# CEREBROSPINAL FLUID AND PLASMA RHEOLOGY IN ALZHEIMER'S DISEASE - INDUCED RATS

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**A PROJECT SUBMITTED TO THE DEPRTMENT OF PHYSIOLOGY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A BACHELOR OF SCIENCE (BSc) DEGREE IN PHYSIOLOGY**

**FEBRUARY, 2025.**

# CERTIFICATION

This is to certify that the project work on "**CEREBROSPINAL FLUID AND PLASMA RHEOLOGY IN ALZHEIMER'S DISEASE-INDUCED RATS"** was carried out by **CHUKS CHIOMA UDEKALU**, with the matriculation number **BMS2001235** in partial fulfilment of the requirement of the award of Bachelor of Science Degree (B.Sc) in the Department of Physiology, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Edo State, Nigeria.

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***External Examiner***

# DEDICATION

I dedicate this project work to God Almighty for His infinite mercies, love, and care toward me. I also want to dedicate this project work to my Irreplaceable family for their maximum care, love, and support. I love you guys and may the blessings of God be with you all forever. Amen.

# ACKNOWLEDGEMENT

I want to bless the name of the Lord for counting me worthy to be alive to experience this moment. I am grateful to my Teacher, Comforter, and Strengthener, the Holy Spirit.

I wish to express my profound gratitude to my project supervisor **PROF. O. I. AJAYI** for being patient and for his encouragement and corrections in my project work. I appreciate you, Sir. Special thanks to **MRS C.N. IGHODARO** for her invaluable contributions and insight. Her dedication and expertise have been instrumental in my success. I am also immensely grateful to my project colleagues, together we have achieved more than I could imagined.

I also wish to express my deepest appreciation to my parents, both biological and spiritual for their words of encouragement, prayers, and finances, who are always there for me. I say a very big thank you all.

# TABLE OF CONTENT

**CONTENTS**

# LIST OF TABLES

# LIST OF FIGURES

# ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive cognitive decline and neuropathological changes. Emerging evidence suggests that AD may also influence cerebrospinal fluid (CSF) composition and plasma rheology, which could play a role in disease progression. This study aimed investigatigating possible alterations in CSF cellular composition and plasma rheological properties such as plasma fibrinogen concentration and plasma viscosity in a rat model of AD. Twelve (12) healthy adult wistar rats weighing between 170-190g were used for this study. The rats were divided into two groups: Group 1 as control (n=6) received water, Group 2 (n=6) were induced with Alzheimer's disease. Aluminum chloride salt (Alcl3) was used to induce Alzheimer's disease, 100mg of $Alcl_3$ salt was dissolved in 10ml of distilled water to achieve a concentration of 100mg/ml. 1ml of this solution was administered intraperitoneally daily for 28 days. Weight of rats were taken weekly, at the end of the experimental period, the rats were sacrificed, blood and CSF samples were collected. Cerebrospinal fluid analysis was performed using microscopy cell counting method, plasma fibrinogen concentration was determined by the clot-weight technique of Ingram and plasma viscosity was determined using the simple viscometer technique. All statistical analysis were carried out using t-test with graph pad prism 10.2.2. Results were presented as standard error of mean (SEM). Analysis of variance (ANOVA) was used to compare the means of tests and control value and a p-value of less than 0.05 was considered statistically significant. Results showed that Alzheimer's disease did not cause significant changes in CSF cellular components. Plasma viscosity remained unchanged between the Alzheimer-induced group and the control. However, plasma fibrinogen concentration was significantly increased in the Alzheimer-induced group, Increased fibrinogen in this study may indicate early-stage neuroinflammation but not enough to alter plasma viscosity. This may lead to hypercoagulability increasing the risk of blood clots, potentially reducing cerebral blood flow and raising the likelihood of stroke and vascular dementia. Additionally, impaired circulation from elevated fibrinogen may decrease oxygen and glucose delivery to the brain, contributing to neuronal stress and cognitive decline. This highlights a crucial link between systemic inflammation and neurodegeneration. In conclusion, this finding suggests that in this Alzheimer's model systemic inflammation was present due to elevated plasma fibrinogen but the lack of CSF cellular changes and stable plasma viscosity indicate minimal neuroinflammation and an intact blood-brain barrier.

# CHAPTER ONE

## 1.1. BACKGROUND OF STUDY

Alzheimer's Disease (AD) is a chronic and progressive neurodegenerative condition marked by cognitive decline, memory impairment, and behavioral changes. The disease is primarily linked to the buildup of amyloid-beta (Aβ) plaques and tau protein tangles in the brain, which contribute to neuronal dysfunction and eventual cell death (Selkoe and Hardy, 2016). While much research has focused on the brain's pathological changes, there is increasing interest in the role of cerebrospinal fluid (CSF) alterations and plasma rheology in the progression of Alzheimer's disease.

Cerebrospinal fluid (CSF) is found within the cerebral ventricles, surrounding and protecting the brain and spinal cord. Because it closely interacts with the brain's interstitial fluid, CSF serves as a valuable medium for assessing neurodegenerative disorders. Changes in its composition often reflect the progression of Alzheimer's disease. Notably, decreased levels of Aβ42—an essential component of amyloid plaques—and increased concentrations of tau protein serve as key biomarkers of the disease. Since these molecular alterations appear in the early stages of AD, analyzing CSF composition is crucial for understanding its pathophysiology (Blennow and Zetterberg, 2018).

Plasma rheology, which examines the flow characteristics of blood—including viscosity, elasticity, and deformation—plays a critical role in maintaining proper circulation. These properties are influenced by systemic inflammation and vascular abnormalities, both of which have been closely associated with the development of Alzheimer's disease (Sundquist *et al*., 2020). Studies suggest that alterations in plasma rheology may exacerbate AD progression by impairing cerebral blood flow and oxygen supply to the brain. A significant feature of plasma

rheology in AD is increased blood viscosity, which can reduce cerebral perfusion and limit the delivery of essential nutrients and oxygen, further contributing to neuronal damage. Additionally, plasma proteins such as fibrinogen interact with Aβ, forming abnormal fibrin clots that are more resistant to breakdown (Cortes-canteli *et al*., 2010). These disruptions in blood flow and clotting mechanisms highlight the significance of plasma rheology in AD pathogenesis.

Animal models of Alzheimer's disease provide a controlled setting for studying these factors. Wistar rats are widely used in biochemical research due to their reliability in simulating disease-related changes in plasma rheology and CSF composition (Salkovic-petrisic and Lackovic, 2021). In experimental AD models, induced rats exhibit similar blood alterations to human patients, including increased viscosity and fibrinogen levels, alongside CSF changes such as elevated Aβ and tau concentrations (Zatta *et al*., 2002).

## 1.2. JUSTIFICATION OF STUDY

Alzheimer's disease remains a major global health concern with profound socio-economic consequences and no established cure. Investigating cerebrospinal fluid (CSF) and plasma rheology in Alzheimer's-induced rat models is essential for gaining deeper insights into the complex pathology of the disease. While most studies emphasize brain-specific markers such as amyloid plaques, emerging research suggests that alterations in CSF and plasma also play a critical role in disease progression.

The Wistar rat model, which exhibits Alzheimer's-like characteristics, serves as a valuable tool for replicating human AD pathology under controlled conditions. Examining both CSF and plasma together in this model may help identify novel biomarkers and potential therapeutic targets, ultimately enhancing early diagnosis and contributing to more effective treatment strategies.

## 1.3. AIM OF RESEARCH

The aim of this study is to investigate the change in Cerebrospinal fluid cellular content and plasma rheology in rats induced with Alzheimer's disease.

## 1.4. RESEARCH QUESTIONS

To address the aim outlined above, the following research questions helped to guide our study:

- Does Alzheimer disease affect the cellular composition of cerebrospinal fluid (CSF) in wistar rats?
- Does Alzheimer disease alter plasma rheology specifically plasma viscosity and fibrinogen concentration in wistar rats?
- Does change in Cerebrospinal fluid cellular composition and plasma rheology serve as potential biomarkers for Alzheimer's disease in wistar rats?
- Is there any relationship between cerebrospinal fluid cellular changes and plasma rheological parameters in wistar rats with Alzheimer's disease?

## 1.5. SPECIFIC OBJECTIVES OF STUDY

- To determine the effect of Alzheimer's disease on the cellular composition in wistar rats.
- To determine plasma viscosity in wistar rats induced with Alzheimer's disease.
- To determine plasma fibrinogen concentration in wistar rats induced with Alzheimer's disease
- To determine if change in cerebrospinal fluid cellular content and plasma rheology serve as a potential biomarkers for Alzheimer's disease in wistar rat
- To determine the relationship between cerebrospinal fluid cellular changes and plasma rheological parameters in wistar rats with Alzheimer's disease

# CHAPTER TWO

## 2.1. LITERATURE REVIEW

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the leading cause of dementia, accounting for approximately 60–80% of dementia cases worldwide (Alzheimer's Association, 2023). The condition is marked by gradual cognitive decline, memory impairment, and difficulties in performing daily tasks. While AD primarily affects individuals over 65 years of age, early-onset cases can develop in people as young as 40 years old. On average, patients survive between 4 and 8 years after diagnosis, though some may live significantly longer. With the global prevalence of Alzheimer's rising, the World Health Organization (WHO) projects that by 2050, dementia cases will triple, placing immense strain on healthcare systems and caregivers (WHO, 2022).

The development of Alzheimer's disease is influenced by multiple factors, including genetic, environmental, and lifestyle components. Genetic mutations in the amyloid precursor protein (APP), presenilin-1 (PSEN1), and presenilin-2 (PSEN2) genes are strongly linked to early-onset familial Alzheimer's disease, whereas the apolipoprotein E (APOE) ε4 allele is the most recognized genetic risk factor for late-onset Alzheimer's disease (Karch and Goate, 2015). Additionally, chronic stress, poor diet, lack of physical activity, and exposure to environmental toxins have been associated with an increased likelihood of developing Alzheimer's (Livingston *et al*., 2020).

At the pathological level, Alzheimer's disease is characterized by the abnormal buildup of amyloid-beta plaques and tau neurofibrillary tangles in the brain. These formations disrupt neuronal communication, impair synaptic function, and eventually lead to neuronal death. Amyloid-beta plaques interfere with neural signaling, while tau tangles destabilize microtubules,

compromising cellular structure and function (Hyman *et al*., 2020). As the disease progresses, initial symptoms such as memory loss extend to language difficulties, attention deficits, and impaired executive function. In advanced stages, patients experience significant behavioral changes, a loss of independence, and ultimately, death.

## 2.2. THE BRAIN AND ALZHEIMER'S DISEASE

### 2.2.1. GROSS ANATOMY OF THE BRAIN

The brain is a highly complex and essential organ responsible for regulating and coordinating most bodily functions. As the central component of the nervous system, it is housed within the cranial cavity and safeguarded by the skull, meninges, and cerebrospinal fluid (CSF).

The cerebrum, the largest region of the brain, constitutes about 85% of its total weight. It is divided into two hemispheres linked by the corpus callosum, with each hemisphere further segmented into four lobes. The frontal lobe governs executive functions such as reasoning, decision-making, and motor control (Kandel *et al*., 2021). The parietal lobe is involved in sensory perception and spatial orientation (Bear *et al*., 2015). The temporal lobe plays a crucial role in auditory processing and memory formation (Purves *et al*., 2018), while the occipital lobe specializes in visual processing (Braak and Braak, 1991). Covering the cerebrum is the cerebral cortex, a thin layer of gray matter responsible for higher cognitive functions, including language, consciousness, and problem-solving (Hardy and Selkoe, 2002).

Beneath the cerebrum lies the diencephalon, which consists of the thalamus and hypothalamus. The thalamus acts as a relay center, directing sensory and motor signals to and from the cerebral cortex (Kandel *et al*., 2021). The hypothalamus is critical for maintaining homeostasis by regulating vital functions such as body temperature, thirst, hunger, and sleep cycles (Purves *et al*., 2018).

The brainstem, which connects the brain to the spinal cord, comprises three key regions: the midbrain, pons, and medulla oblongata. The midbrain is responsible for processing visual and auditory reflexes (Bear *et al*., 2015). The pons facilitates communication between the cerebrum and cerebellum and plays a role in respiration and sleep regulation (Morris *et al*., 2014). The medulla oblongata controls vital autonomic functions, including heart rate, respiration, and blood pressure (Braak and Braak, 1991).

The cerebellum, located at the back of the brain, is essential for coordinating movement, maintaining balance, and ensuring precise motor activity. Although smaller than the cerebrum, it contains more neurons than any other brain region, emphasizing its high computational capacity (Purves *et al*., 2018).

The limbic system is a network of interconnected structures that regulate emotion, memory, and behavior. The hippocampus plays a central role in memory formation and retrieval (Hardy and Selkoe, 2002), while the amygdala is involved in processing emotions such as fear and pleasure (Kandel *et al*., 2021).

Lastly, the ventricular system of the brain consists of interconnected cavities filled with cerebrospinal fluid (CSF). These ventricles cushion the brain, assist in nutrient transport, and aid in the removal of metabolic waste (Morris *et al*., 2014).

## 2.2.2. IMPACT OF ALZHEIMER'S DISEASE ON BRAIN REGION

Alzheimer's disease (AD) is marked by progressive neurodegeneration, with defining pathological alterations such as amyloid-beta plaques, tau tangles, and neuronal loss. These brain regions include;

## Hippocampus

The hippocampus is among the earliest and most severely affected brain regions in Alzheimer's disease (AD). It plays a crucial role in converting short-term memories into long-term storage and is essential for spatial navigation. Research has consistently reported significant hippocampal atrophy in AD patients, which closely correlates with memory decline (Braak and Braak, 1991). Neuropathological analyses indicate that the buildup of neurofibrillary tangles within hippocampal neurons leads to synaptic dysfunction and eventual neuronal death (Jack *et al*., 2018). Functional imaging studies, including MRI and PET scans, have revealed both reduced hippocampal volume and decreased metabolic activity in the early stages of AD (Sperling *et al*., 2011). This progressive structural and functional deterioration disrupts memory networks, leading to the characteristic memory loss observed in AD patients.

## Cerebral Cortex

In Alzheimer's disease (AD), extensive degeneration occurs in the cerebral cortex, particularly in the association areas of the temporal, parietal, and frontal lobes. The temporal lobe, including the entorhinal cortex, is among the first regions affected, leading to disruptions in information processing between the hippocampus and neocortex (Van Hoesen *et al*., 2000). As the disease advances, the parietal lobe deteriorates, impairing visuospatial skills and attention. In later stages, degeneration spreads to the frontal lobe, which is responsible for executive functions like problem-solving and decision-making (Hardy and Selkoe, 2002). The cognitive decline seen in AD patients is closely associated with neuronal loss and synaptic dysfunction in these cortical regions. Positron emission tomography (PET) scans using fluorodeoxyglucose (FDG-PET) have shown reduced metabolic activity in these areas, with the degree of hypometabolism correlating with disease severity (Mosconi *et al*., 2008).

## Basal Forebrain

The basal forebrain, particularly the nucleus basalis of Meynert, serves as the main source of cholinergic input to the cortex and hippocampus. In Alzheimer's disease (AD), the degeneration of cholinergic neurons in this region leads to a significant decline in acetylcholine levels, a neurotransmitter essential for learning and memory (Schliebs and Arendt, 2011). This cholinergic deficit is a key factor in AD pathology and forms the rationale behind cholinesterase inhibitor therapies, which aim to enhance acetylcholine availability in the brain.

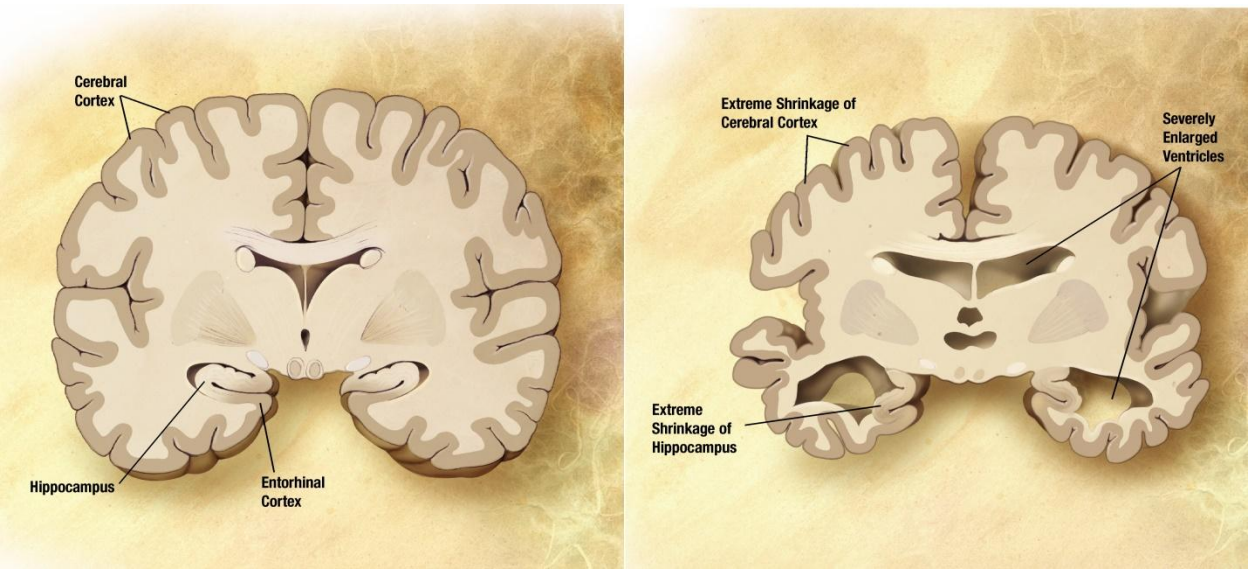


**Figure 2.2: Alzheimer's disease brain comparison**

Source: www.wikipedia.com

https://commons.wikimedia.org/wiki/File:Alzheimer%27s_disease_brain_comparison.jpg

### 2.2.3. HISTOLOGICAL ANALYSIS OF BRAIN TISSUE IN ALZHEIMER'S RESEARCH

Histological examination remains a crucial method for understanding the pathology of Alzheimer's disease (AD). Studies using Wistar rat models have provided essential insights into the cellular and molecular changes associated with the disease. $AlCl_3$-induced AD models mimic key pathological features observed in humans, such as the formation of amyloid plaques and neurofibrillary tangles. These abnormalities are particularly pronounced in the hippocampus, a critical brain region for learning and memory, where extensive neuronal loss and synaptic dysfunction are evident.

Immunohistochemical staining techniques are commonly used to detect amyloid-beta and tau deposits, while hematoxylin and eosin (H&E) staining helps identify neuronal degeneration and gliosis. These histological analyses confirm successful disease induction in experimental models and provide a foundation for assessing the effectiveness of potential therapeutic interventions.

## 2.3. CEREBROSPINAL FLUID (CSF) ANALYSIS IN ALZHEIMER'S RESEARCH

The analysis of cerebrospinal fluid (CSF) has become a crucial aspect of Alzheimer's disease (AD) research, as it provides valuable insights into the pathological mechanisms occurring in the central nervous system (CNS). CSF, a transparent and colorless fluid that surrounds and protects the brain and spinal cord, serves as a vital medium for detecting biochemical and cellular alterations associated with neurodegenerative disorders like AD. Examining the cellular composition of CSF, including neuronal and glial markers, alongside key biomarkers such as amyloid-beta (Aβ) and tau proteins, has significantly contributed to improving the diagnosis, understanding disease mechanisms, and tracking AD progression.

### 2.3.1. CSF CELLULAR MARKERS IN ALZHEIMER'S DISEASE

In addition to measuring soluble biomarkers like amyloid-beta (Aβ) and tau, cerebrospinal fluid (CSF) analysis also involves assessing cellular components, which can provide crucial insights into the disease state. Although CSF typically contains few cells, changes in specific cell types can serve as important indicators of Alzheimer's disease (AD) pathology.

**Neurons**: AD-related neuronal loss and dysfunction contribute to a reduction in neurons within the CSF. Markers such as **neuron-specific enolase (NSE) and neurofilament light chain (NFL)** are commonly used to evaluate neuronal injury and degeneration. Notably, elevated NFL levels have been observed in AD patients, with concentrations correlating with disease progression (Gisslén *et al*., 2016).

**Microglia and Astrocytes**: These glial cells are central to the neuroinflammatory response in AD. Microglia, the brain's resident immune cells, play a role in clearing amyloid plaques and regulating neuronal health. Alterations in microglial activity—such as changes in cytokine levels or shifts in microglial phenotypes—can be detected in CSF, offering insights into neuroinflammation in AD. Similarly, astrocytes, which support neuronal function and help maintain the blood-brain barrier, release **glial fibrillary acidic protein** (GFAP) when activated. Increased GFAP levels in CSF serve as a marker of astrocyte reactivity in AD (Van Wijk *et al*., 2016).

**Extracellular Vesicles (EVs)**: Neurons and glial cells release extracellular vesicles (EVs), including exosomes, in response to stress, injury, or disease. These vesicles contain proteins, RNA, and lipids that reflect the cellular environment from which they originate. The study of EVs in CSF has emerged as a promising area of AD research, offering potential insights into disease mechanisms and novel biomarker discovery (Cheng *et al*., 2018).

### 2.3.3. CHALLENGES AND LIMITATIONS OF CSF CELLULAR ANALYSIS

Despite its value in Alzheimer's disease research, cerebrospinal fluid (CSF) analysis comes with notable challenges and limitations:

- **Low Cell Count**: CSF naturally contains a very small number of cells, making detailed cellular analysis difficult. Detecting and characterizing these cells often requires specialized techniques such as flow cytometry and high-sensitivity assays, which can be both expensive and time-consuming.
- **Heterogeneity of Cellular Sources**: The cells present in CSF may originate from multiple sources, including the brain, blood, and surrounding tissues. This diversity complicates the interpretation of results, as it can be difficult to pinpoint the exact source of certain cellular markers in relation to Alzheimer's pathology.
- **Invasive Nature of Collection**: Obtaining CSF requires a lumbar puncture (spinal tap), an invasive procedure that, while generally safe, carries potential risks such as infection or cerebrospinal fluid leakage. These factors can discourage participation in studies relying on CSF analysis.

## 2.4. PLASMA RHEOLOGY AND ALZHEIMER'S DISEASE

Plasma rheology examines the flow characteristics of blood, including properties such as plasma viscosity, fibrinogen levels, and the movement of blood cells within circulation. In Alzheimer's disease, disturbances in these rheological factors may lead to reduced cerebral blood flow, contributing to vascular dysfunction and impairing brain perfusion. These changes can

exacerbate neuronal damage and cognitive decline by limiting oxygen and nutrient supply to brain tissues.

## Blood-Brain Barrier Dysfunction and Cerebral Blood Flow

The blood-brain barrier (BBB) plays a vital role in preserving the stability of the central nervous system (CNS) by controlling the movement of substances between the bloodstream and brain tissue. In Alzheimer's disease, this barrier becomes compromised, increasing its permeability and allowing harmful molecules, such as amyloid-beta peptides, to enter the brain, thereby worsening neurodegeneration (Zlokovic, 2011). Additionally, abnormalities in cerebral blood flow, often due to increased plasma viscosity and elevated fibrinogen levels, contribute to reduced perfusion in key brain areas like the hippocampus, which accelerates cognitive decline in affected individuals (Ruitenberg *et al*., 2005). Experimental studies using Wistar rats have shown that cerebral blood flow is significantly reduced in Alzheimer's disease models, leading to inadequate perfusion in memory-associated regions like the hippocampus. Research further indicates that increased plasma viscosity and high fibrinogen concentrations are linked to vascular dysfunction in the brain, correlating with cognitive impairment (Van der Willik *et al*., 2018). These findings highlight the potential of plasma rheology as a biomarker for Alzheimer's disease and suggest that targeting blood viscosity may offer a therapeutic approach.

## 2.4.1. EFFECTS OF PLASMA VISCOSITY ON ALZHEIMER'S DISEASE

Plasma viscosity, which describes the blood's resistance to flow, is primarily influenced by components like fibrinogen and hematocrit. In Alzheimer's disease, alterations in plasma viscosity have been observed and are believed to contribute to vascular cognitive impairment (VCI). Increased viscosity slows down blood circulation, especially in smaller vessels, leading to

reduced oxygen supply to neurons. Research has indicated a strong correlation between elevated plasma viscosity and cognitive decline, suggesting that it may serve as an independent risk factor for Alzheimer's disease (Eidelman *et al*., 2016).

Elevated blood viscosity may play a role in the onset of microvascular damage and the formation of blood clots, potentially obstructing blood flow to brain tissue and exacerbating the vascular component of Alzheimer's disease. Studies suggest that regulating plasma viscosity through targeted treatments could help improve cognitive function and support brain health in individuals with Alzheimer's (Paul *et al*., 2017).

### 2.4.2. PLASMA FIBRINOGEN AND NEUROINFLAMMATION IN ALZHEIMER'S

Fibrinogen, a crucial protein involved in blood clotting, also serves as an inflammatory marker linked to a heightened risk of neurodegenerative disorders like Alzheimer's disease. While its primary role is in coagulation, fibrinogen can contribute to inflammation, worsening the neuroinflammatory response observed in Alzheimer's. Increased plasma fibrinogen levels have been associated with a greater accumulation of amyloid plaques and tau tangles, intensifying neuronal damage and disease progression (Paul *et al*., 2017).

Additionally, fibrinogen plays a direct role in amyloid plaque formation by promoting the aggregation of amyloid-beta peptides in the brain. Persistent inflammation, fueled by increased fibrinogen levels and other pro-inflammatory markers, triggers the activation of microglia and astrocytes. This sustained immune response further amplifies neuroinflammation, accelerating neuronal degeneration in Alzheimer's disease (Muller *et al*., 2018).

## 2.5. EXPERIMENTAL MODEL OF ALZHEIMER'S DISEASE

### 2.5.1. LITERATURE REVIEW OF WISTAR RATS AS A MODEL FOR ALZHEIMER'S DISEASE

Wistar rats are widely utilized in Alzheimer's disease research because their physiological characteristics are well-documented, and they can be used to replicate neurodegenerative processes similar to those observed in humans. These albino rats are a preferred choice for experimental studies due to their manageable nature, affordability, and accessibility, making them a valuable model for investigating the pathology and potential treatments of Alzheimer's disease.

**Neuropathological Features of AD in Wistar Rats**

Wistar rats serve as a valuable model for studying neurodegenerative conditions, including Alzheimer's disease. In experimental models of AD, these rats are often subjected to agents like aluminum chloride, which induces hallmark pathological features such as amyloid-beta (Aβ) plaque accumulation, neurofibrillary tangles, oxidative stress, and neuroinflammation.

Several factors make Wistar rats an ideal choice for AD research. Their cognitive function can be evaluated using behavioral tests, such as the Morris water maze, which assesses spatial memory and learning. Additionally, when exposed to amyloid-beta peptides, toxins, or other neurotoxic agents, Wistar rats exhibit characteristic neurodegenerative changes similar to those seen in Alzheimer's patients. Their predictable response to AD-inducing substances, along with their well-documented physiology, reinforces their reliability as a model for studying disease progression and testing potential treatments.

Extensive research has utilized Wistar rats to explore Alzheimer's pathophysiology and assess the effectiveness of therapeutic interventions.

- **Aluminum Chloride-Induced Alzheimer's Disease Model**: Studies have demonstrated that administering aluminum chloride to Wistar rats leads to Alzheimer's-like symptoms, including cognitive deficits, increased oxidative stress, and the accumulation of amyloid plaques. This model is widely used in research to investigate the impact of anti-amyloid therapies and to better understand the underlying mechanisms of Alzheimer's disease (Siddique *et al*., 2021).

- **Amyloid-Beta Infusion Model**: A widely used approach in Alzheimer's research involves the direct infusion of amyloid-beta peptides into the brains of Wistar rats. This method replicates key pathological features of the disease, including cognitive impairment and neuroinflammation. Research has demonstrated that these rats exhibit memory deficits and inflammatory responses similar to those observed in human AD, making this model valuable for studying disease mechanisms and evaluating potential treatments (Sengupta *et al*., 2020).

- **Inflammation and Oxidative Stress**: Wistar rats have been extensively utilized to investigate the link between oxidative stress, inflammation, and the progression of Alzheimer's disease. Studies have shown that persistent inflammation and elevated oxidative stress levels accelerate neuronal damage and cognitive decline in these models (Jing *et al*., 2019).

These studies have provided essential insights into Alzheimer's disease, particularly the influence of inflammation, oxidative stress, and amyloid-beta accumulation in its progression.

Additionally, Wistar rats have served as a valuable model for evaluating the effectiveness of various pharmacological treatments, including antioxidants, anti-inflammatory agents, and therapies targeting amyloid deposition.

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1. MATERIALS

- Distilled water
- Aluminum chloride salt
- Syringes (1ml, 5ml and 10ml)
- Weighing scales
- Disposable gloves
- Cotton wool
- Beaker
- Plastic animal cages
- Dissecting set
- Chloroform
- Centrifuge
- Test tubes
- Lab coats and safety glasses
- Sample bottles
- Spatula

## 3.2. EXPERIMENTAL ANIMALS

A total of twelve (12) healthy adult wistar rats were used for this study, and the animals were obtained from the Animal House of the Department of Pharmacology, University of Benin. The rats were allowed to acclimatize in the new environment (Animal House) for two weeks before the commencement of the study. The rats were separated by the use of body markings for identification and were placed in well ventilated plastic cages of suitable temperature and humidity and kept under natural light/dark cycle. They were allowed free access to standard rodent pellets(ACE Feed PLC Benin, Nigeria) and water ad libitum, in accordance with the guidelines of National Research Council guide for the care of laboratory animals as described by (NRC, 1996) and revised by (Mansour *et al.,* 2017).

## 3.3. EXPERIMENTAL DESIGN

After acclimatization for two weeks, the rats were separated into two groups of six (6) each named control group and experiment group.

- Group 1 consisted of 6 adults female wistar rats with an average weight of 170g and was labeled “control group”.
- Group 2 consisted of 6 adults female wistar rats with an average weight of 170g and was labeled “experiment group”.

### 3.3.1. METHOD OF INDUCING ALZHEIMER’S DISEASE

After a period of Acclimatization for two weeks, Alzheimer’s disease was induced into the experiment group. For this study, Aluminum chloride salt ($Alcl_3$) was used to induce Alzheimer’s disease, a modified guideline was used which was outlined by (Enogieru and Usen, 2023) where 100mg of $Alcl_3$ salt was dissolved in 10ml of distilled water to achieve a

concentration of 100mg/ml. The experimental group was given intraperitoneal injections of aluminum chloride salt.

## 3.4. STUDY DURATION

The experiment lasted for a period of 28 days after which the animals were sacrificed and samples were collected.

### Precautions taken during the course of the study

- The rats were allowed a two weeks acclimatization period before carrying out the study
- The rats were also weighed before the onset of the study
- Proper handling of animals which involved maintenance of hygiene (constant cleaning and changing of beddings, removal of feces and spilled feed from the cages daily).
- Inflicting minimal pain on the animal at any stage of the experiment

## 3.5 ETHICAL CONSIDERATION

Animal management and experimental protocols were carried out in accordance with the recommendation from the ethics and research committee of the University of Benin, Benin City, Edo state, Nigeria.

## 3.6 COLLECTION OF SAMPLES

During the sacrifice, the final weight of the rats were taken using a combat electronic weighing scale calibrated in grams. After recording the final weight, each rat was placed in an enclosed container with cotton wool soaked in about 50 ml of chloroform for anesthesia. After about 3 minutes, the rats were removed from the enclosed container and placed in a supine position on a dissection table. Cerebrospinal fluid was collected through the cisterna magna puncture by

inserting a needle below the occipital bone at the back of the skull, abdomino-thoracic incision was made to expose the abdominal viscera using surgical blade and scissor. Blood was collected from the cardiac puncture, inserting a needle into the heart to collect a large volume of blood from the rats, ideally after CSF collection. Blood is centrifuged to separate the plasma. Sample bottles were properly labeled and used for laboratory analysis.

## 3.7. SAMPLE ANALYSIS

## DETERMINATION OF PLASMA VISCOSITY

## PRINCIPLE

The simple viscometer technique, described by Reid and Ugwu (1987), based on the rate of flow was used for the whole viscosity.

## METHOD

2.0ml of collected blood sample were centrifuged and the plasma was collected into the plain bottles.

The plasma to be tested were drawn up into the vertical syringe, being careful to avoid air bubbles, until the end of the plunger passed the 1ml graduation mark. The plunger was removed slowly and carefully, and a stopwatch started when the lower meniscus of the fluid dropped to the 1ml graduation mark. The time required for 1ml of plasma to flow down the syringe was noted. The same syringe and needle combination was used for the whole series measurements. The plasma viscosity was expressed as the ratio of the flow-time for 1ml of plasma sample to the same volume of distilled water.

## DETERMINATION OF PLASMA FIBRINOGEN CONCENTRATION

## PRINCIPLE

Plasma fibrinogen was determined by the clot-weight technique of Ingram (1961), as modified by Mackie and Machin gravimetric assay method (1989).

## METHOD

2.25ml of blood sample were added to 0.25ml of 3.8% sodium citrate anticoagulant and centrifuged for about 15minutes. 1ml of the clear plasma obtained was introduced into the test tubes containing 1ml of pre warmed calcium chloride and placed in a water bath for 30minutes with an applicator stick dipped into each test tube, after which the cloth formed was collected and compressed to express solution and non-clotted proteins, washed in distilled water and then dried and weighed on a digital weighing balance.

## DETERMINATION OF CEREBROSPINAL FLUID CELLULAR ANALYSIS

Macroscopy;

Describe the appearance; i.e. clear, slightly cloudy, bloodstained, purulent (like pus), or xanthochromia (yellow).

Microscopy

CELL COUNT:

A standard total WBC and RBC cell count was performed on the uncentrifuged CSF sample.

Gram Stain

a. A drop of thoroughly mixed sample with a sterile pipette was placed onto a clean microscope slide.

b. A sterile loop was used to spread and make a thin film for Gram Staining.

c. Slide was allowed to air dry.

d. Slide was briefly heat fixed in a Bunsen flame and, allowed to cool and stained using the Gram Stain method.

e. CSF Gram Stained Smear was examined microscopically for pus cells, bacteria, and yeast cells using the 40x and 100x objectives.

## 3.8. STATISTICAL ANALYSIS

Data were subjected to statistical analysis using t-test with graph pad prism 10.2.2 and relevant statistical values were obtained. One way analysis of variance (ANOVA) was carried out and data were presented as Standard error of Mean (SEM). Values of $P<0.05$ were considered significant. The statistical values obtained were converted into graphical representations in the form of bar charts.

# CHAPTER FOUR

# RESULTS

| | **Control** | **Alzheimer's disease** |
|---|---|---|
| **Visual** | Clear | Clear |
| **No. Of cells** | < 5 cells | < 5 cells |
| | | |

**Table 4.1: showing cerebrospinal fluid microscopy in control and Alzheimer-induced rats**

Result shows no difference in CSF microscopy in control and Alzheimer induced rats as CSF was clear, visible and had less than 5 cells in both control and Alzheimer -induced rats.

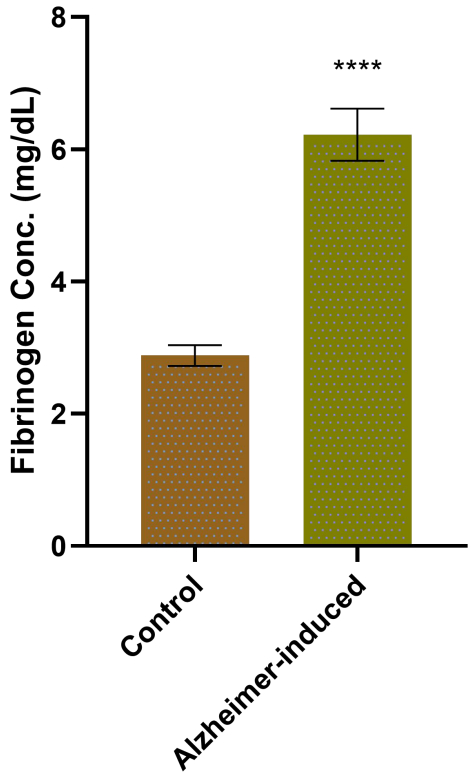


**Figure 4.1: chart showing changes in fibrinogen concentration in rats induced with Alzheimer's disease**

Result shows a statistically significant increase in the Alzheimer-induced group compared with control ($p>0.05$).

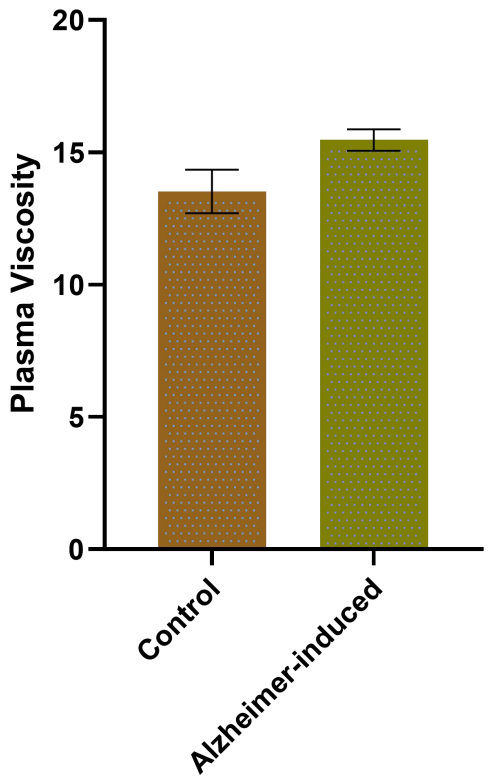


**Figure 4.2: chart showing changes in plasma viscosity in rats induced with Alzheimer's disease**

Result shows no statistically significant difference in the Alzheimer-induced group compared with control ($p<0.05$).

# CHAPTER FIVE

# DISCUSSION AND CONCLUSION

## 5.1. DISCUSSION

The pathologies involved in Alzheimer's disease is multifaceted. The study done highlighted the variations in CSF cellular analysis, plasma fibrinogen concentration, and plasma viscosity in controlled wistar rats and Alzheimer induced wistar rats. The CSF analysis results showed no significant differences between the control and Alzheimer-induced rats. In both groups, the CSF remained clear, indicating the absence of infection, bleeding, or abnormal protein accumulation. If Alzheimer's disease had significantly altered CSF composition, it might have appeared cloudy (suggesting inflammation) or bloody (indicating damage). Both groups had less than 5 cells per field, suggesting no significant cellular infiltration in the CSF. A higher white blood cell (WBC) count would typically indicate inflammation or infection, but since the cell count remained similar in both groups, the Alzheimer's induction did not trigger a noticeable immune response in the CSF. The absence of change in the CSF analysis indicates minimal neuroinflammation in the brain. Also, Alzheimer's disease primarily affect brain tissues rather than causing immune cell infiltration in the CSF indicating that the Blood brain barrier was still intact or was midly compromised preventing significant influx of immune cells into the CSF. These findings align with existing research indicating that Alzheimer's disease primarily affects CSF biochemical markers (such as decreased amyloid-beta and increased tau proteins) rather than cellular composition (Blennow and Zetterberg, 2018). Unlike infections such as meningitis, which trigger an immune response in the CSF, Alzheimer's disease is primarily a neurodegenerative disorder. The key pathological changes, including beta-amyloid plaques and tau tangles, occur within brain tissue rather than in the CSF itself. Since CSF microscopy mainly detects infections or

inflammatory conditions, it may not show major changes in Alzheimer's disease (Heneka *et al*., 2015).

This study also examined how Alzheimer's disease affects plasma fibrinogen concentration and plasma viscosity in rats. The results showed a notable increase in plasma fibrinogen levels in the Alzheimer-induced group compared to controls ($p < 0.05$). However, plasma viscosity did not change significantly between the two groups ($p > 0.05$). These findings suggest that while Alzheimer's disease alters certain blood parameters, its impact on blood flow properties may be more complex than expected. Increased fibrinogen in this study indicates early-stage neuroinflammation. This may lead to hypercoagulability, increasing the risk of blood clots, potentially reducing cerebral blood flow and raising the likelihood of vascular dementia. Additionally, impaired circulation from elevated fibrinogen may decrease oxygen and glucose delivery to the brain, contributing to neuronal stress and cognitive decline. This highlights a crucial link between systemic inflammation and neurodegeneration (Cortes-Canteli *et al*., 2010). Since Alzheimer's disease involves chronic neuroinflammation, the body responds by increasing fibrinogen production in the liver (Van oijen *et al*., 2005). Despite the increase in plasma fibrinogen concentration, plasma viscosity did not show a significant rise. This was unexpected because fibrinogen is known to influence blood viscosity. This implies that the degree of fibrinogen increase in this study did not reach the threshold required to affect plasma viscosity indicating minimal inflammation (Cortes-Canteli *et al*., 2010). Also, plasma viscosity depends on multiple factors, including other plasma proteins (albumin, globulins), hydration levels, and hematocrit. If these remained stable, fibrinogen alone may not have been enough to significantly alter plasma viscosity.

## 5.2. CONCLUSION

The results of this study provide valuable insights into how Alzheimer's disease affects cerebrospinal fluid cellular composition and plasma rheology. In conclusion, this finding suggests that in this Alzheimer's model systemic inflammation was present due to elevated plasma fibrinogen but the lack of CSF cellular changes and stable plasma viscosity indicate minimal neuroinflammation and an intact blood-brain barrier.

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