A COMPARATIVE STUDY ON THE NEURO-PROTECTIVE EFFECTS OF DHA, VITAMIN-E AND MEMANTINE IN THE NEONATAL RAT MODEL OF LPS INDUCED CEREBRAL PALSY

Dissertation submitted to

THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY,

CHENNAI

In partial fulfillment for the award of the degree of

MASTER OF PHARMACY

IN

BRANCH IV – PHARMACOLOGY

Submitted By

N MALATHI

REGISTRATION No:261625005

Under the guidance of

DR.P.MURALIDHARAN, M.Pharm., PhD.

Department of Pharmacology

DEPARTMENT OF PHARMACOLOGY

C.L.BAID METHA COLLEGE OF PHARMACY (AN ISO 9001-2008

CERTIFIED INSTITUTION) CHENNAI– 600097

MAY – 2018

Phone : 24960151, 24962592, 24960425
e-mail : clbaidmethacollege@gmail.com
Website : www.clbaidmethacollege.com

C.L. Baid Metha College of Pharmacy
An ISO 9001:2008 approved institution
Jyothi Nagar, Old Mahabalipuram Road,
Thorapakkam, Chennai - 600 097.

Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai.
Approved by Pharmacy Council of India, New Delhi, and
All India Council for Technical Education, New Delhi

Dr.P.Muralidharan, M.Pharm., Phd.,

Prof & Head,

Department of Pharmacology

CERTIFICATE

This is to certify that Project entitled **A Comparative Study on the Neuro-Protective Effects of DHA, Vitamin-E And Memantine in the Neonatal Rat Model Of LPS Induced Cerebral Palsy** submitted by Regn No: **261625005** in partial fulfillment of the course for the award of the degree of **Master of Pharmacy in Pharmacology.** It was carried out at the Department of Pharmacology in C.L. Baid Metha College of Pharmacy, Chennai-97 under my guidance during the academic year 2017-2018.

Place : Chennai

Date: (**Dr.P.MURALIDHARAN)**

Phone : 24960151, 24962592, 24960425
e-mail : clbaidmethacollege@gmail.com
Website : www.clbaidmethacollege.com

C.L. Baid Metha College of Pharmacy
An ISO 9001:2008 approved institution
Jyothi Nagar, Old Mahabalipuram Road,
Thorapakkam, Chennai - 600 097.

Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai.
Approved by Pharmacy Council of India, New Delhi, and
All India Council for Technical Education, New Delhi

Prof. Dr. GRACE RATHNAM, M. Pharm.,
Ph.D., Principal.

CERTIFICATE

This is to certify that Project entitled **A Comparative Study on the Neuro-Protective Effects of DHA, Vitamin-E and Memantine in the Neonatal Rat Model of LPS Induced Cerebral Palsy** submitted by Regn No: **261625005** in partial fulfillment of the course for the award of the degree of **Master of Pharmacy in Pharmacology.** It was carried out at the Department of Pharmacology in C.L. Baid Metha College of Pharmacy, Chennai-97. Under the supervision of ***Professor Dr .P.Muralidharan*** during the academic year 2017-2018.

Place : Chennai
Date :

(Prof. Dr. GRACE RATHNAM)

.

DECLARATION

Register No: **261625005** hereby declare that this dissertation entitled, **A Comparative Study on the Neuro-Protective Effects of DHA, Vitamin-E And Memantine in the Neonatal Rat Model Of LPS Induced Cerebral Palsy** has been originally carried out by me under the guidance and supervision of **Prof. Dr.P.Muralidharan, M.Pharm,. PhD,** Head of the department of pharmacology C.L. Baid Metha College of Pharmacy, Chennai-97 for the academic year 2017-2018. This work has not been submitted in any other degree at any other university.

Place: Chennai

Date :

Register No: **261625005**

ACKNOWLEDGEMENT

I take pleasure in thanking my respective **guide Dr. P.MURALIDHARAN, M.Pharm., Ph.D., Professor and Head Of Department in Department of Pharmacology**, C. L. BaidMetha College of Pharmacy, Chennai – 97. I am honored to work under his guidance and have his precious guidelines, constant encouragement and support throughout my research work. I am very obliged for the patience with which he guided me at every step of my research work.

I consider it as a great honour to express my deep sense of gratitude and indebtedness to our Principal, **Dr. Grace Rathnam.**, M Pharm., Ph. D, of C.L Baid Metha College of Pharmacy, Chennai-97 for providing the necessary facilities to carry out this work.

I submissively express my deep sense of gratitude and sincere thanks to **Mr. Clement Atlee M.Pharm.,** Assistant Professor and Animal house in-charge, Department of Pharmacology, C.L.Baid Metha College of Pharmacy, Chennai-97, for his encouragement and timely provision of animals to carry out and complete this work.

I extend my special thanks to **Mr.Srinivasa Ragavan, M.Com,** store in-charge and attender **Mr. Anand**, **Mr. Rajaram, Mr. Ruban** C.L.Baid Metha College of Pharmacy, Chennai, for their timely help and supply of all necessary chemicals required for my project work and I also extend my thanks to our security in charge **Mr.GaneshBahadur.**

I would like to express my special thanks of gratitude to my parents Mrs.Vasanthi and Mr.Nagarajan who brought me up in all my endeavors.

I would also like to thank all my friends and well-wishers Mr.Dharmadurai, Mr.Lakshmanabharathy, Ms.Malathi and my brother Mr.Jayasai whose help and moral support contributed to the successful completion of my project work.

My Humble prayers to the Almighty for his choicest blessings at all times.

LIST OF CONTENTS

LIST OF GRAPHS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION [1-4]

The brain is the most complex part of the human body. It is the center of consciousness and controls all the voluntary and involuntary movement and bodily functions. It communicates with each part of the body through the nervous system, a network of channels that carry electrochemical signals.

The belief that the brain is the organ that controls behavior has ancient roots, dating to early civilizations that connected loss of function to damage to parts of the brain and spinal cord. But the modern era of neuroscience began – and continues to progress – with the development of tools, techniques, and methods used to measure in ever more detail and complexity the structure and function of the nervous system. The modern era of neuroscience can be traced to the 1890s, when the Spanish pathologist Santiago Ramón y Cajal used a method developed by the Italian physician Camillo Golgi to stain nerve tissues to visualize the morphology and structure of the neurons and their connections.

A person's unique nervous system develops over the course of their lifespan in a way that resembles the evolution of nervous systems in animals across vast stretches of time. For example, the human nervous system begins developing even before a person is born. It begins as a simple bundle of tissue that forms into a tube and extends along the head-to-tail plane becoming the spinal cord and brain. 25 days into its development, the embryo has a distinct spinal cord, as well as hindbrain, midbrain and forebrain (Stiles & Jernigan, 2010). The brain is the headquarters of the entire nervous system and where most of your sensing, perception, thinking, awareness, emotions, and planning take place. The brain consumes 20% of the total oxygen and calories we consume even though it is only, on average, about 2% of our overall weight.

Human brain development is a protracted process that begins in the third gestational week (GW) with the differentiation of the neural progenitor cells and continues till lifespan. The processes that contribute to brain development range from the molecular events of gene expression to environmental input. Both gene expression and environmental input are essential for normal brain development, and disruption of either can abruptly alter the neuronal outcomes. But neither

genes nor input is determinative of the possible outcome. Hence brain development is characterized as a complex series of dynamic and adaptive processes that operate throughout the process of development to promote the emergence and differentiation of new neural structures and functions. These processes operate within highly constrained and genetically organized, but constantly changes, over time, in order to support the emergence of the complex and dynamic structure of the human brain.

Brain development begins with the foundational changes that occur during the embryonic period, which in humans extends through the eighth week post conception. By the end of the embryonic period the rudimentary structures of the brain and central nervous system are established and the major compartments of the central and peripheral nervous systems are defined.

During this time there is rapid growth and elaboration of both cortical and sub cortical structures, including the rudiments of the major fiber pathways. Neuron production in humans begins on embryonic day E42, i.e. 42 days post conception and is largely complete by the middle of gestation. As neurons are produced they migrate to different areas of the brain where they begin to make connections with other neurons establishing rudimentary neural networks. At the end of the prenatal period major fiber pathways, thalamic-cortical pathway becomes complete.

Structural changes in both the major gray and white matter compartments follows through childhood and adolescence, and these structural changes also parallels with the changes in functional organization that are being reflected in the behavior. During the early postnatal period, level of connectivity throughout the developing brain far exceeds that of adults. This connectivity is gradually pruned back via competitive processes that are influenced by the experience of the organism.

Neurological problems may occur slowly and cause a gradual loss of function (degenerative). Or they may occur suddenly and cause life-threatening problems (acute). Symptoms may be mild or severe. Some serious conditions, diseases, and injuries that can cause nervous system problems include:

- **Epilepsy** – storms of abnormal electrical activity in the brain causing seizures
- **Meningitis** – inflammation of the membrane covering the brain
- **Multiple sclerosis** – the myelin sheaths protecting the electrical cables of the central nervous system are attacked
- **Cerebral palsy** – inability to coordinate and control movement and body posture
- **Sciatica** – pressure on a nerve caused by a slipped disc in the spine or arthritis of the spine and, sometimes, other factors
- **Shingles** – infection of sensory nerves caused by the varicella-zoster virus
- **Stroke** – a lack of blood to part of the brain.

Hundreds of millions of people worldwide are affected by neurological disorders. More than 6 million people die because of stroke each year; over 80% of these deaths take place in low- and middle-income countries. More than 50 million people have epilepsy worldwide. It is estimated that there are globally 47.5 million people with dementia with 7.7 million new cases every year - Alzheimer's disease is the most common cause of dementia and may contribute to 60–70% of cases[5].

1.1. NEURO-DEVELOPMENTALDISORDERS [6-8]

Neurodevelopment is the process of proliferation of the radial glial cells of the brain and the neurons starting from the 2nd month of gestation and continues to develop in the postnatal years.

This process is complete only at the age of 3 years, which occurs within the cerebellum postnatally.

Synapse formation and *myelination* are the two significant processes that are responsible for the complete neurodevelopmental process. Formation of synapse is essential in the last trimester and first 2 years of life, which is significant in functioning and development of the nervous system.

Myelin sheath which acts as the protective covering over the neuronal cells begins in the second half of gestation and continue still adolescence, with different systems myelinating at different period of time.

The brain's center of reasoning and problem solving is among the last to mature. The decade-long MRI study of normal brain development shows that "higher-order" brain centers, such as the prefrontal cortex, don't fully develop until young adulthood.

Cortex areas can be seen maturing at ages in which relevant cognitive and functional developmental milestones occur. The first areas to mature (e.g. extreme front and back of the brain) are those with the most basic functions, such as processing the senses and movement followed by areas involved in spatial orientation and language (parietal lobes).

Areas with more advanced functions such as integrating information from the senses, reasoning and other "executive" functions in the prefrontal cortex matures later.

Tremendous rate of growth in areas of vision and sensation occur in the early school years. In middle school areas in language development shows rapid growth. Late teens exhibit rapid growth in areas controlling inhibition, judgment. Healthy development means an increase and loss of neurological tissue.

Maturation of the central nervous system is critical in the development of neurodevelopmental disorders. Cell pruning or synapse pruning, which occurs between the ages of 5 – 20 years

appears to be a critical process whereby if increased may be linked with childhood onset schizophrenia and if decreased may be linked with autism.

1.2. NEURODEVELOPMENTAL & INTELLECTUAL DISORDERS [9,10]

Neurodevelopmental disorders are disabilities in the functioning of the brain or the nervous system that affect a child's behavior, memory or ability to learn e.g. mental retardation, dyslexia, cerebral palsy, attention deficit hyperactivity disorder (ADHD), learning deficits and autism.

Recent study says that1 in 6 children in the developed countries were affected by,

- Cerebral palsy
- IQ
- Learning disabilities
- Attention deficit hyperactivity disorder (ADHD)
- Autism
- Developmental delay

Neurodevelopmental behavioral disorders were more prevalent in the developing countries and statistics shows that15% of children are described as suffering from learning disabilities, developmental delay, attention deficit hyperactivity disorder, autism, reduced intelligence quotient and cerebral palsy.

In Aboriginal children, the prevalence is often much higher. In some cases etiology was directly linked to identified exposures, e.g. fetal alcohol, tobacco smoke, low birth weight and obstetric complications, in most cases specific etiology is unknown.

Co-morbidity and adult outcomes of failed neurodevelopment are as follows,

- ✦ Anxiety/depression, oppositional defiant disorder, bipolar disorder, Tourette's Syndrome.
- ✦ Substance abuse, antisocial behavior, and even criminality are among the better-known problems persisting into adulthood

1.3. ENVIRONMENTAL LINKAGES OF NEURODEVELOPMENTAL PROCESSES

- **Proliferation**

  Proliferation of neurons from the early development stage and through-out the lifetime of the individual may be affected on consumption of,

  - Alcohol
  - Methyl mercury
  - Chlorpyrifos

- **Migration of Neuroblasts -**

  Neuronal migration is the method by which neurons travel from their origin or birthplace to their final position in the brain.

  - X-ray irradiation
  - Ethanol

- **Differentiation of Neuroblasts -**

  The process of development and differentiation of the neural tube into permanent cellular elements are disrupted by,

  - Ethanol
  - Nicotine
  - Methylmercury
  - Lead

- **Neurotransmission processes -**

  Transmission of neuronal signal or impulses that are transmitted actively by means of neurotransmitters are blocked by,

  - Cholinesterase inhibitors
  - Ethanol
  - Aluminum
  - Pharmaceuticals and pesticides designed to target specific neurotransmitter systems

- **Synaptogenesis -**

  Exuberant synaptogenesis means an explosion of the synapse formation may affect an healthy individual at the early stages of the brain development. It may be caused due to following,

  - Ethanol
  - Lead
  - Polychlorinated biphenyls (PCBs), triethyltin, parathion, permethrin, and

Serotonin antagonist.

- **Apoptosis or cell death**

  Apoptosis is a complex process in which appropriate cells are removed to ensure optimal neurodevelopmental behavioral intellectual development. And this process is adversely affected at critical stages of gestation and postnatal development.

  - Apoptosis is mediated on intake of,
  - Ethanol
  - Lead
  - Mercury and Chlorpyrifos.

1.4. PREVALENCE OF NEURODEVELOPMENTAL DISORDER [4, 5]

The EPIPAGE study –that has been designed to investigate outcomes of preterm children over the past 15 years –

- Identified 5,567 infants born at 22 to 34 weeks' gestation in 2011 in France.
- Rates of survival without severe or moderate neuromotor and sensory disabilities at 2 years of age were 48.5% for children born at 22-26 weeks' gestation, 90% at 27-31 weeks' gestation, and 97.5% at 32-34 weeks' gestation.
- Only one child born at 22-23 weeks' gestation survived.

Upon consideration of the baseline characteristics of infants, survival rate and survival without severe or moderate neuromotor and sensory disabilities at rose between 1997 and 2011 for children born at 22-31 weeks' gestation, but there was no change observed for children born at 24 weeks' gestation.

- Rates of cerebral palsy decreased by 3.3% during 1997-2011 which was statistically significant, at both 24-31 and 32-34 weeks of gestation.

1.4.1. Developmental delay

Upon exclusion of the children suffering from other neurological disorders, 50%, 41%, and 36% of children born at 24-26, 27-31, and 32-34 weeks' gestation, respectively were considered at risk of developmental delay. Delays in language development as well as poorer social-emotional competence most frequently scored below threshold.

Even though the improvements were made in neuromotor and sensory outcomes, a high number of babies born before 34 weeks are prone to developmental delay.

2. LITERATURE REVIEW

2.1. CEREBRAL PALSY [6, 8]

Cerebral palsy is the most common cause of motor disability in children and also affects a large population. The worldwide incidence being 2 to 2.5 per 1000 live births The term cerebral palsy describes a group of movement disorders caused by an injury or infection by micro-organism or disturbance in the early developing brain and specifically the areas involved with creating, coordinating and controlling movement and posture(1).

It may be stated as a static encephalopathy in which, even though the primary lesion, anomaly or injury is static, the clinical pattern of presentation may change with time due to growth and developmental plasticity and maturation of the central nervous system.

2.2. TERMS AND CONCEPTS

Cerebral palsy (CP)[1] describes a group of permanent[2] disorders[3] of the development[4] of movement and posture[5] causing[6] activity limitation[7], that are attributed[8] to non-progressive[9] disturbance[10] that occurred in the developing fetal or infant[11] brain[12].The motor disorders of cerebral palsy are often accompanied by[13] disturbances of sensation[14], perception[15], cognition[16] communication[17], and behavior[18], by epilepsy[19], and by Secondary musculoskeletal problems[20].

1. 'Cerebral palsy (CP)' –

The term describes a prevalent, clinically important and identifiable group of persons with neurodevelopmental disabilities, the term 'cerebral palsy' is established in the literature and is used universally by clinicians, therapists, epidemiologists, researchers, policy makers, health care funding organization and laypersons. The term 'CP has, however, been, indicating the need for an internationally acceptable definition.

2. 'permanent' –

This definition excludes transient disorders, but recognizes that children and adults have changing patterns of clinical manifestations that are permanent.

3. 'disorders' –

This refers to conditions in which there is disruption of the usual orderly stages of child development.

4. 'development' –

The 'developmental' aspect of CP is also important with regard to management strategies that may include interventions that address the developmental consequences. The motor impairments of children eventually diagnosed with CP begin to manifest very early in child development, usually before 18 months of age, with delayed or aberrant motor progress; other neurodevelopmental and functional difficulties may occur. The clinical picture of CP evolves with time, development, learning, activities, therapies, ageing, and other factors.

5. movement and posture–

Abnormal gross and fine motor functioning and organization (reflecting abnormal motor control) are the core features of CP. These motor problems lead to difficulties with walking, feeding and swallowing, coordinated eye movements, articulation of speech, and secondary problems with behaviour, musculoskeletal function, and participation in society

6. 'causing' –

Activity limitations are presumed to be a consequence that are caused of the motor disorder. Thus, disorders of movement and posture that are not associated with activity limitations are not considered to be part of the CP group.

7. 'activity limitation' –

According to World Health Organization's(WHO), International Classification of Functioning, is defined as Disability 'activity' as the execution of a task or
action by an individual", and identifies 'activity limitation' as "difficulties an individual may have in executing activities".

8. 'attributed to' –

Understanding of various forms of developmental neurobiology(including genetic, biochemical, and other influences on brain development) is increasing rapidly, such that
it is becoming possible to identify structural and other evidence of poor brain development in people with CP. Otherwise discovery would have been elusive.

09. 'non-progressive' –

The term non-progressive is used to denote that the pathophysiological mechanisms leading to CP are presumed to arise from a single. The inciting event that results in the partial disruption of the brain functions includes events that are associated with changing or new manifestations that are superimposed on one-another neurodevelopmental process. Not all the motor dysfunctions are considered as Cerebral palsy.

10. 'disturbances' –

Disturbances are the events that may cause interruption, damage, or may affect the normal functioning of the brain development. It poses a major problem

11. 'fetal or infant'-

The disturbances that occur very early in human biological development impact much on the development of motor function than disturbances that occur during later adolescence.

12. 'Brain' –

The term 'brain' includes the cerebrum, the cerebellum and the brain stem. It excludes motor disorders that comprises of spinal, peripheral nerve, muscular or mechanical origin.

13. 'Accompanied by' –

Along with motor and sensory disorders, other neurodevelopmental disorders may also occur.

14. 'Sensation' –

Vision, hearing and other sensory functions are affected, and as a secondary consequence of activity limitations that restrict learning and perceptual development experiences.

15. 'Perception' –

The capacity to incorporate and interpret sensory and/or cognitive information may be impaired both as a function of the 'primary' disturbance(s) to which CP is attributed, and as a secondary consequence of activity limitations that restrict learning and perceptual development experiences.

16. 'Cognition' –

Cognitive processes are affected, both as a function of the 'primary' disturbance and as a secondary consequence of activity that restricts learning and perceptual development experiences.

17. 'communication'-

Expressive and receptive communication may be affected, both as a function of the primary disturbance to which CP is attributed, and as a secondary consequence of activity that restricts learning and perceptual development experiences.

18. 'behaviour' –

This includes psychiatric or behavioural problems such as autistic spectrum disorders, ADHD, sleep disturbances, mood and anxiety disorders

19. 'epilepsy'-

Epilepsies are seen in persons with cerebral palsy as multitude of neuron firing.

20. 'secondary musculoskeletal problems' –

People with CP may develop a variety of musculoskeletal problems, such as muscle/tendon contractures, bony torsion, hip displacement,
spinal deformity. Many of these problems develop throughout life and are related to physical growth, muscle spasticity, ageing and other factors.

2.3. HISTORY OF CEREBRAL PALSY [9]

Since the 1830s, cerebral palsy's physiological details and some of its cause were uncovered by numerous doctors, clinical investigators, and other experts though several mysteries about its causes still remain.

1830's: British surgeon William John Little defined what is now known to be as "cerebral palsy." He was partially disabled by polio, and spent most of his medical career studying various disabling conditions, including spastic diplegia, which was identified as a form of cerebral palsy.

1853: William John little published a research titled "On the Nature and Treatment of the Deformities of the Human Frame." He described various neonatal injuries and congenital disabilities that affect the muscular system. He also wrote the first description of pseudo hypertrophic muscular dystrophy.

1861: William John little created the first definition of cerebral palsy, referring to the birth injuryas a result of difficulties during labor in which "the child has been partially suffocated." Since no one else had written about spasticity or variation in muscle tone, the term "cerebral palsy" still didn't exist. Instead, the disability is referred to as Little's Disease. Little's research paper also discussed the importance of early treatment and intervention. Little wrote about how the children have been "restored to considerable activity and enjoyment of life."

1889: Sir William Osler wrote a book titled *Cerebral Palsies of Children.* Osler didn't refer to the disability as Little's Disease, but instead built upon Little's work and added to it from his

own scientific findings. Osler chose to base the term "cerebral palsy" on the Latin words for "brain" and "paralysis" rather than use common English vernacular.

1892: William Osler became the Chief of Medicine at Baltimore's Johns Hopkins Medical School. He published a text titled *The Principles and Practice of Medicine: Designed for the Use of Practitioners of Students of Medicine.* The textbook became a standard work that was studied for the next 40 years. Osler's work also helped establish credibility for his studies on cerebral palsy..

1897: Austrian neurologist and psychiatrist Sigmund Freud is the first medical researcher to disagree with the theories put forward by Drs. Little and Osler. Freud suggested that cerebral palsy was a brain-related disease that affects children before birth (not during or after as Little proposed). Freud also associated various disorders, including intellectual disabilities, visual disturbances, and seizures, with cerebral palsy .

1949: The United Cerebral Palsy Foundation (UCP) is founded by Leonard Goldenson, president of United Paramount Theaters and the ABC television network, and his wife Isabelle in a joint effort with prominent New York businessman Jack Hausman and his wife Ethel. Both couples are parents of children with cerebral palsy and use their influence to create a foundation to help others affected by the disability.

1952: Marie Killilea wrote *Karen*, the first of two nonfiction books about the author's experiences while caring for her daughter Karen, who was born with cerebral palsy. *Karen* and its sequel, *With Love From Karen* (1963) became best sellers. Killilea also wrote *Wren* (1954), a children's version of Karen's story.

1963: President John F. Kennedy signed the Community Mental Health Act. The law stipulates that children with mental disabilities can be cared for in the community instead of having to be institutionalized.

1980's: Medical researchers prove Freud's theory that cerebral palsy can happen before birth. In addition, clinical studies confirm that cerebral palsy can also be caused by birth injuries, though it only makes up less than 10% of all diagnosed cases.

1990: Congress passed the Americans with Disabilities Act (ADA), a federal law that prohibits employers from carrying out discriminatory hiring practices against individuals with disabilities.

2.4. ETIOLOGY OF CEREBRAL PALSY [9, 10]

Congenital Cerebral Palsy Causes

If an infant develops cerebral palsy before or during childbirth, it's considered congenital CP. This form of CP is marked by development before birth. Per the Centers for Disease Control and Prevention (CDC), congenital CP is the most common form of cerebral palsy.

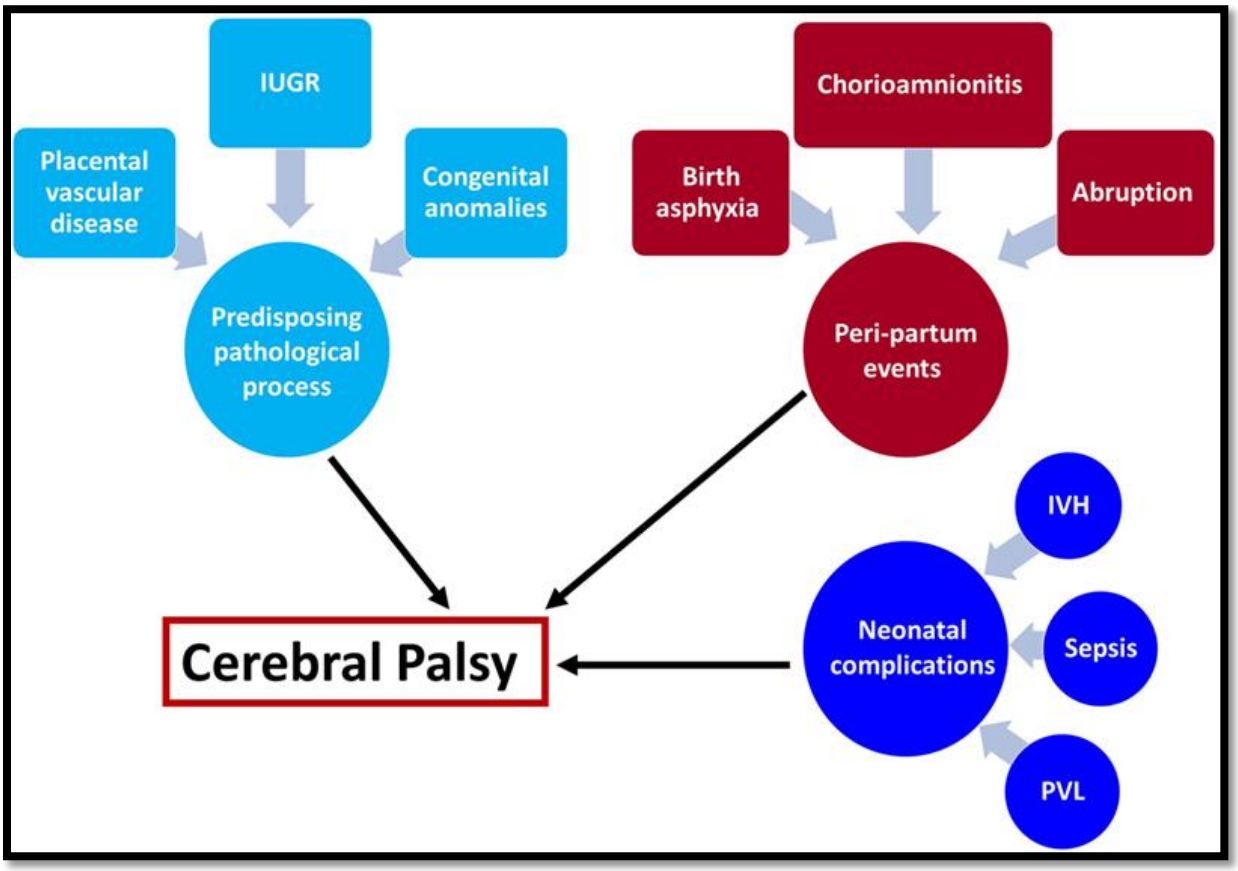


Fig no. 01 Pathogenesis of Cerebral palsy

The most common causes of congenital CP include:

- **Infections While Pregnant:** Infections such as cytomegalovirus (CMV), chicken pox, and rubella may increase the chances of an infant developing CP as these diseases increase cytokines, tiny proteins that act as receptors in the body. When cytokines are released in substantial amounts, it can lead to infant brain damage, which in turn can lead

to CP. Mothers with high fevers and/or a urinary tract infection also run the risk of their infant developing CP.

- **Twins or Multiple Births:** The birth of more than one baby increases the chances of CP due to, in most cases, low birth weight.
- **Low Birth Weight:** Infants under five pounds are at an increased risk of developing CP, with infants under three pounds being the highest risk group. Since most infants who are born prematurely typically weigh five pounds of less, premature babies are also at risk.
- **Infertility Treatments:** Since many infants born after infertility treatments tend to be born premature with low birth weights, the risk for CP is increased.
- **Jaundice:** There is a common misconception that jaundice is a minor medical that won't pose any long-term medical problems. However, if left untreated, jaundice can cause a host of serious problems, including a heightened risk of developing CP.
- **Birth Problems:** Uterine rupture, placental problems, and umbilical cord problems can all lead to an increased risk of the infant developing CP.
- **Chorioamnionitis:** A study performed by San Diego's University of California revealed that chorioamnionitis present in the womb during pregnancy increases the risk of an infant developing CP by up four times.
- **Incompatible Blood:** Incompatibility of blood types between a mother and infant can lead to Rh factor diseases, increasing the risk of CP. However, if a physician detects the blood incompatibility in time, the mother can be given a Rh immune globulin injection starting at around the 28 week of pregnancy.
- Periventricular leukomalacia (PVL)

PVL is a kind of damage that affects the brain's white matter because of a lack of oxygen in the womb.

It may occur if the mother has an infection during pregnancy, such as rubella or German measles, low blood pressure, preterm delivery, or if she uses an illegal drug.

- **Asphyxia during labor and delivery** – Until recently, it was widely believed that asphyxia (lack of oxygen) during a difficult delivery was the cause of most cases of

cerebral palsy. The ACOG/AAP report shows that fewer than 10 percent of the types of brain injuries that can result in cerebral palsy are caused by asphyxia.

Acquired Cerebral Palsy Causes:

Acquired CP occurs when an infant develops brain damages at least 28 days or more after birth. Acquired CP happens much less often when compared to congenital CP. In fact, acquired CP affects around 20% of all people who've developed the disorder. The most common causes include:

- **Low Birth Weight:** Similar to congenital CP, low birth also increases the risk of acquired CP.
- **Blood Flow Problems:** Blood flow problems, particularly to the brain, heightens the risk of acquired CP. Common blood flow problems to brain can occur because of blood clotting, unformed blood vessels, sickle cell diseases, and/or heart defects.
- **Traumatic Head Injuries:** Traumatic head injuries can happen when an infant is dropped, is in an accident, or falls. If brain damage occurs after a traumatic brain injury, acquired CP may follow.

Brain Damage

Brain damage whether before, during, or after birth can lead to CP. In the past, many physicians believed that CP was related to brain damage that occurred during birth because of asphyxiation. This in part is true, with at least 1 out of every 10 infants developing CP after suffering from asphyxiation. However, recent research shows that brain damage that occurs before birth can also lead to CP. There are a few different ways that brain damage occurs during birth that heightens the risk of developing CP.

Mothers must be monitored frequently during pregnancy, especially if they have any infections or high blood pressure. Both infections and high blood pressure may lead to infant brain damage, which in turn can lead to CP. In addition, abnormal brain development during pregnancy can lead to CP. Abnormal brain development can occur due to a maternal infection, mutations in the genes, or trauma to the baby's head.

Medical Negligence

Even though several cases of CP are caused without reason, there are many instances in which physicians failed to properly identify medicals issues and/or failed to use the correct actions during and birth birth to reduce the risk. The most common reasons CP occurs because of medical negligence include:

- Failure to properly monitor and asses the fetal heartbeat during and after delivery
- Failure to schedule and provide a timely C-section
- Failure to detect and treat maternal infections
- Failure to use birth-assisting tools correctly, such as forceps or a vacuum extraction tool
- Failure to correct umbilical cord problems, such as a prolapsed cord
- Failure to supply oxygen in a timely manner to an asphyxiated infant
- Failure to monitor respiratory and oxygen treatments

Intracranial hemorrhage

Sometimes, bleeding inside the brain happens when a fetus experiences a stroke.

Bleeding in the brain can stop the supply of blood to vital brain tissue, and this tissue can become damaged or die. The escaped blood can clot and damage surrounding tissue.

Several factors can cause a stroke in a fetus during pregnancy:

- a blood clot in the placenta that blocks the flow of blood
- a clotting disorder in the fetus
- interruptions in arterial blood flow to the fetal brain
- untreated pre-eclampsia in the mother
- inflammation of the placenta
- pelvic inflammatory infection in the mother

During delivery, the risk is increased by the following factors:

- emergency cesarean
- the second stage of labor is prolonged
- vacuum extraction is used during delivery
- fetal or neonatal heart anomalies
- umbilical cord abnormalities

2.5. SYMPTOMS [11]

An infant with cerebral palsy may have muscular and movement problems, including poor muscle tone. Muscle tone refers to a person's automatic ability to tighten and relax muscle when required.

Features can include:

- overdeveloped or underdeveloped muscles, leading to stiff or floppy movements
- poor coordination and balance, known as ataxia
- involuntary, slow writhing movements, or athetosis
- stiff muscles that contract abnormally, known as spastic paralysis
- crawling in an unusual way
- lying down in awkward positions
- favoring one side of the body over the other
- a limited range of movement

Other signs and symptoms include:

- late achievement of developmental milestones such as crawling, walking, or speaking
- hearing and eyesight problems

- problems controlling bladder and bowel movements
- seizures
- drooling, and problems with feeding, sucking, and swallowing
- being easily startled

Symptoms normally start to show during the first 3 years of life.

2.6. CLASSIFICATION OF CEREBRAL PALSY [12]

Below are the most commonly used classification systems understood and used by qualified practitioners.

- Classification based on severity level
- Classification based on topographical distribution
- Classification based on motor function
- Classification based on gross motor function classification system
-

1. Classification based on severity level

Cerebral Palsy is often classified by severity level as mild, moderate, severe, or no CP. These are broad generalizations that lack a specific set of criteria.

Even when doctors agree on the level of severity, the classification provides little specific information, especially when compared to the GMFCS. Still, this method is common and offers a simple method of communicating the scope of impairment, which can be useful when accuracy is not necessary.

Mild –

Mild Cerebral Palsy means a child can move without assistance; his or her daily activities are not limited.

Moderate –

Moderate Cerebral Palsy means a child will need braces, medications, and adaptive technology to accomplish daily activities.

Severe –

Severe Cerebral Palsy means a child will require a wheelchair and will have significant challenges in accomplishing daily activities.

No CP –

No CP means the child has Cerebral Palsy signs, but the impairment was acquired after completion of brain development and is therefore classified under the incident that caused the Cerebral Palsy, such as traumatic brain injury or encephalopathy.

2. Classification based on topographical distribution

Topographical classification describes body parts affected. The words are a combination of phrases combined for one single meaning. When used with Motor Function Classification System, it provides a description of where and to what extent a child is affected by Cerebral Palsy. This method is useful in ascertaining treatment protocol.

- Paresis means weakened
- Plegia/Plegic means paralyzed

The prefixes and root words are combined to yield the topographical classifications commonly used in practice today.

1. **Monoplegia/monoparesis**

Only one limb is affected. It is believed this may be a form of hemiplegia/hemiparesis where one limb is significantly impaired.

2. **Diplegia/diparesis**

Indicates the legs are affected more than the arms; primarily affects the lower body.

3. **Hemiplegia/hemiparesis**

   Indicates the arm and leg on one side of the body are affected.

4. **Paraplegia/paraparesis**

   The lower half of the body, including both legs, is affected.

5. **Triplegia/triparesis**

   Indicates three limbs are affected. This could be both arms and a leg, or both legs and an arm. Or, it could refer to one upper and one lower extremity and the face.

6. **Double hemiplegia/double hemiparesis** 3

   Indicates all four limbs are involved, but one side of the body is more affected than the other.

7. **Tetraplegia/tetraparesis**

   Indicates that all four limbs are involved, but three limbs are more affected than the fourth.

8. **Quadriplegia/quadriparesis**

   Means that all four limbs are involved.

9. **Pentaplegia/pentaparesis**

   Means all four limbs are involved, with neck and head paralysis often accompanied by eating and breathing complications

3. Classification based on motor function

The brain injury that causes Cerebral Palsy affects motor function, the ability to control the body in a desired matter. Two main groupings include spastic and non-spastic. Each has multiple variations and it is possible to have a mixture of both types.

1. **Spastic Cerebral Palsy** is characterized by increased muscle tone.
2. **Non-spastic Cerebral Palsy** will exhibit decreased or fluctuating muscle tone.

Motor function classification provides both a description of how a child's body is affected and the area of the brain injury.

Muscle tone

Many motor function terms describe Cerebral Palsy's effect on muscle tone and how muscles work together. Proper muscle tone when bending an arm requires the bicep to contract and the triceps to relax. When muscle tone is impaired, muscles do not work together and can even work in opposition to one another.

Two terms used to describe muscle tone are:

1. **Hypertonia/Hypertonic** — increased muscle tone, often resulting in very stiff limbs. Hypertonia is associated with spastic Cerebral Palsy

2. **Hypotonia/Hypotonic** — decreased muscle tone, often resulting in loose, floppy limbs. Hypotonia is associated with non-spastic Cerebral Palsy

Two classifications by motor function: **pyramidal (spastic)** and **extrapyramidal (non-spastic)** When referring to location of the brain injury, spastic and non-spastic Cerebral Palsy is referred to in the medical community as pyramidal and extrapyramidal Cerebral Palsy.

1. Pyramidal, or spastic Cerebral Palsy

   The pyramidal tract consists of two groups of nerve fibers responsible for voluntary movements. They descend from the cortex into the brain stem.

2. Extrapyramidal, or non-spastic Cerebral Palsy

   Indicates the injury is outside the tract in areas such as the basal ganglia, thalamus, and cerebellum. Pyramidal and extrapyramidal are key components to movement impairments. Non-spastic Cerebral Palsy is decreased and/or fluctuating muscle tone. Multiple forms of non-spastic Non-spastic Cerebral Palsy is divided into two groups, ataxic and dyskinetic.

- ❖ Ataxia
- ❖ Dyskinetic

1. Ataxic/ataxia

Ataxic Cerebral Palsy affects coordinated movements. Balance and posture are involved. Walking gait is often very wide and sometimes irregular. Control of eye movements and depth perception can be impaired. Often, fine motor skills requiring coordination of the eyes and

hands, such as writing, are difficult. Does not produce involuntary movements, but instead indicates impaired balance and coordination

2. Dyskinetic

Dyskinetic Cerebral Palsy is separated further into two different groups; athetoid and dystonic.

a) **Athetoid** Cerebral Palsy includes cases with involuntary movement, especially in the arms, legs, and hands.
b) **Dystonia/Dystonic** Cerebral Palsy encompasses cases that affect the trunk muscles more than the limbs and results in fixed, twisted posture.Because non-spastic Cerebral Palsy is predominantly associated with involuntary movements, some may classify Cerebral Palsy by the specific movement dysfunction, such as:
c) **Athetosis** — slow, writhing movements that are often repetitive, sinuous, and rhythmic
d) **Chorea** — irregular movements that are not repetitive or rhythmic, and tend to be more jerky and shaky
e) **Choreoathetoid** — a combination of chorea and athetosis; movements are irregular, but twisting and curving
f) **Dystonia** — involuntary movements accompanied by an abnormal, sustained posture

3. Mixed

A child's impairments can fall into both categories, spastic and non-spastic, referred to as mixed Cerebral Palsy. The most common form of mixed Cerebral Palsy involves some limbs affected by spasticity and others by athetosis

04. Classification based on Gross Motor Function Classification System

Gross Motor Function Classification System, or GMFS, uses a five-level system that corresponds to the extent of ability and impairment limitation. A higher number indicates a higher degree of severity. Each level is determined by an age range and a set of activities the child can achieve on his or her own.

The GMFCS is a universal classification system applicable to all forms of Cerebral Palsy. When the child fits in multiple levels, the lower of the two classification levels is chosen. The GMFCS

classification system recognizes that children with impairments have age-appropriate developmental factors. GMFCS is able to chart by age group (0-2; 2-4; 4-6; 6-12; and 12-18) a developmental guideline appropriate for the assigned GMFCS level. It emphasizes sitting, movement transfers and mobility, charting independence and reliance on adaptive technology.

Cerebral Palsy is often classified by severity level as mild, moderate, severe, or no CP. These are broad generalizations that lack a specific set of criteria.

GMFCS classification,

1. **GMFCS Level I** – walks without limitations.
2. **GMFCS Level II** – walks with limitations. Limitations include walking long distances and balancing, but not as able as Level I to run or jump; may require use of mobility devices when first learning to walk, usually prior to age 4; and may rely on wheeled mobility equipment when outside of home for traveling long distances.
3. **GMFCS Level III** – walks with adaptive equipment assistance. Requires hand-held mobility assistance to walk indoors, while utilizing wheeled mobility outdoors, in the community and at school; can sit on own or with limited external support; and has some independence in standing transfers.
4. **GMFCS Level IV** – self-mobility with use of powered mobility assistance. Usually supported when sitting; self-mobility is limited; and likely to be transported in manual wheelchair or powered mobility.
5. **GMFCS Level V** – severe head and trunk control limitations. Requires extensive use of assisted technology and physical assistance; and transported in a manual wheelchair, unless self-mobility can be achieved by learning to operate a powered wheelchair.

2.7.PATHOPHYSIOLOGY OF CEREBRAL PALSY [13]

The causal factors for CP are multiple and are linked. CP can follow a brain abnormality that occurs pre, peri, or postnatally. Even when the injury occurs at a defined time, moderating factors may also exist. The risk factors for CP include: preterm birth, intra-uterine growth restriction, maternal/fetal infection, inflammation, perinatal and intrapartum difficulties (e.g., hypoxic–ischemic insult), and genetic predispositions. Remarkably, intrapartum hypoxia–ischemia (HI) is thought to account for only 14.5% of CP cases.

2.7.1. PRETERM BIRTH [14]

The premature neonatal brain is susceptible to two main pathologies: intraventricular hemorrhage (IVH) and periventricular leukomalacia (PVL). Although both pathologies increase the risk of CP, PVL is more closely related to CP and is the leading cause in preterm infants. The term PVL describes white matter in the periventricular region that is underdeveloped or damaged ("*leukomalacia*"). Both IVH and PVL cause CP because the **corticospinaltracts**, composed of descending motor axons, course through the periventricular region.

A. Intraventricular hemorrhage (IVH)

IVH describes bleeding from the sub-ependymal matrix (the origin of fetal brain cells) into the ventricles of the brain. The blood vessels around the ventricles develop late in the third trimester, thus preterm infants have underdeveloped periventricular blood vessels, resulting in the increased risk of IVH. The risk of cerebral palsy increases with the severity of IVH.

B. Periventricular leukomalacia (PVL)

IVH is a risk factor for PVL, but PVL is a separate pathological process. The pathogenesis of PVL arises from two important factors:

(1) Ischemia/hypoxia

(2) infection/inflammation

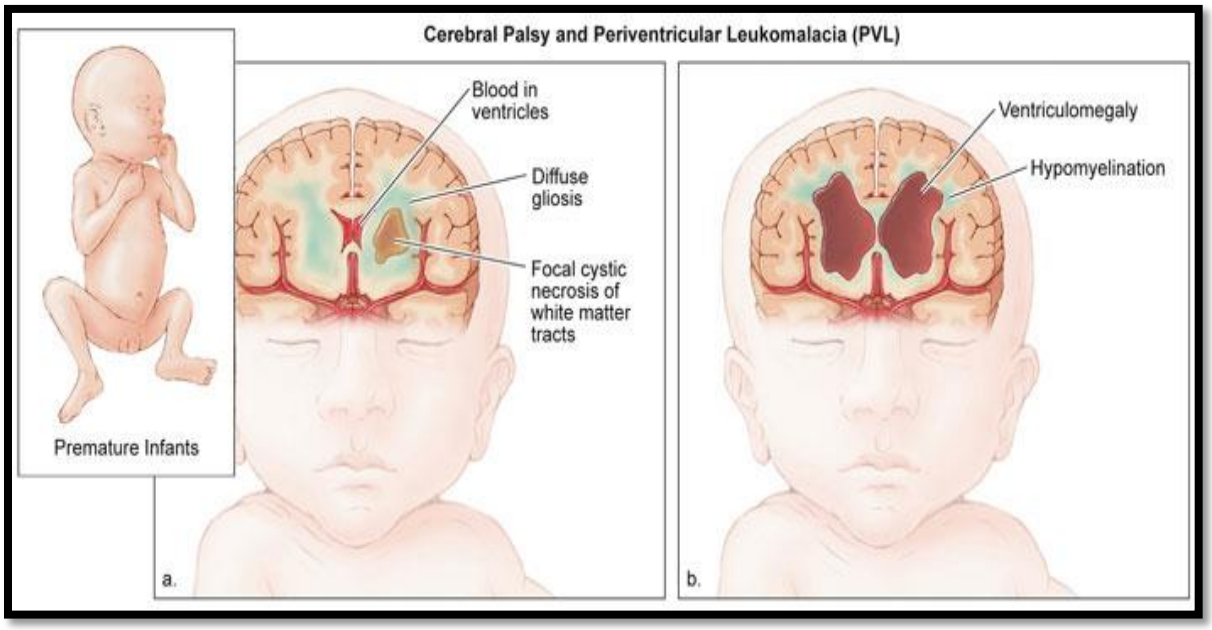


Fig no. 02 Cerebral palsy and Periventricular Leukomalacia

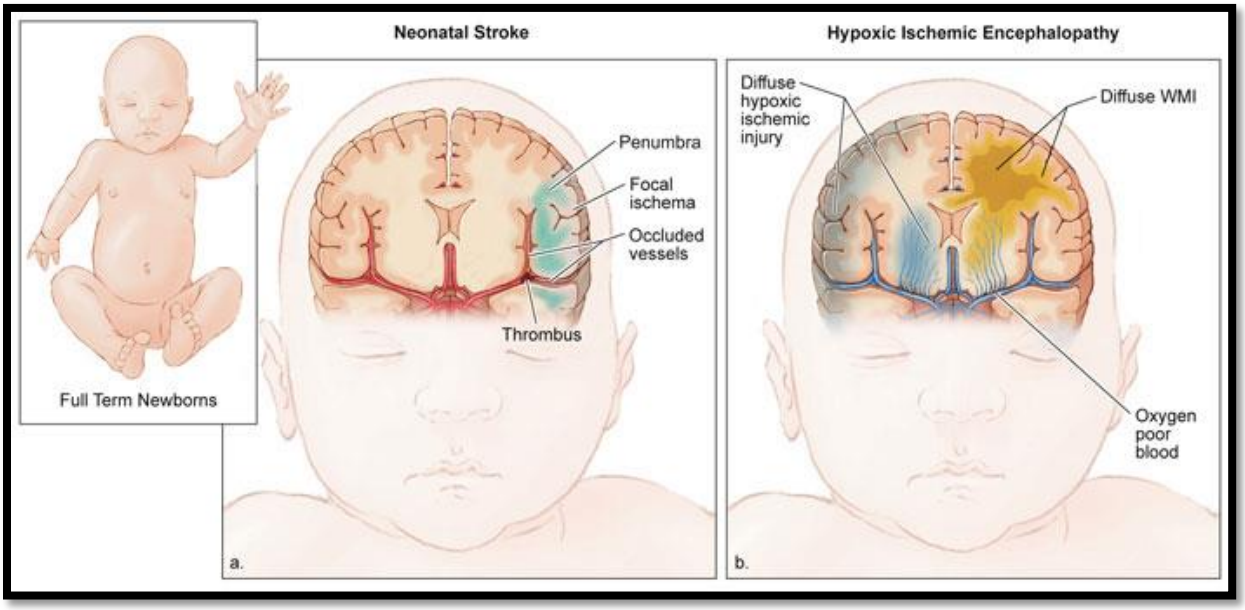


Fig no. 03 Neonatal stroke and H/I encephalopathy

1. Hypoxia/ischemia

   The periventricular white matter of the neonatal brain is supplied by the distal segments of adjacent cerebral arteries. Blood flow from two arterial sources protects the white mater when one artery is blocked, this watershed zone is susceptible to damage from cerebral hypoperfusion i.e., decreased cerebral blood flow in the brain.

   Since preterm and even term neonates have low cerebral blood flow, the periventricular white matter is susceptible to ischemic damage.

2. Infection and inflammation

   Process of infection and inflammation involves microglial (brain macrophage)cellactivation and cytokine release**,** which causes damage to a specific cell type in the developing brain called the oligodendrocyte. The oligodendrocytes are a type of supportive brain cell that wraps around neurons to form the myelin sheath, which is essential for white matter development.

   Intrauterine infections activate the fetal immune system, which produces cytokines (e.g., interferon γ and TNF-α) that are toxic to premyelinatingoligodendrocytes. Infections also activate microglial cells, which release free radicals. Premyelinatingoligodendrocytes have immature immunity against reactive oxygen species (e.g., low production of *glutathione*, an important antioxidant). IVH is hypothesized to cause PVL because iron-rich blood causes iron-mediated conversion of hydrogen peroxide to hydroxyl radical, contributing to oxidative damage.

2.**7.2. EXCITOTOXICITY** [15]

It is a process where increased extracellular glutamate levels stimulate oligodendrocytes to increase calcium influx, which stimulates reactive oxidative species release. Glutamate is increased because hypoxia causes white matter cells to reduce reuptake of glutamate due to lack of energy to operate glutamate pumps. Glutamate is also released from microglial cells during the inflammatory response.

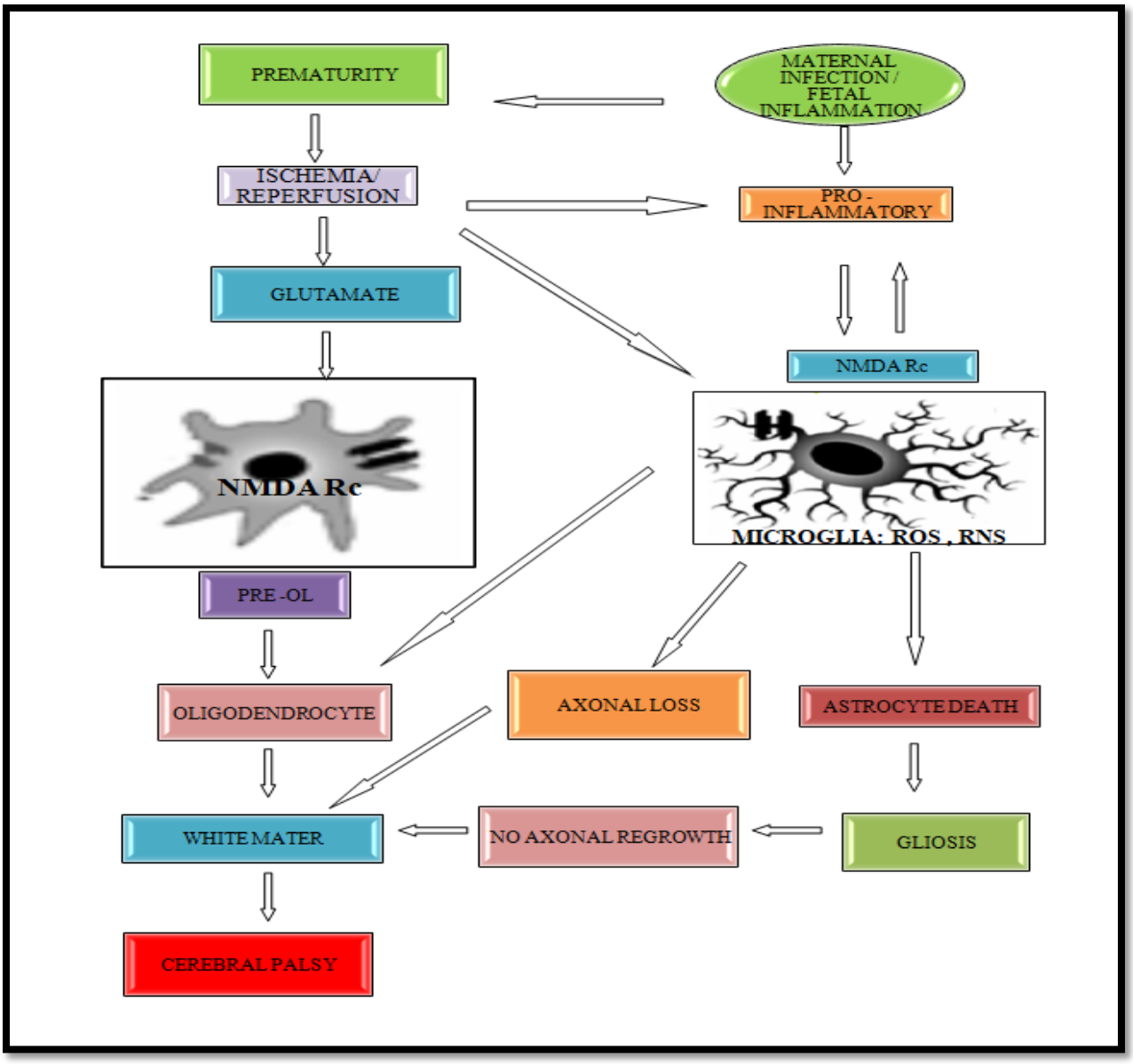


Fig no. 04 Events that leads to Cerebral palsy

2.8. TREATMENT [16]

Treating Cerebral Palsy is almost as complex as the condition is, and there's no specific approach because each individual is affected differently. Although the brain injury that causes Cerebral Palsy cannot be healed, the resulting physical impairment can be managed with a wide range of treatments and therapies. Although there is no universal protocol developed for all cases, a person's form of Cerebral Palsy, extent of impairment, and severity level help to determine care.

There is no present approach to totally prevent or cure CP but many treatments have been developed that can help reduce its impact on the patient's quality of life. As with many diseases, it is best that treatments are initiated early in the course of the disease.

This is especially true with a progressive disease such as CP with limited windows for interventions. Current treatments include physical and occupational therapy and device or equipment to help with mobility and coordination, medications to alleviate motor symptoms.

While therapy and adaptive equipment are the primary treatment protocol for Cerebral Palsy, an individual may also require drug therapy and surgical interventions.

2.8.1. DRUGS IN TREATMENT OF CP [17]

Children who experience seizures, spasticity, and unwanted or uncontrolled movements, such as athetosis and chorea, are prescribed drugs to minimize the movements. Some medications are used to relax muscles, increase comfort and facilitate better posture.

The most common medications used to treat Cerebral Palsy include medications within these categories:

- Anticholinergics (uncontrolled body movements)
- Anticonvulsants (seizure medications)
- Antidepressants (depression medications)
- Antispastic (muscle relaxers)
- Anti-inflammatories (pain management)
- Stool softeners

01. Anticholinergics

Anticholinergic medications are used to treat uncontrolled body movements such as muscle stiffness, tremors and spasms, as well as drooling associated with non-spastic Cerebral Palsy. In large doses, some anticholinergics can stimulate the nervous system; in small doses the drug can act as a depressant.

Some forms of Cerebral Palsy where anticholinergics may also be prescribed include:

- Athetosis – slow, writhing movements that are often repetitive and involuntary
- Chorea – jerky, involuntary, irregular and uncontrollable movements
- Choreoathetoid – a combination of chorea and athetosis, where movements are jerky, twitching, slow and writhing
- Dystonia – prolonged, involuntary contractions that result in twisting motions, tremors and abnormal posture

These drugs work by blocking the neurotransmitter that causes muscles to move. Excessive movement is often caused by excessive flexing of muscles. Nerve impulses cause acetylcholine to be produced and travel across the gaps between nerves. When it binds to the second nerve, it fires, eventually stimulating the prospective muscle. Anticholinergics prevent the acetylcholine from binding to the second nerve, thus limiting muscle stimulation.

Common anticholinergic medications are:Benztropinemesylate, Carbidopa-levodopa, Glycopyrrolate, Procyclidine hydrochloride, Trihexyphenidyl hydrochloride

02. Anticonvulsants

Anticonvulsants are prescribed to reduce or prevent seizure activitythat may occur. They also stabilize mood. Anticonvulsants reduce, or depress, excessive stimulation to the brain without affecting respiration or causing drowsiness. Different drugs control different types of seizures.

Common anticonvulsants include:Gabapentin, Lamotrigine, Oxcarbazepine, Topiramate, Zonisamide.

03. Antidepressants

Antidepressants are psychiatric medications with mood altering and analgesic qualities used to treat depression, anxiety, and in some cases seizures. One-third of those with a chronic illness, such as Cerebral Palsy, may be depressed. Depression is a persistent sense of sadness, helplessness, and hopelessness.

In addition, depression can worsen the medical condition by:

- Aggravating the Cerebral Palsy
- Decreasing confidence
- Increasing fatigue
- Intensifying pain
- Worsening ability to cope

Treatment for depression is multi-faceted. Focus is on gaining emotional support, guidance, counseling, and perspective. Antidepressants can also be harmful during pregnancy. Drug therapy can be used exclusively or in combination with any of the following:

- Behavioral therapy – to identify and address perception and gain new perspective
- Biofeedback and massage therapy – to relax, meditate, calm, peacefully escape, and energize
- Healthy diet and exercise – to provide the body with essentials to cope
- Sleep – to rest properly and re-energize

Side effects from antidepressants may include:

- Agitations, blurred vision, constipation, dizziness, dry mouth, headache, insomnia, nausea, nervousness, stomach upset,

Some commonly prescribed antidepressants include:

- Citalopram, lexapro, Prozac, paxil, zoloff.

04. Anti-Spastic Drugs

Antispastics, also known as muscles relaxers, are prescribed to relax contracted, overactive, or stiff muscles. Antispastic medications are often the first treatment choice for reducing tremors or controlling widespread spasticity.

Oral medications are usually prescribed for overall spasticity, while injections target a specific muscle. The benefits of oral medications and injections are usually short-term, requiring refills or further injections.

Alcohol wash injections target specific nerves and can provide relief for a few months to several years. Botox is usually administered in three-month intervals. Other injectable antispasmodics typically remain effective for about three to eight months. The intrathecal baclofen pump is found to be most effective on those with chronic, severe stiffness and those with uncontrolled muscle movements.

Some benefits derived from antispastic medications include:

- Control muscle contractions
- Increase range of motion
- Reduce tremors and muscle spasms
- Relax overactive muscles
- Relax tight muscles

Possible side effects to anti-spastic medications may include: Confusion, constipation, diarrhea, dizziness, drowsiness, flu-like symptoms, headache, high blood pressure liver damage, slurred speech.

Medications vary in the way they relax muscles. Some, like baclofen, relax the muscle directly, while others, like diazepam, act on brain chemistry. Botox (botulinium toxin) causes mild muscle paralysis. Commonly prescribed anti-spastic medications include:

Botulinum toxin, or Botox, diazepam, dantrolene, tizanidine.

05. Anti-Inflammatory Drugs

Medscape reports 67%-84% of individuals with Cerebral Palsy experience pain lasting one hour or longer per episode. Anti-inflammatory agents alleviatepain by reducing inflammation.

Many medications and alternative treatments are available to manage pain often associated with Cerebral Palsy. Pain medication includes non-steroidal, anti-inflammatory drugs (NSAIDs) or anti-inflammatory corticosteroids. Many of these medications play a significant role in reducing pain as they alleviate the primary causes of discomfort.

Pain can result from the actual health conditions involved with Cerebral Palsy, or can be experienced when exercising, strengthening and expanding range-of-motion during physical therapy.

Some common origins of pain in those with Cerebral Palsy, include:

- Gastrointestinal pain – abdominal pain related to digestive complications from malnutrition, malabsorption, impaired orofacial functioning, esophageal issues, aspiration, incontinence, constipation and flatulence.
- Orthopedic pain – deformity, compensation, subluxation, degeneration, and dislocation found in the face, fingers, hands, arms, shoulders, back, spine, hips, pelvis, legs, ankles, feet or toes. Procedures performed on the bones, ligaments, joints, tendons, muscles, and nerves. Orthotic overuse, underuse, skin irritation, and rubbing can cause pain, as well.
- Rehabilitative therapies – pain worsened by therapy sessions involving assisted stretching and range of motion exercises, including occupational, physical, speech and language therapy.
- Surgical pain – pain derived from surgical procedure, post-operative healing, and needle injections such as blood draws, pain medication, and intravenous feeds.

Pain medication can be helpful in reducing or alleviating pain. Four main categories of anti-inflammatories include:

- Aspirin – suppresses prostaglandins, regulates body temperature, and constricts blood.

- Corticosteroids – anti-inflammatory agent with a large number of other functions, such as glucose utilization, fat metabolism, and bone development.
- Nonsteroidal anti-inflammatory drugs (NSAIDs) – a non-steroid substance which has analgesic, antipyretic, and platelet-producing capabilities to primarily treat mild to moderate pain, and fever.
- Steroids – decrease inflammation and reduce immune system activity.

06. Stool Softeners

Constipation is common in children with Cerebral Palsy. The condition can be caused by medications, lack of activity, inadequate fluid intake, dietary complications, and difficulty in accessing bathrooms and labored breathing.

The primary way to treat constipation is with a healthy diet rich in high-fiber foods. Laxatives, which can be very useful, come in several forms. Stool softeners help to maintain water content to prevent stool from becoming hard and painful. Various stimulants induce bowel contractions and move stool along the intestinal tract.

2.8.2. CONVENTIONAL & COMPLEMENTARY TREATMENT [18]

Conventional treatment methods involve systems, practices and products that have been researched, tested and approved by the medical community as acceptable forms of treatment. Complementary medicine, when used under doctor supervision, can be used as a complement to an existing treatment plan. Alternative medicine is a treatment method that is used to replace conventional medicine.

Six forms of complementary and alternative medicine

The field of complementary medicine and alternative medicine is divided into six categories. They are:

A. Biological-based therapies

Biological-based alternative treatments use substances that are found in nature. Echinacea and fish oil with omega 3s are common biological based therapies. These include amino acid, animal-derived extracts, botanicals, fatty acides, minerals, prebiotics, probiotics, proteins and vitamins.

B. Energy therapies

Energy therapies involve manipulation of energy fields (electromagnetic fields and biofields/putative fields) by channeling energy fields from the practitioner to the client to affect health and well-being. Energy therapy techniques include magnet therapy, light therapy, healing touch and Reiki.

C. Manipulative and body-based methods

In manipulative and body-based methods, the body systems and structures (bones, joints, soft tissues, circulatory system and lymphatic systems) are manipulated beyond their passive range of motion and with appropriate use of force. This method is commonly part of chiropractic and osteopathic medicine. Methods in this category include massage therapy and spinal manipulation.

Mind and body methods attempt to use the mind techniques to affect physical function and to promote health. The concept of using the mind to treat illness is an important approach used in traditional Chinese medicine, Ayurvedic medicine and referenced in moral and spiritual aspects of the healing process by Hippocrates, the father of Western medicine. Mind-body interventions include acupuncture, deep-breathing exercises, guided imagery, hypnotherapy, meditation, progressive relaxation, tai chi and yoga.

D. Movement therapies

Movement therapies use body movement control in promoting emotional, mental, physical and spiritual balance and well-being. There are a broad range of Eastern and Western movement

methods including Alexander Technique, Feldenkrais Method, Pilates, Rolfing Structural Integration and Trager Psychophysical Integration.

E. Whole medical systems

Whole medical systems are complete medical systems of theory and practice evolved in various cultures over time. They do not include Western medicine or conventional medicine. They instead include ancient whole medical systems such as Ayurvedic medicine and traditional Chinese medicine, as well as modern whole medical systems such as homeopathy and naturopathy.

3. ANIMAL MODELS FOR CERBRAL PALSY [19-21]

Animal models are commonly used to investigate the various pathophysiological mechanisms underlying the development of diseases. A critical aspect for the modeling of CP is the timing of the brain insult as there are species differences in the timing of key brain maturation events. It has been reported that in rodents the period between embryonic day 17 and postnatal day 7-10 replicates many features seen in the third trimester in humans. Animal models of CP have been developed in a variety of species including mice, rats, rabbits, sheep and non-human primates. The most commonly used models include,

1) Models of hypo-perfusion or HI model
2) Models using either infectious agents or bacterial products
3) Excitotoxic insults.
4) Models of stroke

3.1. HYPOPERFUSION MODEL

- In the Rice-Vannucci HI model, rats undergo unilateral ligation of the carotid artery followed by exposure to 8% oxygen hypoxic air.
- Although these animals are used to study CP since the animals show brain damage, hypoxia/ischemia, inflammation and motor and cognitive deficits.
- Many modifications of the HI model have been made to try to get a model that better recapitulates features of human CP.
- Some of these modifications included varying the day of the animals used and the example of hypoxic conditions.
- In the Rice-Vannucci model, there is extensive damage to the gray matter.
- Another model reduced the hypoxic conditions to 6% oxygen in an attempt to produce less severe brain damage.

3.2. INDUCTION USING INFECTIOUS AGENTS

- One of the models used in the study of CP is based on lipopolysaccharide (LPS) as brain inflammation-inducing agent.
- This toxin is a potent inducer of inflammation and has different effects on cells of the immune system, as microglial cells.
- This produces model of brain inflammation in rats based on the unilateral stereotaxic injection of LPS, which mimics the inflammatory reaction produced in brain diseases such as Parkinson's, Alzheimer disease and CP.
- For establishment of chronic neuroinflammation such as Parkinson's disease (PD) potent inflammatory agent, lipopolysaccharide (LPS) is used in order to gain better understanding of immune-mediated events in PD.
- However, the effect of intra-cerebral LPS on neuroinflammation and neurodegeneration and its impact on motor function has been less well studied.

3.3. FETAL SHEEP MODEL

- There are several larger animal models besides mice and rats that might be more applicable to humans.
- Fetal sheep have shown to have advantages over rodent models. Repeated measurements can be performed in-utero and they exhibit similarities to the third trimester in humans that can be accurately replicated.

3.4. RABBIT IN-VIVO UTERINE MODEL

- In rabbits, in vivo uterine ischemia by inflation of an aortic balloon at a level proximal to uterine arteries resulted in hypertonia and neurobehavioral findings in rabbits mimicking those found in CP.

3.5. PERINATAL ASPHYXIA MODEL

- In pigtailed macaques CP was modeled using an acute perinatal asphyxia model. Occlusion of the umbilical cord for 12-18 minutes before birth induced hypoxic-ischemic

conditions resulting in neurodevelopmental and long term physical and cognitive deficits associated with CP.

3.6. RAT MODELS OF STROKE

- Animal models of stroke show many features of CP and have been used to study the disease.
- These models have reproduced the hypoxic-ischemic (HI) conditions, the apoptotic-necrotic pattern of white and gray matter damage and the neuromotor impairments.

These models are important to develop early treatments and to determine if there are factors that worsen the condition. Another way to try to replicate human CP is to introduce inflammatory agents in animal models since these agents play a role in brain injury. However, it has been found that only some aspects of the human condition are replicated by this approach. Additionally, these studies were performed at any early point in the gestation of the animal models and do not correlate with the timing of the development of CP. It has also been found in the animal models that gestational stress worsens the condition.

4. TREATMENT DRUGS UNDER STUDY

Presently there is no systematic approach to totally prevent or cure CP but treatments have been developed that can help reduce itsimpact on the patient's quality of life. As with many diseases with varied symptoms, it isbest that treatments are initiated early in the course of the disease.Despite various therapies available to alleviate symptoms of CP, there remains an unmet need for better preventive and earlytherapies for rescue. The following class of drugs was chosen to study on the treatment of cerebral palsy,

- VITAMIN- E TOCOPHEROL
- DHA – DOCOSA HEXANOIC ACID
- MEMANTINE

5. OXIDATIVE STRESS & VITAMIN-E [24-28]

Oxidative stress may result in neuroinflammation, which is a highly recognized part of cerebral ischemia as well as many neurodegenerative diseases including cerebral palsy, Parkinson's disease, Alzheimer's disease, and Amyotrophic Lateral Sclerosis.

A target in neuroprotective treatments was to reduce the induced oxidative stress because of their role in causing neuron apoptosis. Oxidative stress can directly cause neuronal cell death or it can trigger a cascade of events that may result in protein misfolding, proteasomal malfunction, mitochondrial dysfunction, or glial cell activation. Further neurodegradation is caused as each of these events causes' neuron cell apoptosis. Neuroprotectives acts by eliminating this oxidative stress, therefore further neurodegradation can be inhibited. In order to eliminate the oxidative stress Anti-oxidants were employed.

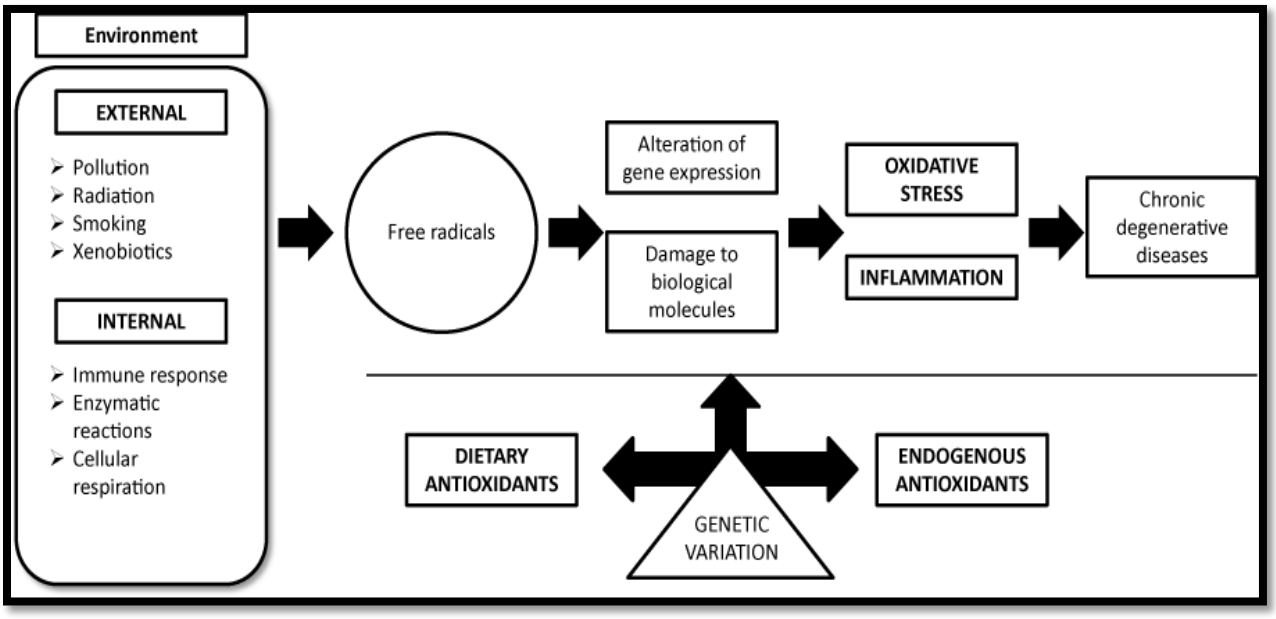


Fig. No. 05 Free radicals and Oxidative stress

Antioxidants are the primary treatment used to control oxidative stress levels. Antioxidants work to eliminate reactive oxygen species, which are the prime cause of neurodegradation. The effectiveness of antioxidants in preventing further neurodegradation is not only disease dependent but can also depend on gender, ethnicity, and age.

Dietary antioxidants protect neurons against a variety of experimental neurodegenerative conditions. Several natural components and vitamins have potential against a variety of oxidative stress-induced neurodegenerative diseases.

5.1. VITAMIN – E (Tocopherol)

Vitamin E is a group of fat-soluble compounds that contain antioxidant distinctive activities. Vitamin E exists in 8 chemical forms; alpha, beta, and delta-tocopherol and alpha-, beta-, gamma-, and delta-tocotrienol. In general, Vitamin E is a fat-soluble antioxidant that prevents the production of ROS formed during the process of the oxidation of fat. Scientists are currently investigating whether vitamin E could help prevent or slow the chronic diseases associated with free radicals.

Deficiency in vitamin E can cause nerve and muscle damage to the point of loss of feeling in arms and legs, muscle weakness, and vision problems. It can also result in a weakened immune system.

Vitamin E has many important biological functions which include:

1. Antioxidant.
2. Enzymatic Activities
3. Gene Expression
4. Neurological Functions
5. Cell Signaling
6. Protects Lipids and Prevents Oxidation of Polyunsaturated Fatty Acids

5.2. SOURCES

- PLANT FOODS:

  Sunflower seeds, wheat germ, almonds, hazelnuts, peanuts, vegetable oils, dark green leafy vegetables, taro, sweet potato, pumpkin, avocado, kiwifruit, fortified ready-to-eat cereals, nutrition bars, margarines, peanut butter and beverages.

- ANIMAL FOODS:

  Oily fish (sardines, salmon, herring, trout, mackerel), mollusks (abalone, conch, snails), shrimps.

5.3. FORMS OF VITAMIN-E

Vitamin E can be classified into two types - Tocopherol s and Tocotrienols. Each of these forms is named with prefixes such as alpha, beta, gamma, and delta. Among these forms, α-Tocopherol is of biological and physiological importance.

- **α-Tocopherol**

  Alpha Tocopherol mainly serve as an antioxidant in humans. Other functions of alpha-tocopherol is to inhibit the activity of protein kinase C. Protein kinase C is an important molecule for cell-signaling. Alpha-tocopherol also affects the activity of enzymes in immune and inflammatory cells.

- **γ-Tocopherol**

  Gamma Tocopherol is the most common form of Vitamin E in North American diet. However, its function is not as well-known as alpha tocopherol .It appears that alpha-tocopherol lowers gamma-tocopherol levels

5.4. MECHANISM OF ACTION

- α - Tocopherol exhibits significant inhibition of neurotoxicity induced by a non-specific protein kinase inhibitor.
- This PK inhibitor is known to induce apoptosis in many types of cells including neurons.
- It is also known that α-tocopherol protects striatal neurons by the reduction of oxidative stress gradually by decreasing intracellular $O_2^{\cdot -}$ levels, and by inhibition of apoptosis.
- α - Tocotrienol prevents glutamate-induced death of immature and mature primary cortical neurons. Also it reduces the production of IL-1β, IL-8 and TNF-α by leukocytes.
- It also protects the integrity of the membrane by inhibiting the lipid peroxidation.

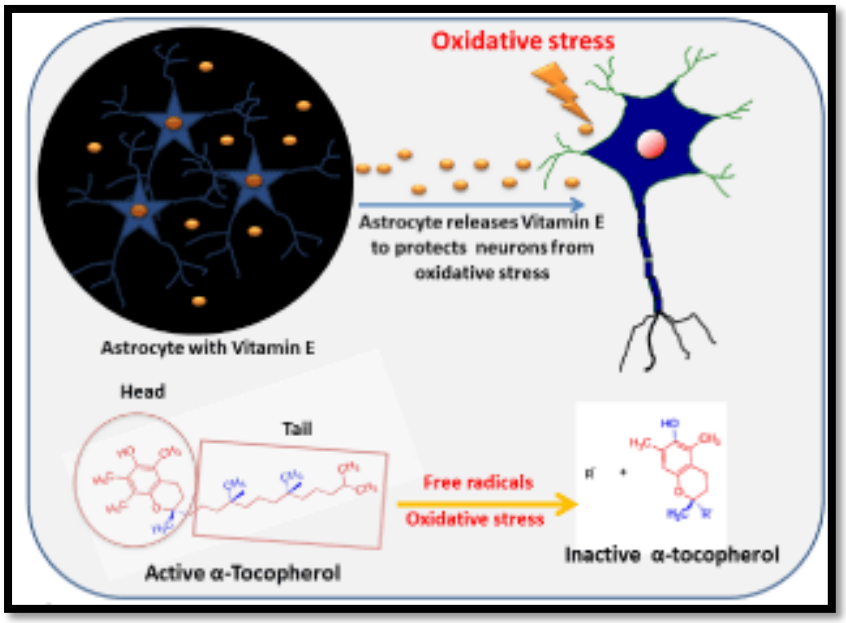


Fig. No. 06 Vitamin – E in oxidative stress

5.5. NEUROPROTECTIVE EFFECTS

Vitamin- E is capable of participating in the reduction of oxidative stress in diabetic patients by its antioxidant activity.

It is also known that the use of vitamin-E after ischemic injury not only triggered the oxidative injury of the muscle cells but also reduced the formation of edema. It has protective effects on the retina during retinal ischemia-reperfusion injury. Also patients receiving cisplatin chemotherapy with vitamin-Edecreased the incidence and severity of peripheral neurotoxicity.

It is most effective in Alzheimer's disease and has been shown to haveneuroprotection effects when treating ALS. A meta-analysis involving 135,967 participants showed there is a significant relationship between vitamin E dosage and all-cause mortality, with dosages equal to or greater than 400 IU per day showing an increase in all-cause mortality. However, there is a decrease in all-cause mortality at lower doses, optimum being 150 IU per day. Vitamin E is ineffective for neuroprotection in Parkinson's disease.

It also protects the integrity of the membrane by inhibiting the lipid peroxidation.

6. DHA – AN ESSENTIAL FATTY ACID FOR THE BRAIN [29-32]

DHA, an essential fatty acid for the central nervous system DHA is the major n-3 PUFA constituent in the neuronal membranes, enhances synaptic activities in neuronal cells present in approximately 30-40% of the phospholipids of the gray matter of cerebral cortex and

Its effects on neuronal lipid composition, neurochemical signaling and cerebrovascular pathobiology, docosahexaenoic acid (DHA) emerges it as a neuroprotective agent against cerebrovascular disease.

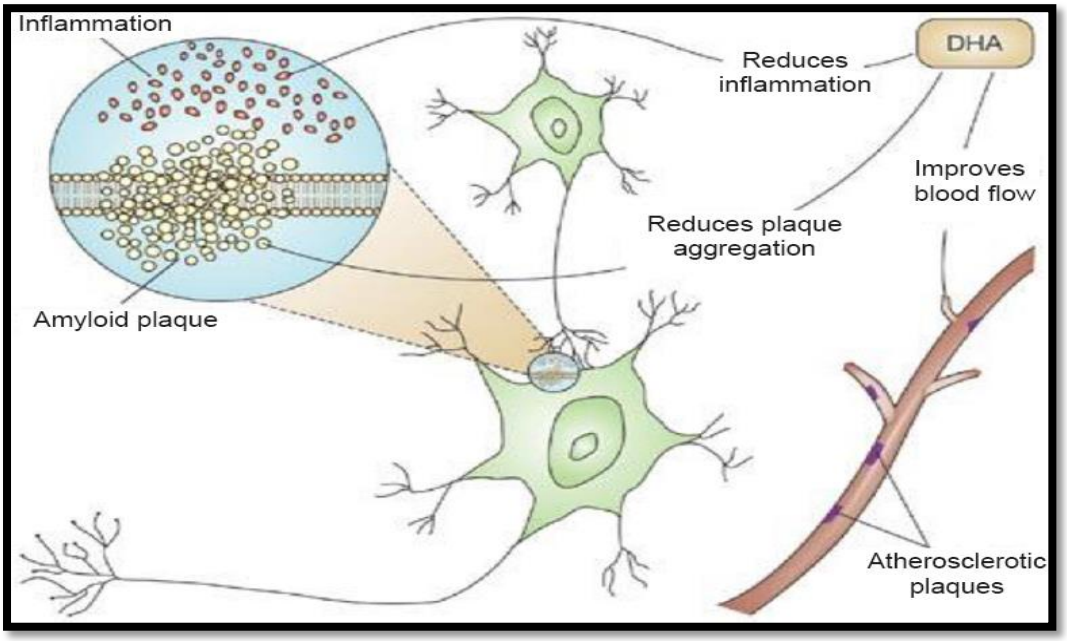


Fig. No. 07 Mechanism of action of DHA

Brain tissue has the highest concentration of DHA in the body, suggesting that brain function relies in unique ways on this powerful fatty acid. Within the brain itself, regions most closely related to memory show the greatest DHA concentrations. And even at the cellular level, DHA concentrates in the structures involved in forming new memories, such as synaptic membranes and tiny outgrowths called "neurites."

DHA resides almost exclusively in cell membranes, which undergo continuous degradation and renewal.That means in turn that the body needs a constant dietary supply of DHA to avoid depletion and neuronal injury.

6.1. DIETARY INTAKE

DHA can be obtained through diet or from α-linolenic acid. Other than diet neurons lack the enzymes necessary for de novo DHA and arachidonic acid synthesis, hence DHA gets synthesized from the dietary precursors, a- to a limited extent via conversion from its precursor, α-linolenic acid (α-LNA) linoleic acid in liver and in a minor way in cerebral endothelium from where they are exported to neuronal cells.

DHA supplementation in humans at 2g/day provides sufficient DHA to maintain healthy function, but levels drop when the patients stopped taking the supplements.

6.2. FUNCTIONS OF DHA

DHA influences how the brain develops and functions,

- ✓ It helps to determine brain structure, and it protects brain tissue from damage.
- ✓ DHA's protective effects come from three distinct mechanisms: DHA protects brain tissue from inflammatory damage. It accomplishes this by promoting development of anti-inflammatory molecules while suppressing pro-inflammatory molecules in brain cell membranes.
- ✓ DHA stimulates physical changes that underlie learning and memory.
- ✓ DHA promotes outgrowth of neurites, the tiny projections that form intimate connections between cells as memories form.
- ✓ DHA promotes rapid signal transduction across synapses and helps membranes maintain their fluidity, a condition required for rapid changes in shape and function we recognize as memory.
- ✓ DHA promotes healing after injury to brain tissue. Immediately after such an injury, cell membranes release DHA in massive amounts for conversion into compounds called protectins.

- ✓ DHA is necessary to maintain proper physical conformation of ion channels, receptors and transporters in membranes in cells involved in memory, synaptic membrane biogenesis and function and neuroprotection .
- ✓ Depletion of DHA induces extensive damage of sensory, behavioral and cognitive function
- ✓ DHA improves behavioral function, decreases infarct volume, promotes cell survival in the ischemic penumbra as well as resolution of cerebral edema photoreceptor cells in the retina.

6.3. REDUCED DHA LEVELS

Reductions in perinatal brain DHA accrual are associated with deficits in neuronal arborisation, multiple indices of synaptic pathology including deficits in serotonin and mesocorticolimbic dopamine neurotransmission, neurocognitive deficits, elevated behavioral indices of anxiety, aggression and depression and decreased visual acuity. In primates and humans, preterm delivery has been shown to be associated with the same troubles which can be reverted by n-3 PUFA supplementation [9]. After the perinatal brain development, DHA intake remains essential for the normal maintenance of brain functions including synaptic plasticity, neurotransmission and vision.

6.4. EFFECTS OF PERINATAL DHA

In the last trimester of fetal life and the first two years of childhood, the brain undergoes a period of rapid growth termed the "brain growth spurt". During this period, the need in this PUFA is dramatically elevated because of the increase in brain size and in relative DHA contents. The polyunsaturated fatty acid docosahexaenoic acid (DHA) has intrinsic neuroprotective properties and investigated for utilizing it as an oral supplement for pregnant women.

Animal studies have demonstrated that reductions in perinatal brain DHA accrual are associated with deficits in neuronal arborisation, multiple indices of synaptic pathology including deficits in serotonin and mesocorticolimbic dopamine neurotransmission and neurocognitive deficits. In humans, preterm delivery is associated with disorders those which can be reverted by providing

DHA supplementation. DHA intake remains essential even after the complete brain development for the normal functioning of the brain that includes synaptic plasticity, neurotransmission and vision.

7. EXCITOTOXICITY AND MEMANTINE [33-35]

Glutamate excitotoxicity is one of the most important mechanisms known to trigger neuronal cell death in cerebral palsy. Excitotoxicity is involved in many types of acute and chronic CNS neurodegenerative disorders and is connected with Ca2+ overload. Disturbance of glutamate homeostasis probably plays a pivotal role in neuropathology triggered by other factors such as: energy deficits, free radicals formation, etc. that facilitate the neurotoxic potential of endogenous glutamate.

Although glutamate is a crucial mediator of physiological communication between neuronal cells, under certain conditions activation of glutamate receptors kills neurones - a term called "excitotoxicity".

7.1. GLUTAMATE RECEPTORS

Glutamate is the fast excitatory neurotransmitter in the mammalian CNS. It activates three major types of ionotropic receptors, namely c-amino-3- hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate and N-methyl-D-aspartate (NMDA) and several types of metabotropic receptors.

AMPA receptors are probably involved in all forms of fast glutamatergic neurotransmission. All AMPA receptors are permeable to Na + and K + while complexes lacking GluR2 subunits are permeable to Ca 2+. NMDA-sensitive ionotropic glutamate receptors are permeable to Na +, K+, and Ca 2+. NMDA receptors are only activated following depolarisation of the postsynaptic membrane which physiologically follows AMPA receptor stimulation. This unique feature and the high Ca 2+ permeability renders NMDA receptors inherently suitable as mediators of synaptic plasticity (e.g. learning and memory). Uncompetitive NMDA receptor antagonist memantine block the NMDA channel in the open state.

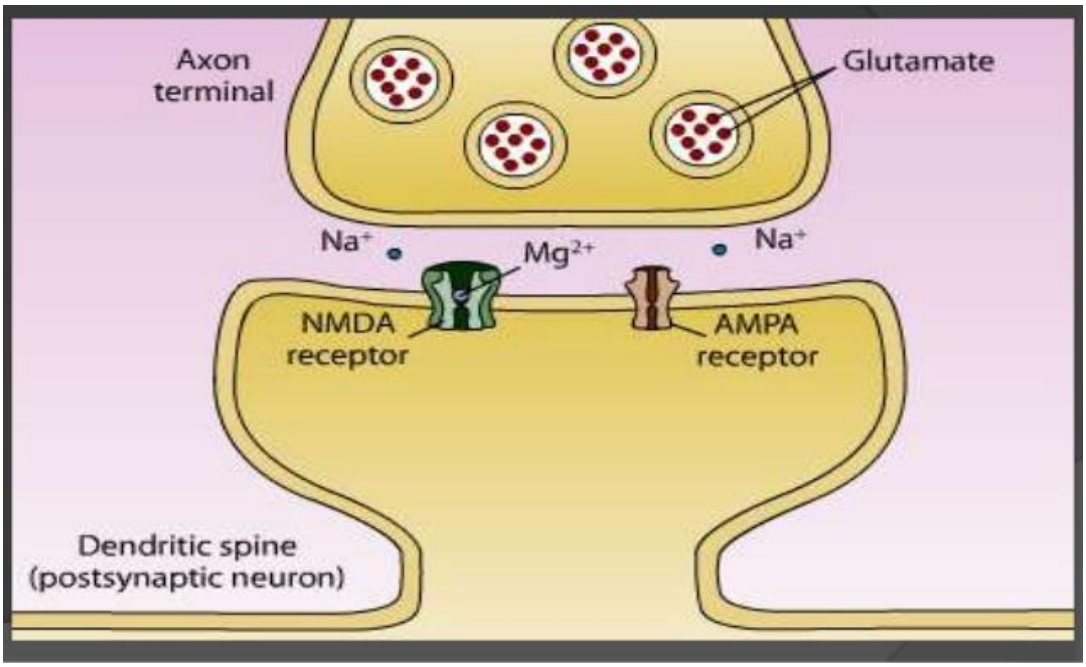


FIG. NO. 08 GLUTAMATE RECEPTOR

Glutamate is involved to some degree in virtually all CNS functions from primary sensory perception to cognition. The hippocampus is the structure most often connected with memory formation. All fast excitatory projection pathways to, within and from the hippocampus utilise glutamate as a transmitter. This brain region, due to its highly organised structure, is ideally suited for studies on long term potentiation (LTP) which is believed to represent an elementary feature of memory formation at the neuronal level. LTP refers to a persistent increase in synaptic sensitivity following high frequency stimulation of input neurones. NMDA receptors are involved in mediating the postsynaptic components of LTP in the hippocampus e.g. in the Schaffer collateral projection.

7.2. PATHOGENESIS

The neurotransmitter glutamateactivates several classes of metabotropic receptor and three major types of ionotropic receptor—α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kinates and N-methyl-d-aspartate (NMDA).

Over-excitation of NMDA (Glutamate) receptors, results in an increase in calcium ion (Ca^{2+}) influx due to the lack of specificity in the ion channel opened upon glutamate binding. As Ca^{2+} accumulates in the neuron, the buffering levels of mitochondrial Ca^{2+} sequestration are exceeded, which has major consequences for the neuron. Because Ca^{2+} is a secondary messenger and regulates a large number of downstream processes, accumulation of Ca^{2+} causes improper regulation of these processes, eventually leading to cell death. Ca^{2+} is also thought to trigger neuroinflammation, a key component in all CNS disorders.

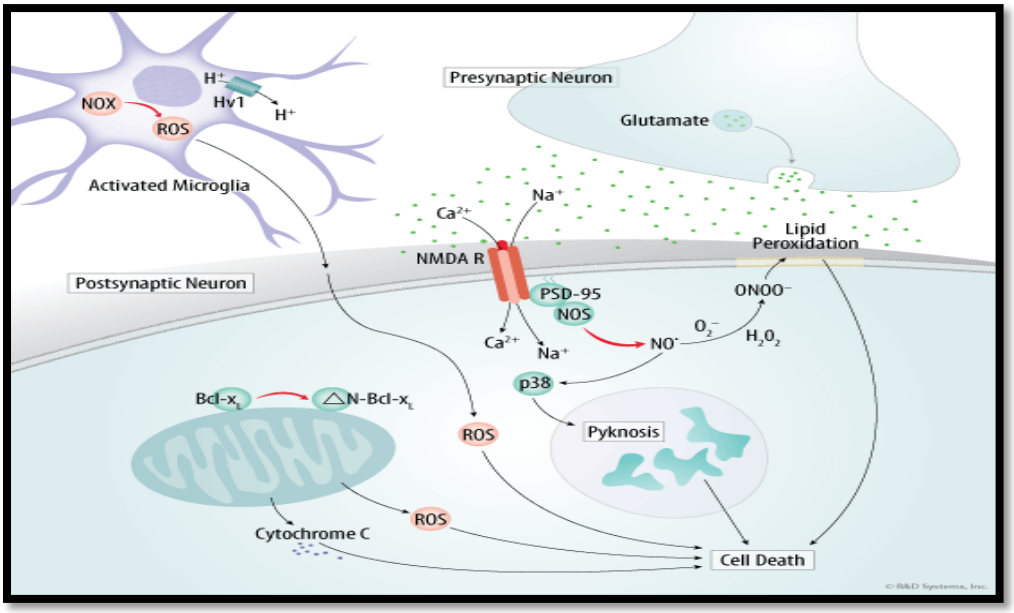


Fig. No. 09 Excitotoxicity & Neuronal Death

7.3. GLUTAMATE ANTAGONISTS

Glutamate antagonists are the primary treatment used to prevent or control excitotoxicity. The goal of these antagonists is to inhibit the binding of glutamate to NMDA receptors such that accumulation of Ca^{2+} and therefore excitotoxicity can be avoided.

Memantine is a moderate affinity, uncompetitive NMDA receptor antagonist with strong voltage-dependency and fast kinetics.The mechanism of action (MOA) of memantine is to provide both neuroprotection and reverse deficits in learning/memory by the same MOA.

7.4. MECHANISM OF ACTION

Memantine'sneuroprotective effect also increases brain levels of the neuronal marker, *N*-acetyl aspartate (NAA). Because NAA is found primarily on neuronal axons in the brain, hence the neuroprotective effect of memantine can be measured by quantifying the change in NAA concentrations in brain tissue.

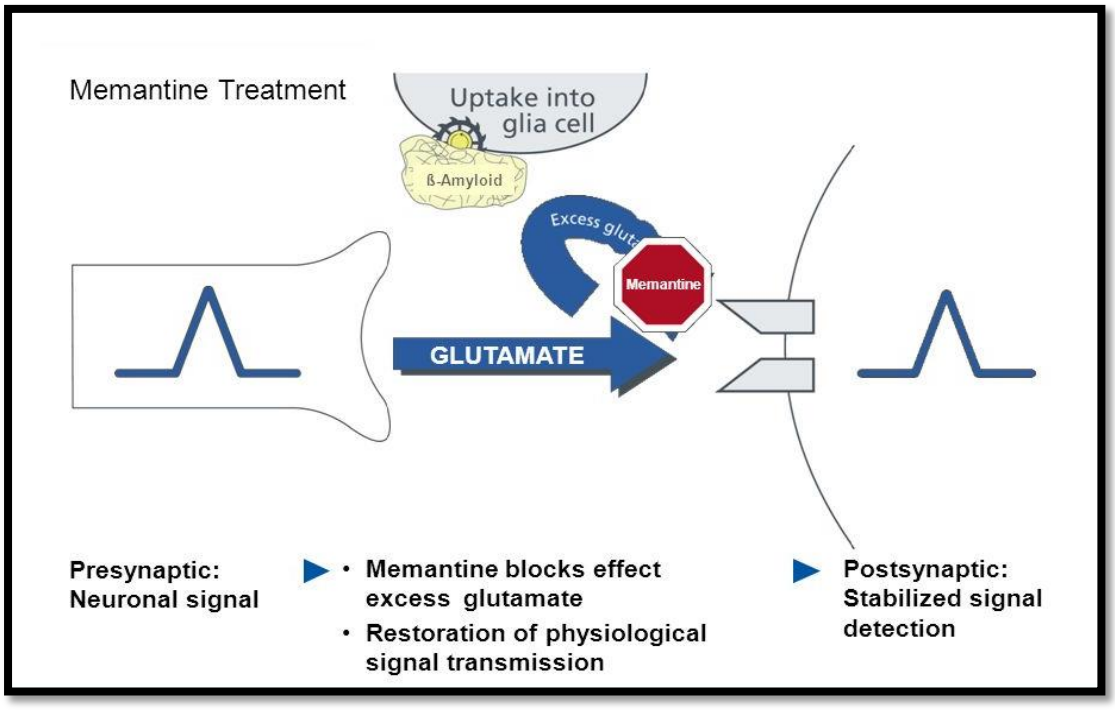


Fig. No. 10 MOA of Glutamate receptor

Memantine can interact with a variety of ligand gated ion channels. However, NMDA receptors appear to be a key target of memantine at therapeutic concentrations. Memantine is an uncompetitive (channel blocking) NMDA receptor antagonist. Memantine at high concentrations can inhibit mechanisms of synaptic plasticity that are responsible learning and memory. At lower concentrations memantine promotes synaptic plasticity and preserve or enhance memory. In addition, memantine can protect against the excitotoxic destruction of cholinergic neurons.

8. LITERATURE REVIEW FOR CEREBRAL PALSY

1. **Establishing a rat model of spastic cerebral palsy by targeted ethanol injection**

Yadong Yu *et al.*, reported that ethanol as a chemical ablation agent specifically and thoroughly damages the pyramidal tract, and therefore, the animals display flexion spasms, which are a typical symptom of the cerebral palsy.

Neural regeneration research, 2013; 8(34): 3255-3262.

2. **Animal Models and Treatments for Cerebral Palsy**

Fran norflus *et al.,* reported the therapies available to alleviate symptoms of cerebral palsy that remains as an unmet need for better preventive and early rescue therapies. Also discussed about the animal models that are being developed that mimic some aspects of the disease. Small animal models are best at reproducing the molecular events and large animals might be closer anatomically to model the human form of the disease.

SciMedCentral, 2016; 1(1): 1008.

3. Rodent Hypoxia–ischemia Models for Cerebral Palsy Research: A Systematic Review

Prakasham Rumajogee *et al.,* compared and discussed the advantages, limitations, and the translational value for Cerebral palsy research of HI models of perinatal brain injury.

Frontiers in Neurology, 2016; 7: 57.

4. Animal Models of Cerebral Palsy: Hypoxic Brain Injury in the Newborn.

Wilson*et al.,* reported the Hypoxic insults that are implicated in the spectrum of fetal disorders, including cerebral palsy (CP). In view of the major contribution of intrapartum risk factorsand prematurity to subsequent neurological morbidity and mortality in humans, it aimed to clarify the pathophysiology of brain injury, especially periventricular white matter damage (WMD), that occur in utero to the immature and near-term fetal CNS.

Iran J Child Neurol. 2015; 9(2): 9–16.

5. Treadmill exercise improves motor and memory functions in cerebral palsy rats through activation of PI3K-Akt pathway

Sun-Young Jung *et al.,* evaluated the effects of treadmill exercise on motor and memory functions in relation with phosphatidylinositol 3-kinase (PI3K)-Akt pathway using CP rat model. Memory functions improvements were evaluated using Rota-rod test, step-down avoidance task, 5-bromo-2'-deoxyuridine (BrdU) immunohistochemistry, and western blot for synapsin I, postsynaptic density-95 (PSD-95), PI3K, Akt, and glycogen synthase kinase-3β (GSK-3β).
Journal of Exercise Rehabilitation 2017; 13(2): 136-142.

6. Erythropoietin Attenuates Lipopolysaccharide-Induced White Matter Injury in the Neonatal Rat Brain

Kumral *et. al.,* demonstrated that a protective effect of EPO on LPS-induced WM injury in the developing brain. Regarding the wide use of EPO in premature newborns, this agent may be potentially beneficial in treating LPS-induced brain injury in the perinatal period.

Neonatology. 2007; 92(4): 269-78.

7. IGF-1 Can Either Protect Against or Increase LPS-Induced Damage in the Developing Rat Brain

Yi pang *et. al.,* tested whether IGF-1 can prevent PVL-like brain damage induced by lipopolysaccharide (LPS) in the neonatal rat. Intraventricular delivery of LPS resulted in an acute brain inflammatory response, *i.e.*, rapid recruitment of polymorphonuclear leukocytes (PMNs), activation of microglia and astrocytes, and induction of IL-1β (IL1β) expression. Brain inflammation was associated with the loss of O4+ preoligodendrocytes (preOLs), a decrease of myelin basic protein (MBP) in the white matter and an increase of pyknotic cells in the cortex. IGF-1 at a low dose significantly prevented LPS-induced deleterious effects without alteration of IL-1β expression and microglia/astrocytes activation.

Pediatr. Res. 2010 Jun; 67(6): 579–584.

9. SCOPE OF WORK

Cerebral palsy is the most common childhood physical disability. There is no known cure for cerebral palsy, but fortunately there are many treatments that can help both children and adults with cerebral palsy.

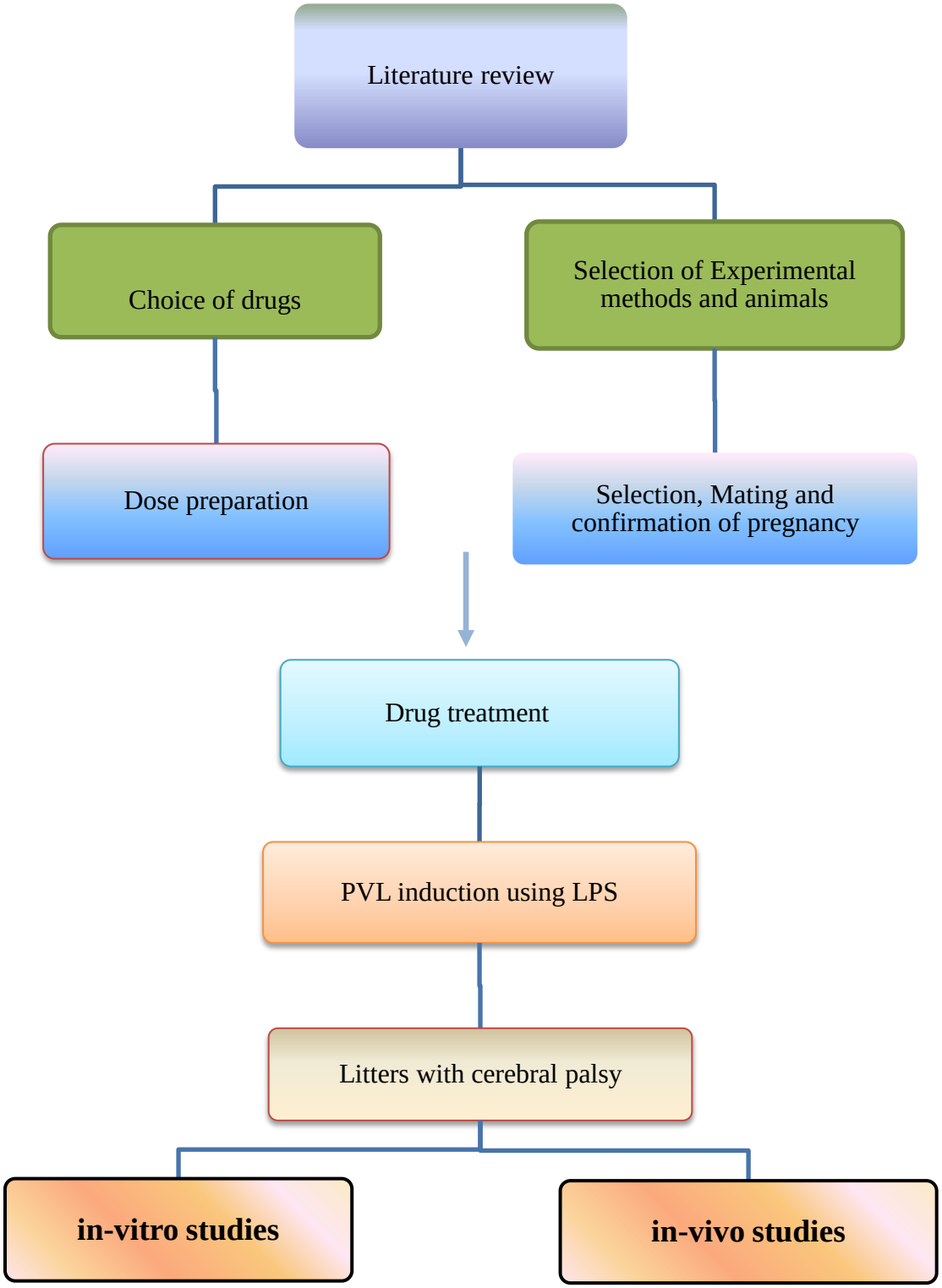
Premature delivery may result in the brain damage of the new born, and causes impairments such as loss of senses and permanent cognitive impairment. Early treatment for children with cerebral palsy is important because the developing brain and the body are more resilient. Multi-disciplinary team is operational in treating cerebral palsy, which involves localized treatment specific for the particular symptoms.

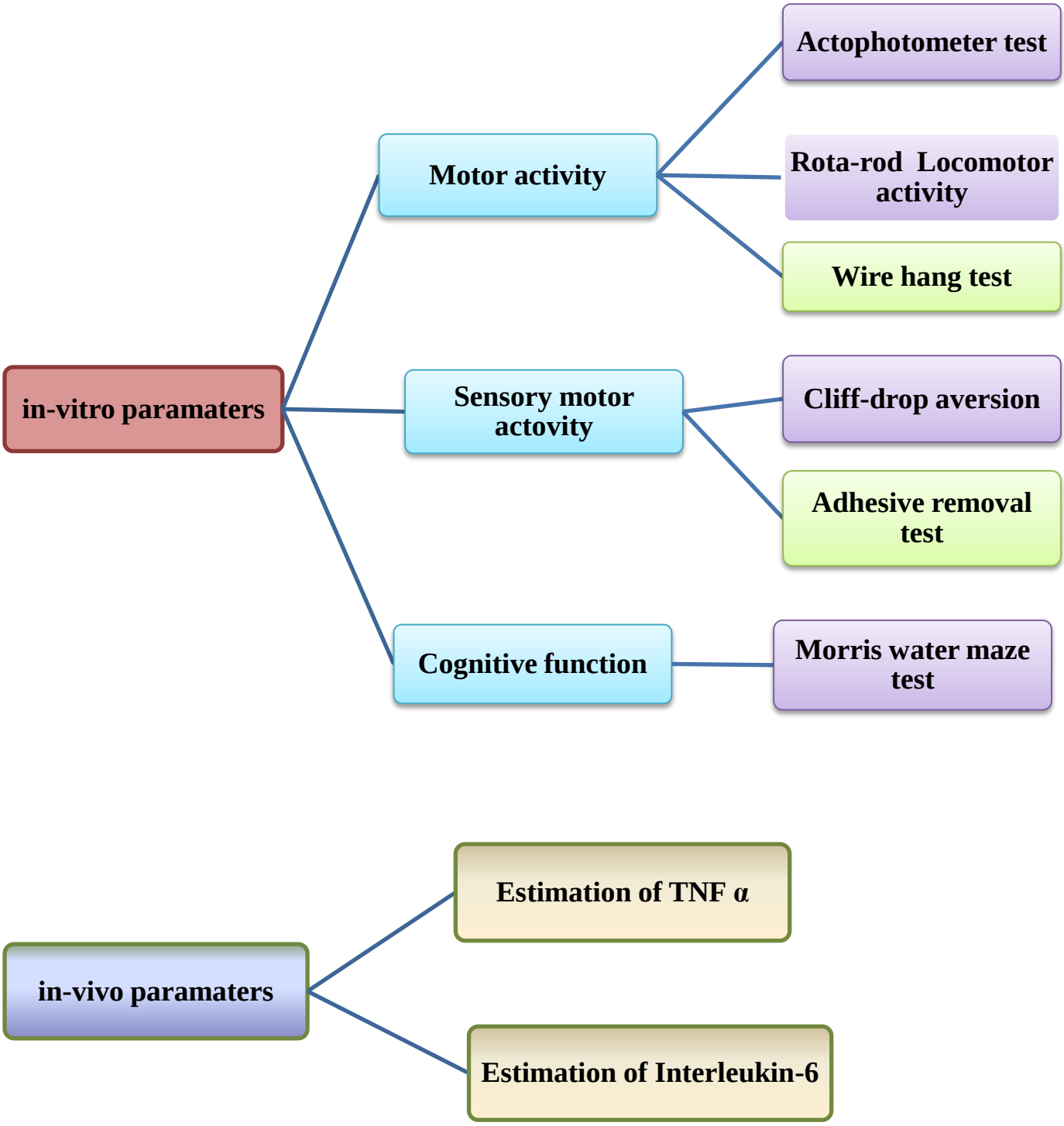
The scope of this work is to bring up the treatment regimen that prevents the fetus being affected by the maternal antibodies; there is currently only symptomatic relief. But currently some category of drugs like stimulants and non-stimulants were used along with some antioxidants and some other supportive therapy. Therefore there is a lot of promising scope in the development of drug therapy for this chronic and debilitating disorder.

The present study is carried out in order to prevent the neuro-degrading conditions that need to be avoided during the gestation period. Treating cerebral palsy after the initiation of neuro-degeneration of the nervous system will not fetch any results as this complete process is irreversible and can never be cured completely. Hence, prenatal treatment with energy-rich neuronal supplementation will be the choice for preventing CP and for brain development

.

10. PLAN OF WORK




in-vitro paramaters
Motor activity
Actophotometer test
Rota-rod Locomotor activity
Wire hang test
Sensory motor actovity
Cliff-drop aversion
Adhesive removal test
Cognitive function
Morris water maze test
in-vivo paramaters
Estimation of TNF α
Estimation of Interleukin-6

11. MATERIALS AND METHODS

11.1. EXPERIMENTAL ANIMALS:

All experimental protocols were approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and Institutional Animal Ethical Committee (IAEC).

11.2. ANIMAL SELECTION

Female Sprague dawley rats weighing 200 – 270 g of around 8-9 weeks of age were used for this study. Male SD rats will reach sexual maturity at approximately 10-12 weeks of age, although females may have their first estrus as early as 8-9 weeks of age. The animals were procured from the animal house of King Institute of Preventive Medicine and Research is a medicine research institute located in Guindy, Chennai.

11.3. ANIMAL HUSBANDRY

They were housed six per cage under standard laboratory conditions at a temperature 22±2°C with 12:12 hrs light and dark circle. Rats were housed in the ratio of 3 females for 1 male in standard polypropylene cages (size: approximately L 410 x B 280 x H 140 mm), with stainless steel top grill having facilities for pellet food and drinking water in glass bottle. The animals were provided with standard animal feed, water and ad libitum. The animals were adapted to laboratory conditions one week prior to initiation of experiments. All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The study was approved by Institutional Animal Ethical Committee (IAEC).

11.4. MATING OF RATS [36, 37]

The duration of the estrous cycle of rats is 4–5 days, it is on these days mating occurs. Mating is usually nocturnal. One male and up to three females are housed together for mating. In order to confirm pregnancy, vaginal smears must be taken at the same time each day, preferably in the morning, for a minimum of 7 consecutive days.

11.4.1. EXAMINATION OF THE MORPHOLOGY OF VAGINAL SMEARS

Vaginal smears must be taken at the same time each day, preferably in the morning, for a minimum of 7 consecutive days.

Wet smears:

- Lift the female by the base of her tail
- Use a blunt-tipped disposable pipette to flush then aspirate approximately 0.2 ml of saline into the vaginal cavity, repeat 2 times. Place fluid onto a clean microscope slide and cover using a cover slip.

11.4.2. EXAMINE SMEARS UNDER A MICROSCOPE AT LOW POWER

- Proestrus: lasts approximately 12 hours and has abundant nucleated non-cornified epithelial cells.
- Estrus: lasts up to 12 hours and is indicated by the presence of large cornified cells in the vaginal smear.
- Metestrus: lasts approximately 21 hours and usually has many neutrophils in the smear and scattered squamous epithelial cells.
- Diestrus: lasts up to 57 hours and there are abundant neutrophils and a few nucleated non-cornified epithelial cells.

11.5. EXPERIMENTAL DESIGN:

10.5.1. INTRODUCTION:

According to Catherine Rousset., et. Al., cerebral palsy in the neonates can be induced using lipopolysaccharide injected intraperitoneally to the pregnant S.D rats.

11.5.2. PROCEDURE:

- Sprague dawley rats were allowed to mate at 22±1^{o}c under a 12 hour light and 12 hour dark cycle. They were provided free access to food and water ad libitum.
- After confirming pregnancy by vaginal smear test, drug treatment is initiated for the respective treatment arms.

- From Day 5 of gestation period, treatment is initiated with DHA, Memantine and Tocopherol for Groups C, D and E respectively.
- Doses of drugs are as below:
    - DHA - 2.5mg/kg
    - Memantine - 20 mg/kg
    - α- tocopherol - 150mg/day
- Treatment is followed till 20th day of Gestation. On 19th dayof gestation LPS 500 µg/kg i.p. was administered.
- Once the pups were born, they follow weaning period of up to 21 to 23 days. After which they are separated for analyzing their motor, sensory and cognitive functions.

11.5.3. TREATMENT GROUPS

Table 01: Grouping of Animals

GROUP	**TREATMENT REGIME**	**ANIMALS**
Group A	Control	3 Female SD rats
Group B	Negative control – LPS induced Periventricular leukomalacia (PVL)	3 Female SD rats
Group C – DHA	LPS induced PVL and treated with intraperitoneal injections of DHA at 2.5mg/kg	3 Female SD rats
Group D – Memantine	LPS induced PVL and treated with intraperitoneal injections of Memantine 20 mg/kg	3 Female SD rats
Group E – α–Tocopherol	LPS induced PVL and treated with intraperitoneal injections of 150mg/day α- tocopherol .	3 Female SD rats

12. METHODS OF ASSESSMENT (in-vitro)

All the parameters were assessed on Day 21 of the newborn rat pups.

12.1. ASSESSMENT OF MOTOR ACTIVITY

12.1.1. Actophotometer test

1. Spontaneous locomotor activity of each group of the pups was measured using actophotometer with infrared sensitive photocells.
2. Before locomotor test, each animals were placed individually in the actophotometer cage for 2 min for habituation.
3. Thereafter, locomotor activity was recorded for a period of 5 min for pups from group A, B, C, D and E.
4. During this period, locomotor activity and immobility time were recorded in secs.
5. The difference in the activity was recorded considering control Group A and after treatment groups i.e. B, C, D and E.
6. Finally percentage decrease in locomotor activity was calculated at the 21st day of birth.

12.1.2. Wire Hang test

1. The Wire Hang test is used to evaluate motor function and deficit in rodent models of CNS disorders. It detects neuroprotective effects of different treatments at early and late time points (up to 3 weeks) in 30- and 60-minute transient MCAo.(Middle cerebral artery occlusion
2. The test begins with the animal hanging from an elevated wire cage top.
3. The distance of 37 cm is sufficient to encourage mice to remain hanging, but also low enough to prevent mice from injuries when falling down.
4. The animal from each group is placed on the cage top, which is then inverted and suspended above the home cage.
5. When the rat falls off the wire, timer is stopped and the hanging time is recorded. When rats are able to hang for 600 sec, they are returned to the respective cages.
6. Rat pups that fall before this limit are given a maximum of two more tries. Maximum hanging time (*i.e.* the longest of the trials) is recorded for each group of rat pups.
7. The average performance is presented as the average of the three trials.

8. Total 5 groups were involved and their average hanging time was concluded using MEAN±SEM.
9. Motor activity was assessed on day 21.

12.1.3. Rotarod Test

1. Motor coordination and balance was assessed using a rotarod apparatus.
2. In total, four training trials per day with an interval trial time of one hour were performed. Rats falling off during a training trial were put back on the rotating rod. Following the training days, a one day test of three trials was performed using two speed levels (10 & 20 R.P.M) mode of the apparatus over 5-min.
3. Animals are placed individually in separate lanes on rod rotating at 5rpm such that animals may walk forward to keep balance.
4. The rotating rod is allowed to rotate at 10 rpm and animals from each group are placed individually on the rod and the fall off time was recorded for each animal.
5. Similar procedure is followed by increasing the speed to 20 rpm.
6. Total 5 groups were involved and their average hanging duration was concluded using MEAN±SEM.
7. Skeletal muscle activity was assessed on day 21.

12.2. ASSESMENT OF SENSORY MOTOR ACTIVITY

12.2.1. Cliff-drop aversion:

Cliff aversion tests the labyrinth reflexes, as well as strength and coordination and can be used to test pups from neuro-developmental disorder.

A pre-scented box with a flat elevated ledge is used and the pup is placed with the digits only of their forepaws and their snout positioned over the edge. Scoring is performed by counting the total time it takes the pup to turn away from the cliff and move its paws and snout away from the edge. If no response is seen after 30 sec, the test is terminated.

Pups are placed on the edge of the box, making sure that the forepaws, digits and snout are the only parts over the edge.

1. The pup is released and timer is started.
2. Each pup is placed on the edge of a cliff with the forelimbs partially on and partially off this surface.
3. The mature response is that the animal quickly turns its head and forelimbs and avoids dropping. This test allows us the evaluate the somatosensory function.
4. Once both the snout and paws have been removed from the edge, the timer and record time is stopped
5. Similar procedure is repeated for pups in Group B, C, D and E.

8. Total 5 groups were involved and their average head turn, arm turn and complete avoidance rate was concluded using MEAN±SEM.

6. Sensory motor activity was assessed on day 21

12.2.2. Adhesive Removal Test

1. The test rats are restrained and using a pair of small forceps one adhesive label is placed onto the snout of the rats.
2. The label is pressed on the snout with the forceps and released. And the lid is placed on the cage and time is noted.
3. The time when the rat pup attempts to remove the label with its forepaws is recorded. If the animal makes contact but does not remove the label,time is extended until it removes it.
4. Contact time and time of removal of the adhesive is noted for all the Groups.
5. If the rat does not contact or remove the sticker within 60 sec then the trial is ended and the sticker is removed manually.
6. After completion, the animal is placed back in the clean cage and next ratfrom another group is tested.
7. Total 5 groups were involved and their average time of adhesive removal was concluded using MEAN ± SEM.

12.3. ASSESMENT OF MEMORY AND LEARNING

12.3.1. Spatial Memory Performance in Water Maze Probe Trial

- The spatial learning and memory test was performed by the method of Morris water-maze..
- Water maze test was consisted of a place navigation test and a probe test. The place navigation test was performed as two trials daily for 5 consecutive days.
- Rats were trained to locate and escape onto the platform during the training session.
- A different starting position for each rat was used in each trial.
- The pups were allowed to swim freely to find the hidden platform within 60 s.
- Animals that are failed to find the location within the given time were guided to the platform and were allowed to stay on it for 20 to 40 sec and then returned to the cage.
- For each individual treatment group, the position of the platform was fixed throughout the test.
- The escape latency and platform crosses of each rat per day were calculated.
- On the memory retention session (probe test), a single test was conducted in which the platform was removed.
- Total 5 groups were involved and their average time in target platform was concluded using MEAN ± SEM
- Cognitive function is analyzed on 21st day of rat pups.

13. STATISTICAL ANALYSIS:

Data are reported as mean ± SEM and subjected to one-way analysis of variance (ANOVA). The statistical analysis was carried by one way ANOVA followed by Dunnett's test.

The P value< 0.05 (95% confidence limit) was considered statistically significant.

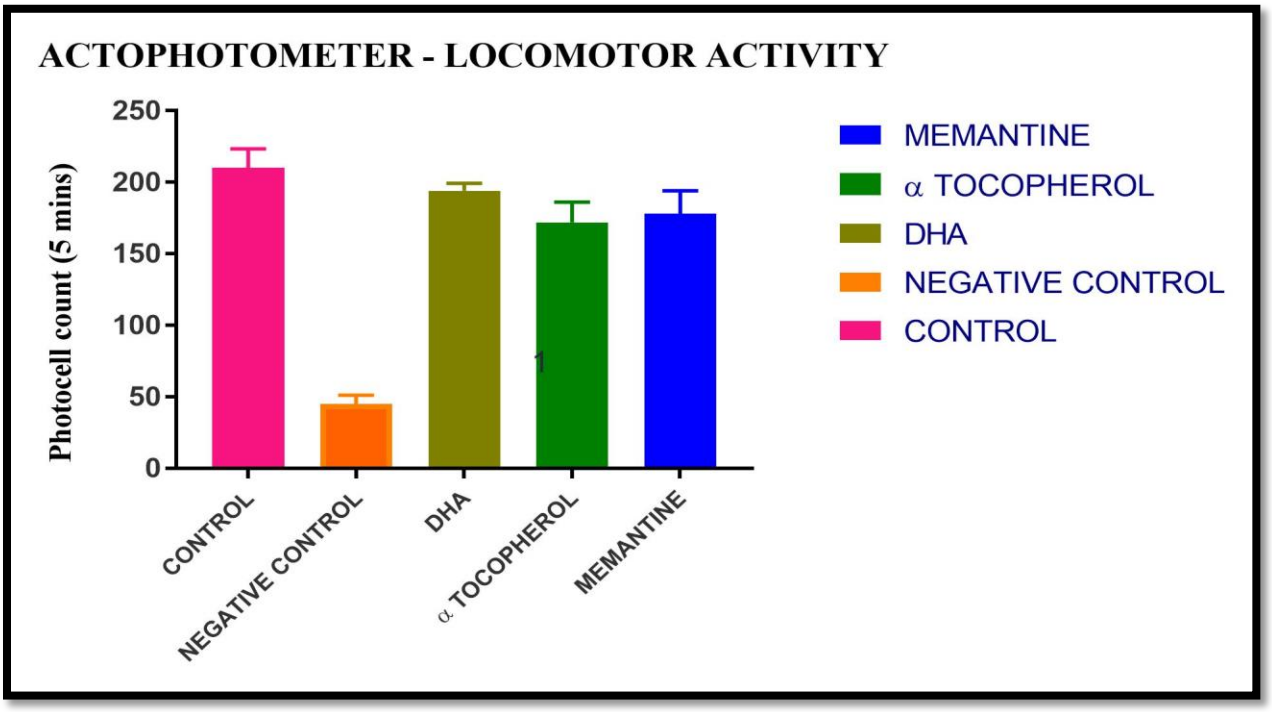
Statistical analysis were done using Software Graph pad Prism 6.0.

13.1. Effect of DHA, Tocopherol and Memantine In Neonatal Rats Using Actophotometer

S.NO	GROUP	PHOTOCELL COUNTS (5 MINS)
01	Control	210 ± 13.34
02	Negative control	45 ± 6.13
03	DHA	194 ± 5.26^{A*B***}
04	Tocopherol	172 ± 14.10 ^{A*B****}
05	Memantine	178 ± 16.11 ^{A*B****}

Table 01: Effect of DHA, Tocopherol and Memantine In Neonatal Rats Using Actophotometer

Values are presented as mean ± SEM, (n=5). Comparison: Group II vs. Group III, IV, V. Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's't' test. (ns- Non significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001)



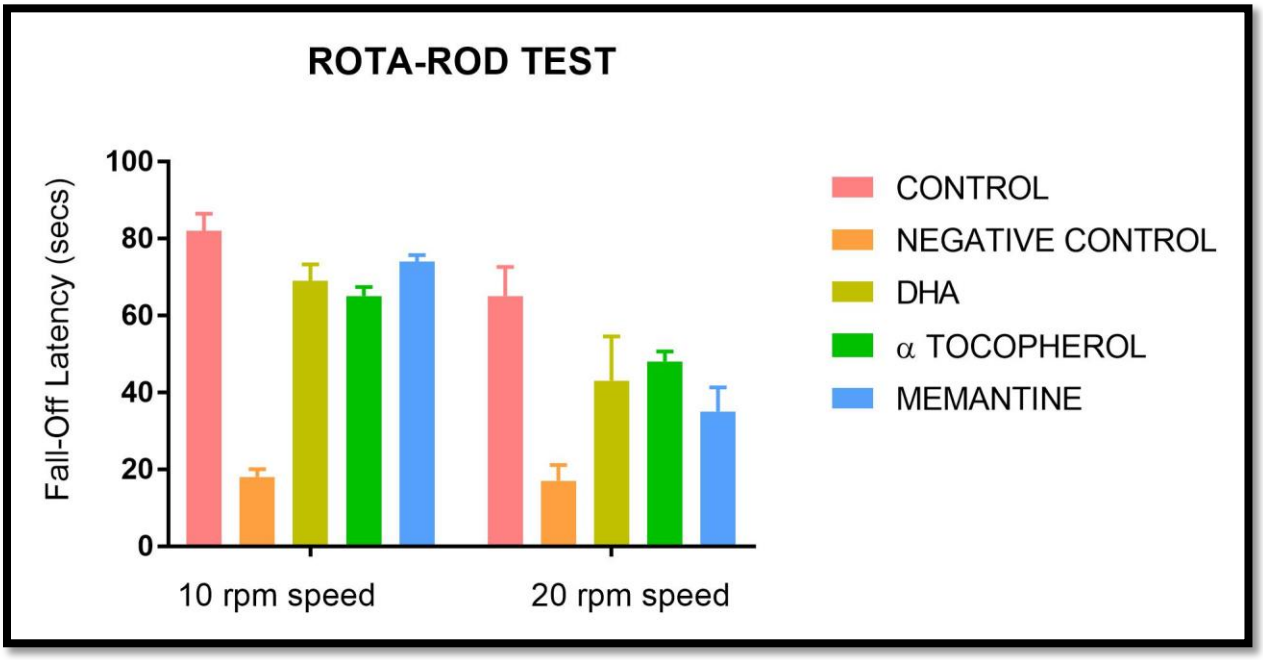
Graph 01: Actophotometer – Locometer activity

13.2. EFFECT OF DHA, TOCOPHEROL AND MEMANTINE IN NEONATAL RATS USING ROTAROD TEST

S.NO	GROUP	Fall off latency (10 RPM)	Fall off Latency (20 RPM)
01	Control	82 ± 4.42	65 ± 7.6
02	Negative control	18 ± 2.08	17 ± 4.12
03	DHA	79 ± 4.3	43 ± 11.5
04	Tocopherol	85 ± 2.40	48 ± 2.64
05	Memantine	74 ± 1.69	35 ± 6.25

Table 02: Effect of DHA, Tocopherol and Memantine In Neonatal Rats Using Rota-rod test

Values are presented as mean ± SEM, (n=5). Comparison: Group II vs. Group III, IV,V Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's't' test. (ns- Non significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001)



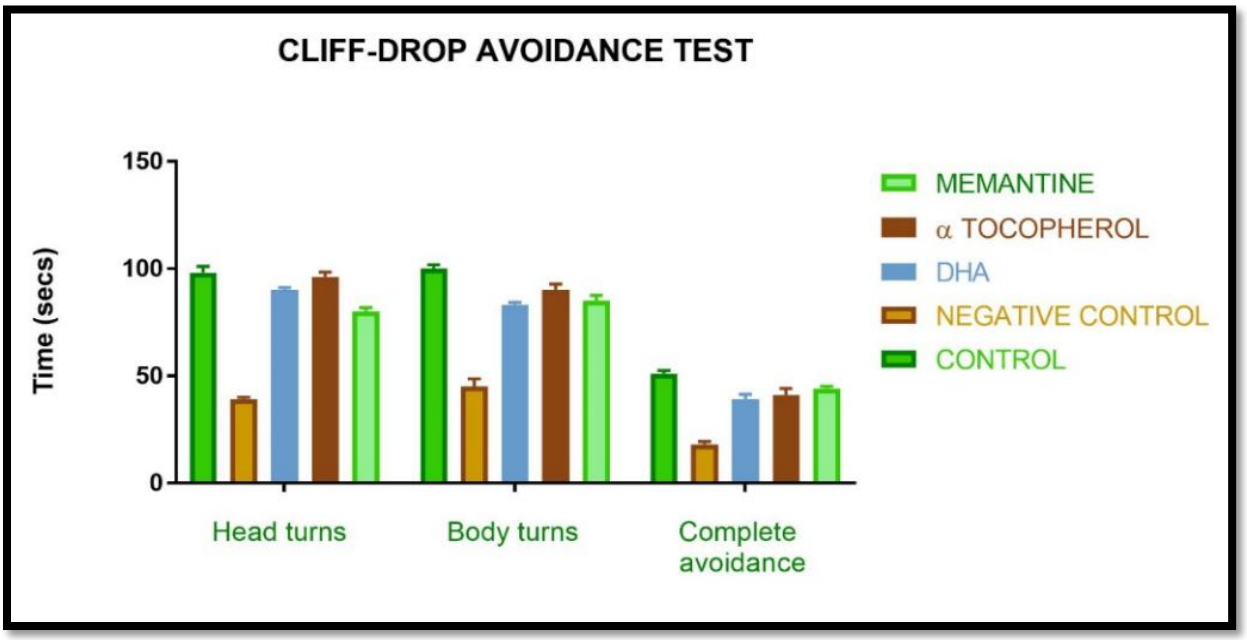
Graph 02: Rota-rod test

13.3. EFFECT OF DHA, TOCOPHEROL AND MEMANTINE IN NEONATAL RATS USING CLIFF-DROP AVOIDANCE TEST

S.NO.	GROUPS	HEAD TURN	ARM TURN	COMPLETE AVOIDANCE
01	Control	98 ± 3.06	100 ± 1.84	51 ± 1.49
02	Negative control	39 ± 0.91	45 ± 3.56	18 ±1.31
03	DHA	90 ± 1.04	83 ± 1.19	39 ± 2.25
04	Tocopherol	96 ± 2.25	90 ± 2.71	41 ± 3.02
05	Memantine	80 ± 1.67	85± 2.48	44 ± 1.10

Table 03: Effect of DHA, Tocopherol and Memantine In Neonatal Rats Using Cliff-drop avoidance test

Values are presented as mean ± SEM, (n=5). Comparison: Group II vs. Group III, IV, V. Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's't' test. (ns- Non significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001)



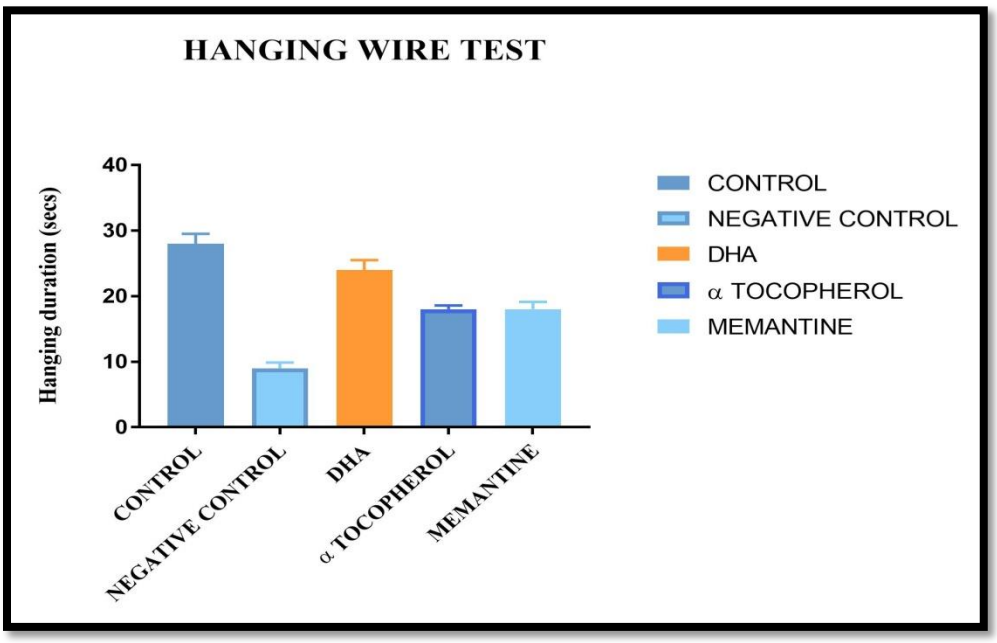
Graph 03: Cliff-drop Avoidance test

13.4. EFFECT OF DHA, TOCOPHEROL AND MEMANTINE IN NEONATAL RATS USING WIRE HANG TEST

S.NO	GROUP	HANGING DURATION (secs)
01	Control	28 ± 1.5
02	Negative control	9 ± 0.88
03	DHA	24 ± 1.52
04	Tocopherol	18 ± 0.577
05	Memantine	18 ± 1.15

Table 04: Effect of DHA, Tocopherol and Memantine In Neonatal Rats Using wire-hang test

Values are presented as mean ± SEM, (n=5). Comparison: Group II vs. Group III, IV, V. Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's't' test. (ns- Non significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001)



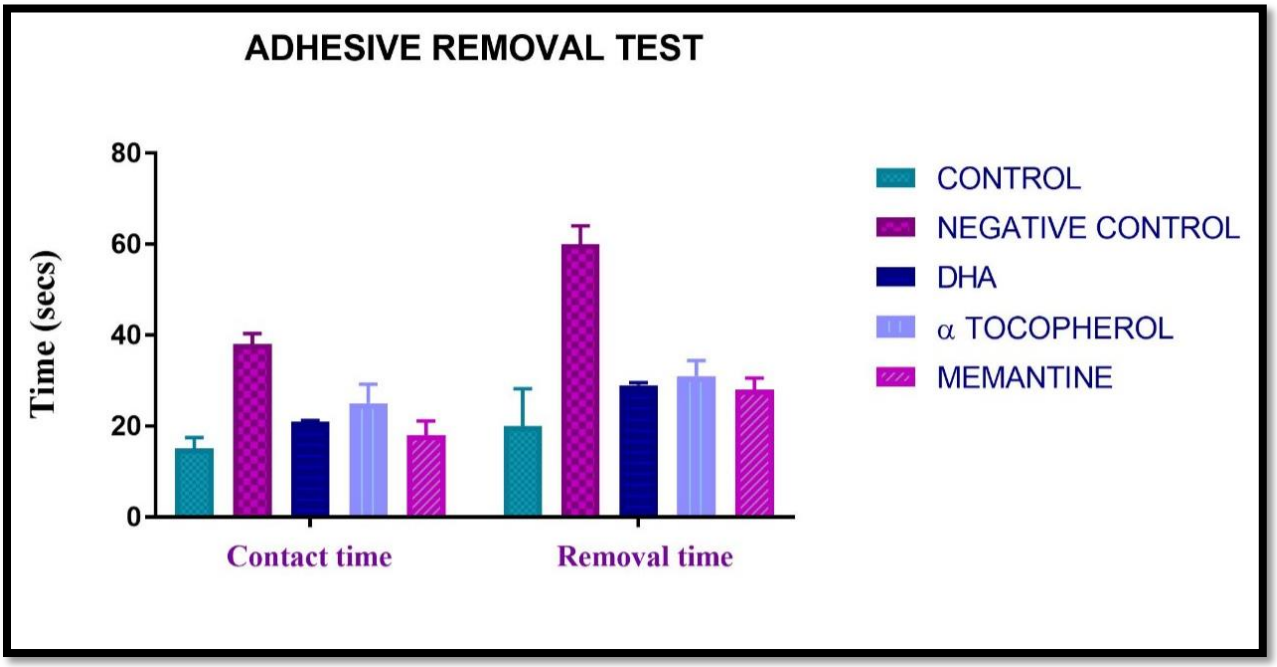
Graph 04: Hanging wire test

13.5. EFFECT OF DHA, TOCOPHEROL AND MEMANTINE IN NEONATAL RATS USING ADHESIVE REMOVAL TEST

S.NO	GROUP	Contact time (secs)	Removal time (secs)
01	Control	15 ± 2.51	20 ± 8.23
02	Negative control	38 ± 2.36	60 ± 4.03
03	DHA	21 ± 0.24	29 ± 0.57
04	Tocopherol	25 ± 4.20	31 ± 3.43
05	Memantine	18 ± 3.18	28 ± 2.56

Table 05: Effect of DHA, Tocopherol and Memantine In Neonatal Rats Using Adhesive removal test

Values are presented as mean ± SEM, (n=5). Comparison: Group II vs. Group III, IV, V. Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's't' test. (ns- Non significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001)



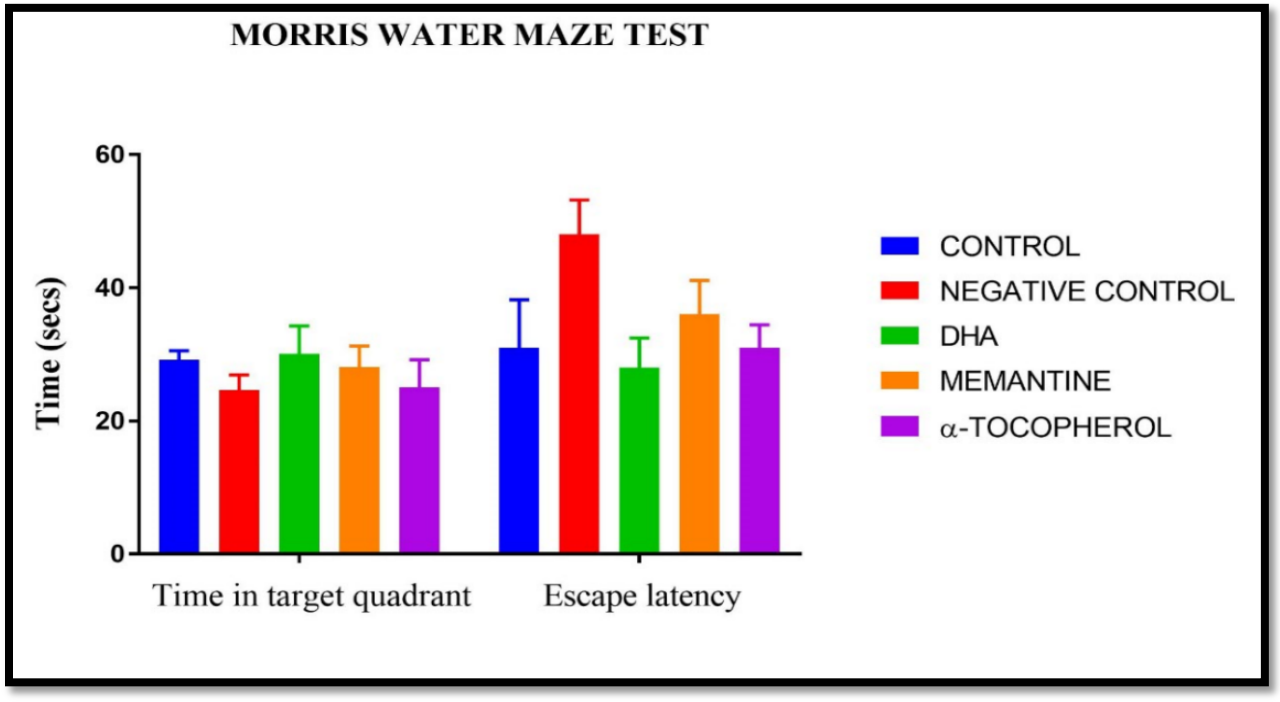
Graph 05: Adhesive Removal test

13.6. EFFECT OF DHA, TOCOPHEROL AND MEMANTINE IN NEONATAL RATS USING SPATIAL LEARNING TEST

S.NO	GROUP	TIME IN TARGET QUADRANT (%)	ESCAPE LATENCY
01	Control	29.2 ± 1.36	31±7.21
02	Negative control	24.6 ± 2.3	48±5.21
03	DHA	30.1 ± 4.32	28±4.47
04	Tocopherol	29.5 ± 2.6	34±3.35
05	Memantine	28.1 ± 3.16	36±5.12

Table 06: Effect of DHA, Tocopherol and Memantine In Neonatal Rats Using Spatial learning test

Values are presented as mean ± SEM, (n=5). Comparison: Group II vs. Group III, IV, V. Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's't' test. (ns- Non significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001)



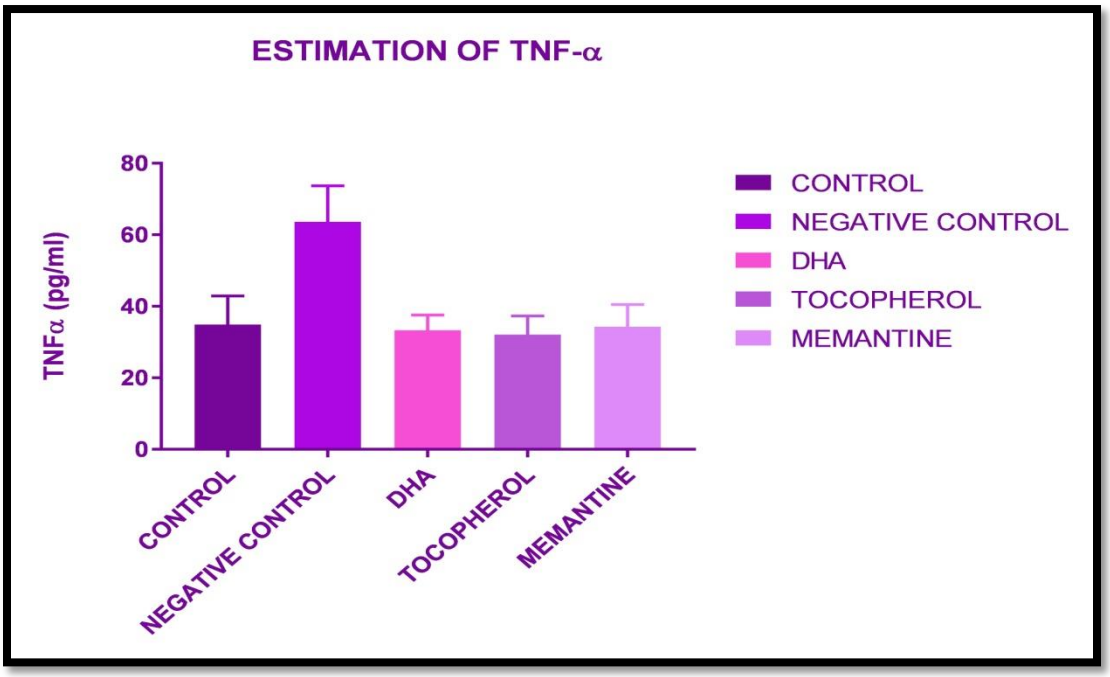
Graph 06: Morris Water-maze test

13.7. EFFECT OF DHA, TOCOPHEROL AND MEMANTINE IN INDUCING TNF - α

S.NO	GROUP	TNF – α (pg/ml)
01	Control	34.9 ± 8
02	Negative control	50.4 ± 10.1
03	DHA	33.3 ± 4.3**
04	Tocopherol	40.12 ± 5.2***
05	Memantine	34.32 ± 6.2**

Table 07: Effect of DHA, Tocopherol and Memantine In Inducing TNF - α

Values are presented as mean ± SEM, (n=5). Comparison: Group II vs. Group III, IV, V. Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test. (ns- Non significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001)



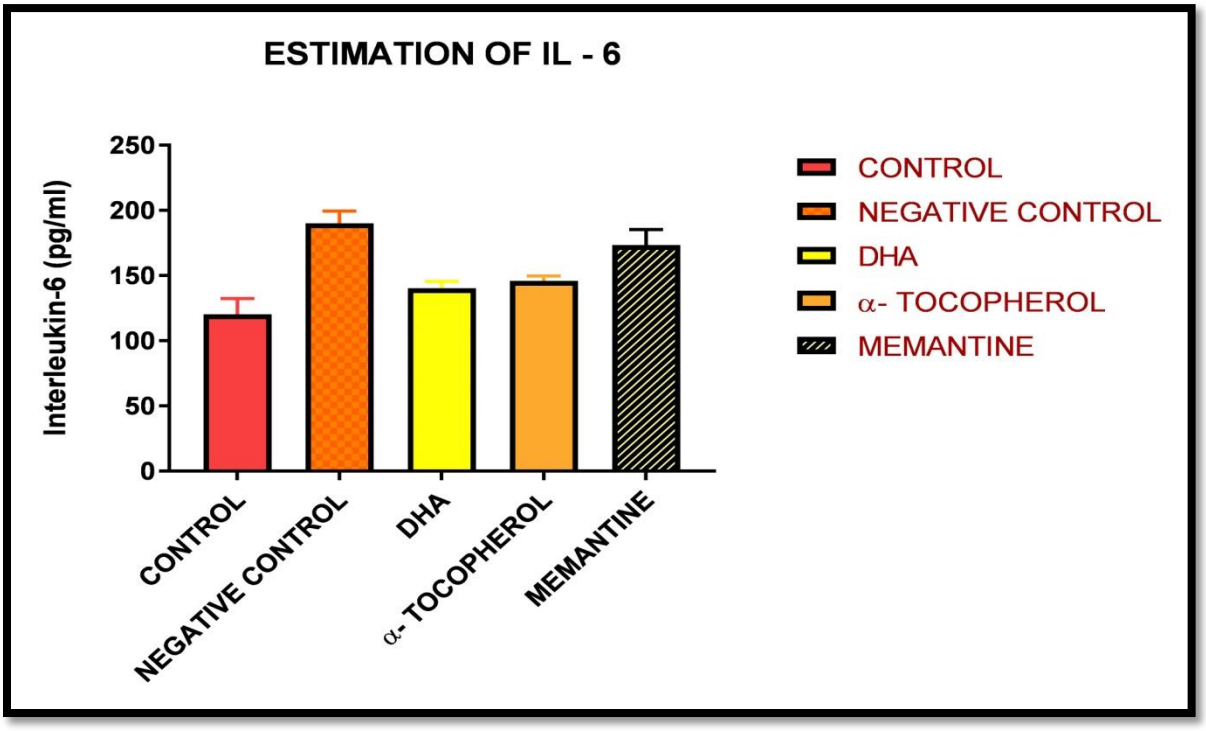
Graph 07: Estimation of TNF α

13.8. EFFECT OF DHA, TOCOPHEROL AND MEMANTINE IN INDUCING IL-6

S.NO	GROUP	INTERLEUKIN – 6 (pg/ml)
01	Control	120.23 ± 12.1
02	Negative control	190.17 ± 9.23
03	DHA	140.23 ± 5.36 **
04	Tocopherol	146 ± 3.63**
05	Memantine	173.36 ± 12***

Table 08: Effect of DHA, Tocopherol and Memantine In Inducing IL-6

Values are presented as mean ± SEM, (n=5) Comparison: Group II vs. Group III, IV, V. Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's't' test. (ns- Non significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001)



Graph 08: Estimation of IL-6

14. RESULTS

14.1. INDUCTION OF PVL

Periventricular Leukomalacia, which is known to cause cerebral palsy, was induced in the pregnant rats at the Gestation day of 19. It was successfully induced by intraperitoneal injection of LPS (Bacterial product) 500 µg/kg of the G-19 female rats. Abrupt rise in TNF-α and Interleukin – 6 after 4 hours of LPS administration confirms the inflammatory response induced in the brain.

14.2. ASSESMENT OF MOTOR ACTIVITY

14.2.1. Effect of DHA, Tocopherol and Memantine in neonatal rats using Actophotometer

- The Group II animals showed significant decrease in locomotor activity when compared with Group I animals.
- Treatment with DHA, Tocopherol and Memantine showed significant increase in ($p<0.001$, $p<0.01$, $p<0.001$ for Group III, IV, V respectively) locomotor activity when compared with group II.

14.2.2. Effect of DHA, Tocopherol and Memantine in neonatal rats using Rota-rod apparatus

- The fall off time of Group II animals has decreased when compared withGroup I animals.
- Treatment with DHA, Tocopherol and Memantine showed significant increase ($p<0.01$, $p<0.01$, $p<0.01$ for Group III, IV, V respectively) in fall off timewhen compared with Group II animals.

14.2.3. Effect of DHA, Tocopherol and Memantine in neonatal rats using Hanging wire test

- The hanging duration of Group II animals was remarkably decreased when compared withGroup I animals.
- Treatment with DHA, Tocopherol and Memantine showed significant increase in hanging duration ($p<0.001$, $p<0.01$, $p<0.01$ for Group III, IV, V respectively) when compared with Group II animals.
-

14.3. ASSESMENT OF SENSORIMOTOR ACTIVITY

14.3.1. Effect of DHA, Tocopherol and Memantine in neonatal rats using Cliff-Drop aversion test

- The Group II animals showed significant decrease in the number of head turns and body turns when compared with Group I animals.
- Complete avoidance of the Group II animal when being placed at the cliff also seems to be less when compared with Group I.
- Treatment with DHA, Tocopherol and Memantine showed significant increase in ($p<0.001$, $p<0.0001$, $p<0.001$ for Group III, IV, V respectively) locomotor activity when compared with group II.

14.3.2. Effect of DHA, Tocopherol and Memantine in neonatal rats using Adhesive Removal test

- The Group II animals showed significant increase in the time taken to remove the adhesive when compared with Group I animals.
- Time taken to sense and contact the adhesive is found to be more in Group II when compared with Group I.
- Treatment with DHA, Tocopherol and Memantine showed significant decrease in latency to remove the adhesive ($p<0.01$, $p<0.001$, $p<0.01$ for Group III, IV, V respectively) when compared with group II.

14.4. ASSESMENT OF COGNITION AND LEARNING

14.4.1. Effect of DHA, Tocopherol and Memantine in neonatal rats using Morris-water maze test

- The Group II animals showed significant increase in the time taken to escape and reach the target quadrant when compared with Group I animals.
- Time spent in the target quadrant is found to be less in Group II when compared with Group I.

- Treatment with DHA, Tocopherol and Memantine showed significant increase in the time spent in the target quadrant ($p<0.0001$, $p<0.001$, $p<0.0001$ for Group III, IV, V respectively) when compared with group II.

14.5. ESTIMATION OF TNF α & INTERLEUKIN

Expression of tumor necrosis factor-α and interleukin-6 was observed more frequently in brain lesions with periventricular leukomalacia. TNF α is known to be responsible for the neuronal pain in most of the degenerative conditions. Hence it was found that, increase in the measure of TNF α & Interleukin 6 is the marker of cerebral palsy. Pro-inflammatory cytokines plays a major role in the development of PVL.

Estimation of TNF α and IL-6 was carried out using ELISA type assay.

15. DISCUSSION

Brain injury induced remains a major cause of cerebral palsy. Although therapeutic hypothermia is now established to improve recovery from hypoxia–ischemia (HI) at term, many infants continue to survive with disability, and hypothermia has not yet been tested in preterm infants. There is increasing evidence from in vitro and in vivo preclinical studies that stem/progenitor cells may have multiple beneficial effects on outcome after hypoxic–ischemic injury. Stem/progenitor cells have shown great promise in animal studies in decreasing neurological impairment; however, the mechanisms of action of stem cells, and the optimal type, dose, and method of administration remain surprisingly unclear.

In this study we investigated the underlining cellular mechanisms responsible for the neuroprotective effects of DHA, Vitamin-E and Memantine treatment in prenatal white matter injury in the developing rat brain. We have demonstrated the behavioral phenomena by means of in-vitro tests that are being carried out in the neonatal CP induced rats.

As per existing studies, neuroprotective effects were limited to TG-enriched only in DHA, but not EPA. Also, neuroprotection was evident when n-3 TG were injected 2 hr after the H/I injury. The present study has revealed the ameliorative effect of DHA, Memantine and Tocopherol on LPS-induced cerebral palsy in SD neonates.

LPS induced sensory motor and memory impairments was assessed by using various behavioral parameters like Rota-rod apparatus and Morris water maze test. It was found that treatment with DHA and Tocopherol protects the cognitive deficits of the neonatal rats.

Locomotor activity was assessed using Actophotometer based on locomotion in closed field and treatment with DHA, Tocopherol and Memantine reported that the increase in the locomoter activity when compared to –ve control group which exhibited considerably lesser locomoter count and time spent duration in the Rota-rod.

.

Skeletal muscle activity was assessed by rota-rod apparatus. LPS induced PVL animals showed high fall off time in rota-rod apparatus. Treatment with DHA, Tocopherol and Memantine in

deficit groups greatly reduced fall off time. This indicated that DHA and Tocopherol has muscle relaxant property.

Morris water maze task represents more specific for spatial memory. The essential feature of this technique is that rats are placed into large circular pool of water and can escape into a hidden platform. Thus, the platform offers no local cues to guide escape behavior and the rat can escape from swimming by climbing on to the platform apparently learns the spatial location of the platform any starting position at the circumference of the pool. The only spatial cues are those outside water tank are primarily visual cues. Thus, the versatility of the task makes it a widely acceptable experimental model for the assessment of cognitive tests.

Typically, LPS induced animals exhibited an increase time for escape latency indicating loss of visual cues to escape to the platform. Such a diminished cognition was reversed by the administration of the DHA, Tocopherol and Memantine at the specified dosage levels and exhibited escape latency (EL), indicating the well-developed spatial memory.

Sensorimotor tests are carried out to determine the active sensory perception by the LPS induced PVL neonatal rats. These includes Cliff drop aversion test and adhesive removal test, which determines the sensory and motor activity of the CP induced animals. The active avoidance by DHA, Tocopherol and Memantine Group in the cliff drop aversion test indicates better sensory and motor actions.

Comparatively, prenatal treatment with DHA, an N-3 fatty acid rich triglycerides found to be more promising in ameliorating the effects of brain damage induced by LPS. DHA administered group experienced normal functioning in terms of locomotor, sensory and cognitive functions. Vitamin-E or Tocopherol also exhibited effects similar to DHA but to lesser extent in terms of cognitive functions.

NMDA receptor blockade with memantine provided an effective pharmacological prevention of PVL in the premature neonates, by protecting the degradation of myelin sheath and lowering the effects of white mater damage on the neuronal development in the neonatal pups.

16. CONCLUSION

In conclusion, the derived results demonstrate that DHA, TOCOPHEROL and MEMANTINE could protect neuronal cells, and thereby preventing brain damage in adverse conditions. The neuroprotective pathway appears to implicate NMDA receptors and inflammatory modulation, leading to a promotion of oligodendrocyte maturation. The present study also delineates the cellular mechanisms of Memantine's neuroprotective benefits.

Furthermore, our data strongly suggest that DHA, TOCOPHEROL and MEMANTINE could be of great interest not only in perinatal white matter damage but also as a potential neuroprotective strategy for myelinopathy diseases observed in adults.

The data provides the proof of concept that antioxidant therapy with DHA and TOCOPHEROL may antagonize the effects of free-radicals that may lead to neuronal loss. The results provide new insights into the potential of employing n-3 TG specifically DHA as a unique long chain FA aiding in neonatal H/I brain injury. A number of pathways are likely involved in n-3 TG neuroprotection. For example, chronic administration of DHA resulted in increases of DHA levels in brain mitochondria. My findings suggest a need for further studies to determine if acute injection of DHA could be neuroprotective after stroke injury in humans.

I hypothesize that n-3 fatty acids, specifically DHA, will prove to be a "novel" and important therapy to treat various neuro-degenerative disorders and could decrease mortality and increase long-term functional recovery.

REFERENCES

1. Biswas-Diener, R. The brain and nervous system. In R. Biswas-Diener & E. Diener (Eds), Noba textbook series: Psychology. (2018).
2. Brodal, P *et. al.,* The central nervous system: Structure and function. New York: Oxford University Press. 1992; 115:10, 2424-2425.
3. Fred H. Gage. *et. al.,* Neuroscience: The Study of the Nervous System & Its Functions 2017.
4. Joan Stiles *et, al.,* The Basics of Brain Development. Neuropsychology Rev. 2010; 20(4): 327–348.
5. WHO. What are neurological disorders, 2018.
6. Rosenbaum P *et. al.,* A report: the definition and classification of cerebral palsy. Dev Med Child Neurol. 2007. Jun; 49(6):480.
7. Giovanni cioni *et. al.,* Early intervention in neurodevelopmental disorders: underlying neural mechanisms. Developmental Medicine & Child Neurology 2016; (4): 61–66
8. http://www.ucpmobile.org/what-is-cp/
9. http://www.birthinjuryguide.org/cerebral-palsy/causes/
10. https://www.cdc.gov/ncbddd/cp/causes.html
11. https://www.medicalnewstoday.com/articles/152712.php
12. http://www.cerebralpalsy.org/about-cerebral-palsy/types-and-forms
13. Laura rogers *et. al.,* Cerebral palsy. McMaster Pathophysiology review.
14. Hüppi PS *et. al.,* Quantitative magnetic resonance imaging of brain development in premature and mature newborns. Ann Neurol. 1998 Feb; 43(2):224-35.
15. Kahlert S *et. al.,* Glutamate-mediated influx of extracellular Ca2+ is coupled with reactive oxygen species generation in cultured hippocampal neurons but not in astrocytes. J Neurosci Res. 2005 Jan 1-15; 79(1-2):262-71.

16. http://www.cerebralpalsy.org/about-cerebral-palsy/treatment/medication

17. https://www.webmd.com/children/tc/cerebral-palsy-medications

18. http://www.cerebralpalsy.org/about-cerebral-palsy/treatment/complementary-medicine

19. Rumajogee P, Bregman T, Miller SP, Yager JY, Fehlings MG. Rodent Hypoxia-Ischemia Models for Cerebral Palsy Research: A Systematic Review. Front Neurol. 2016; 7: 57. 6

20. Wilson MD *et. al.,* Animal Models of Cerebral Palsy: Hypoxic Brain Injury in the Newborn. Iran J Child Neurol. 2015; 9: 9-16. 8.

21. Prakasham Rumajogee *et. al.,* Rodent Hypoxia–ischemia Models for Cerebral Palsy Research: A Systematic Review Front Neurol. 2016; 7: 57.

22. Yanrong Hu et, al., A rat pup model of cerebral palsy induced by prenatal inflammation and hypoxia.Neural Regen Res. 2013; 8: 817-824.

24. https://en.wikipedia.org/wiki/Neuroprotection

25. Morani, Aashish & Bodhankar, Subhash. (2008). Neuroprotective effect of vitamin E acetate in models of mononeuropathy in rats. Neuroanatomy. 7.

26. Savita Khanna *et. al.,* Characterization of the potent neuroprotective properties of the natural vitamin E α- tocotrienol. 2006; 98 (5): 1474-1486

27. Behl C. *et. al.,* Antioxidant neuroprotection in Alzheimer's disease as preventive and therapeutic approach. 2010.

28. Mohamed D Morsy *et. al.,* A potential protective effect of α-tocopherol on vascular complication in spinal cord reperfusion injury in rats. J Biomed Sci. 2010; 17(1): 55.

29. Sung-Ha Hong. *et. al.,* Docosahexaenoic acid confers enduring neuroprotection in experimental stroke**.** J Neurol Sci. 2014 Mar 15; 338(0): 135–141.

30. Mayurasakorn K. *et. al.,* Docosahexaenoic acid: brain accretion and roles in neuroprotection after brain hypoxia and ischemia. Curr Opin Clin Nutr Metab Care. 2011 Mar; 14(2):158-67.

31. Kirk Stoke. *et. al.,* DHA: An Essential Brain Food, Life extension magazine ; November 2012.

32. Sabrina FLORENT-BÉCHARD *et. al.,* Neuroprotective effects of DHA in Alzheimer's disease models. 2007; 14: 3(4).

33. Kewal K Jain *et. al.,* Evaluation of memantine for neuroprotection in dementia. 2005; 1397-1406

34. Wojciech danysz *et. al.,* Neuroprotective and Symptomatological Action of Memantine Relevant for Alzheimer's Disease - A Unified Glutamatergic Hypothesis on the Mechanism of Action. Eckenheimer Landstrasse 100-104.

35. Ken S. Ota. *et. al.,* Memantine: The Next Trend in Academic Performance Enhancement. The Journal of the American Osteopathic Association. June 2006; 106, 358-359.

36. C.C. Paccola *et. al.,* The rat estrous cycle revisited: a quantitative and qualitative analysis. Animal Reproduction. 2013; 4: 677-683.

37. Standard operating procedure #609 rat breeding colony management, McGill SOP 609.01.

38. Hind Benammi *et. al.,* Disturbed sensorimotor and electrophysiological patterns in lead intoxicated rats during development are restored by curcumin I, 2017.

39. Koopmans GC, Deumens R, Brook G, Gerver J, Honig WM, *et al*., Strain and locomotor speed affect over-ground locomotion in intact rats. Physiol Behav. 2007; 92, 993–1001.

40. Santillán ME, Vincenti LM, Martini AC, de Cuneo MF, Ruiz RD, Mangeaud A, *et al*. Developmental and neurobehavioral effects of perinatal exposure to diets with different omega-6:omega-3 ratios in mice. Nutrition.2010; 26:423–31.

41. Petrovas C *et. al.,* Determination of tumor necrosis factor-alpha (TNF-alpha) in serum by a highly sensitive enzyme amplified lanthanide luminescence immunoassay. ClinBiochem. 1999 Jun;32 (4):241-7.

42. Laura Bennet PhD *et. al.,* Cell therapy for neonatal hypoxia–ischemia and cerebral palsy. Neurological Progress. 2012; 71(5): 589-600.

43. Jill J. Williams *et. al.,* N-3 Fatty Acid Rich Triglyceride Emulsions Are Neuroprotective after Cerebral Hypoxic-Ischemic Injury in Neonatal Mice, 2013.