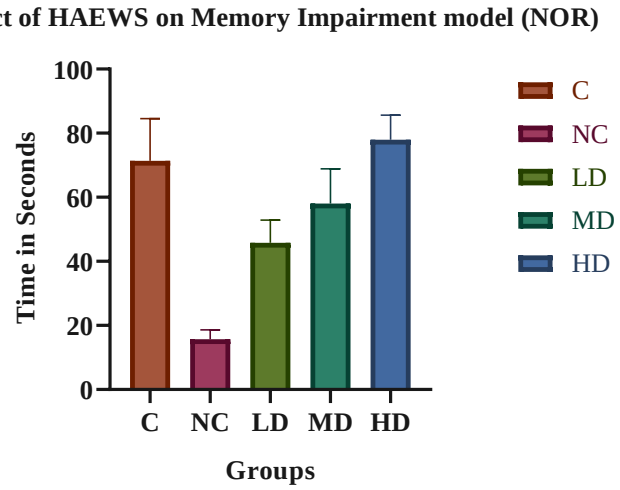


Figure-4

5.5 Effect of HAEWS 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 HAEWS (Groups III, IV, and V) demonstrated a dose-dependent enhancement in spatial memory. Notably, the higher dose of HAEWS 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 HAEWS dose suggests a potential reversal of memory impairment induced by LPS infusion, indicating a pronounced memory-improving characteristic associated with ashwagandha.Results were illustrated in Figure-5.

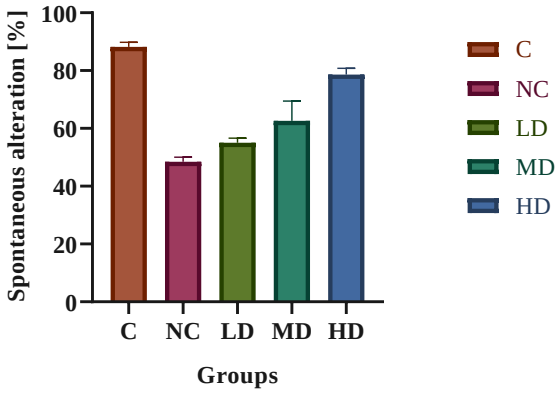


Figure-5

5.6 Effect of HAEWS on Neuronal viability

The cell viability-boosting capacity of the plant extract (HAEWS) 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 (HAEWS) 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 HAEWS extract showed

intense result with 95.32 % cell viability at a concentration of 100 µg/ml, Cell viability enhancing capability of HAEWS is given in Figure-6.

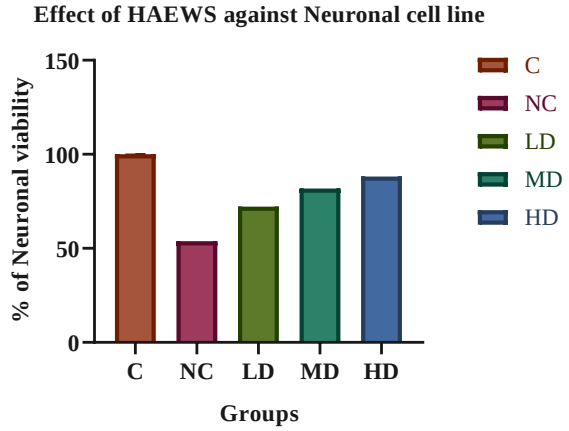


Figure-6

5.7 Effect of HAEWS 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, HAEWS 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 HAEWS treatment in a dose-dependent manner (Group, III, IV and V), the findings are illustrated in Figure-7.

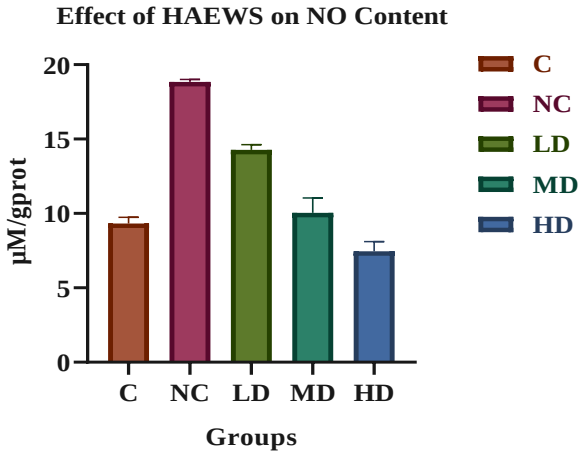


Figure-7

5.8 Effect of HAEWS on NF-κ B, and TNFα

A single ICV LPS dose administered to rats significantly ($P<0.0001$) elevated NF-κ B levels compared to Group I animals. Notably, animals treated with HAEWS exhibited a significant ($p<0.0001$) reduction in NF-κ B expression in dose dependent manner in compared to the LPS group. Results were illustrated in Figure-8.

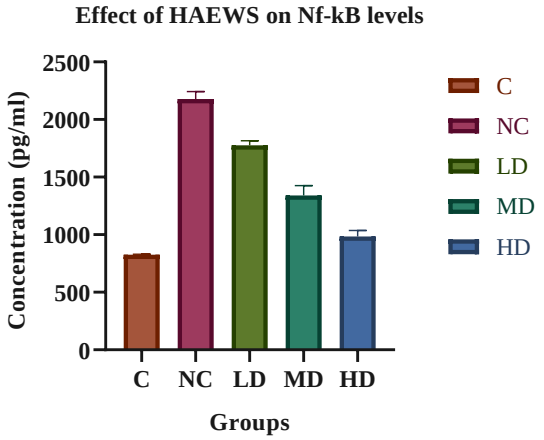


Figure-8

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 HAEWS 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.

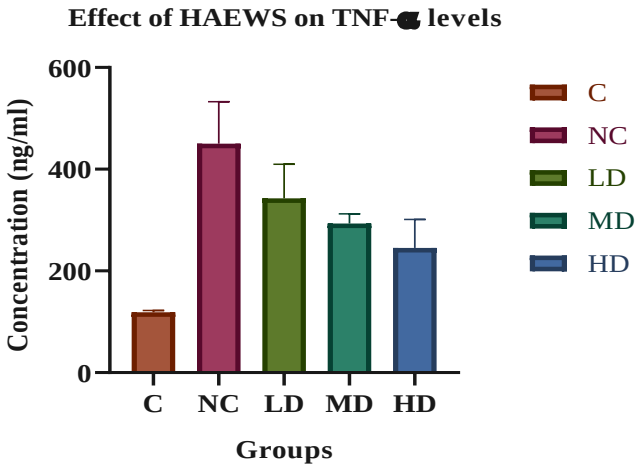


Figure-9

6. CONCLUSION

The findings of this study highlight the neuroprotective attributes, significant anti-inflammatory, and antioxidant effects of the hydroalcoholic root extract of Withania somnifera L. These positive outcomes are closely linked to the modulation of glutamate levels in the rat brain during LPS-induced cerebral palsy. Initial phytochemical analysis and High-Resolution Liquid Chromatography–Mass Spectrometer (HR LCMS) examination of the ethanolic root extract of Withania somnifera identified diverse compounds, including Withanolide A, Withanolide B, 12-deoxy-withastramonolide, and Withanosides, known for their neuroprotective and memory-enhancing effects. However, a comprehensive exploration of Withania somnifera is essential to isolate potential phytoconstituents and elucidate their specific mechanisms contributing to neuropharmacological effects.

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