ASSESSMENT OF ROS GENERATION AND IL-1β EXPRESSION IN LN229 GLIOMA CELLS CHALLENGED WITH EXOSOMES DERIVED FROM OKADAIC ACID EXPOSED NEURO2A CELLS

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This is to certify that the dissertation entitled "**ASSESSMENT OF ROS GENERATION AND IL-1β EXPRESSION IN LN229 GLIOMA CELLS CHALLENGED WITH EXOSOMES DERIVED FROM OKADAIC ACID EXPOSED NEURO2A CELLS**" submitted to the University of Kerala in partial fulfilment of the requirements for the award of degree of Master of Science in Biochemistry, is a record of original research work carried out by **ARYAMOL (Candidate code: 63824142003)** of the Department of Biochemistry, T.K.M. College of Arts and Science, Kollam, under the guidance and supervision of **Dr. Viji Vijayan, Assistant Professor, Department of Biochemistry, University of Kerala, Karyavattom**. It is also certified that no part of this work has been presented for the award of any other degree, diploma, or similar title at any other university.

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Dr. Viji Vijayan
Supervising Guide

Thiruvananthapuram,
July 2026

DECLARATION

I, hereby declare that the project work entitled **"ASSESSMENT OF ROS GENERATION AND IL-1β EXPRESSION IN LN229 GLIOMA CELLS CHALLENGED WITH EXOSOMES DERIVED FROM OKADAIC ACID EXPOSED NEURO2A CELLS"** submitted to the University of Kerala, is a record of an original work done by me under the guidance of Dr. Viji Vijayan, Assistant Professor, Department of Biochemistry, University of Kerala, Thiruvananthapuram and this project work is submitted in the partial fulfilment of the requirements for the degree of Master of Science in Biochemistry. The results embodied in this thesis have not been submitted to any other University or Institute for the award of any degree or diploma.

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LIST OF ABBREVIATIONS

AD: Alzheimer’s Disease

APS: Ammonium Persulfate

Aβ: Amyloid Beta

BR Assay: Broad Range Assay

BSA: Bovine Serum Albumin

CD63: Cluster of Differentiation 63

CD81: Cluster of Differentiation 81

CD9: Cluster of Differentiation 9

CNS: Central Nervous System

CO_2: Carbon dioxide

CSFIR: Colony Stimulating Factor 1 Receptor

CTE: Chronic Traumatic Encephalopathy

DCF: Dichloro Flurescein

DCFDA: 2',7'-dichlorodihydrofluorescein diacetate

DMEM: Dulbecco’s Modified Eagle Medium

DNA: Deoxyribonucleic Acid

GSK-3β: Glycogen Synthase Kinase-3beta

Hsp: Heat Shock Protein

IFN: Interferon

IL-1β: Interleukin-1beta

IRF8: Interferon Regulatory Factor 8

JNK: c-Jun N-terminal Kinase

lncRNA: Long non-coding RNA

MA: Massachusetts

MAPT: Microtubule Associated Protein Tau

MFI: Mean Fluorescence Intensity

miRNA: MicroRNA

mRNA: Messenger RNA

MS: Multiple sclerosis

N2A: Neuro2A

NCCS: National Centre for Cell Science

NG2: Neuron-Glial antigen-2

NIH: National Institute of Health

OA: Okadaic Acid

PBS: Phosphate Buffered Saline

PP1: Protein Phosphatase 1

PP2A: Protein Phosphatase 2A

PU.1: Purine-rich Box1

RIPA: Radioimmunoprecipitation Assay Buffer

RNA: Ribonucleic Acid

ROS: Reactive Oxygen species

rRNA: Ribosomal RNA

SDS-PAGE: Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis

TBS: Tris-Buffered Saline

TBST: Tris-Buffered Saline with Tween-20

TEMED: N,N,N',N'-Tetramethylethylenediamine

Tris-HCl: Tris-Hydrochloric Acid

TSG101: Tumor Susceptibility Gene 101

USA: United States of America

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ABSTRACT

Alzheimer’s disease is a progressive neurodegenerative disorder in which oxidative stress and neuroinflammation contribute to disease progression. Exosomes released from neuronal cells may mediate the transfer of pathological signals to recipient glial cells. The present study investigated the effects of exosomes derived from untreated and okadaic acid-treated Neuro2A cells on oxidative stress and inflammatory responses in LN229 cells. Neuro2A cells were exposed to okadaic acid (100 nM) for 4 h, and exosomes were isolated from untreated (Exo-Basal) and okadaic acid-treated cells (Exo -OA). LN229 cells were treated with these exosomes, followed by assessment of intracellular reactive oxygen species using the DCFDA assay and IL-1β protein expression by Western blotting. Both Exo-Basal and Exo-OA increased ROS generation compared with PBS control, with greater ROS induction by Exo-Basal. In contrast, Exo-OA markedly increased IL-1β expression, suggesting enhanced inflammatory signaling under tauopathy-like conditions.

1. INTRODUCTION

The brain is the central organ of the nervous system that regulates cognition, memory, emotion, sensory perception, and motor coordination (Purves et al., 2018). Its normal function depends on the coordinated activity of neurons and glial cells. Neurons are the functional units that transmit electrical and chemical signals, whereas glial cells, including astrocytes, microglia, and oligodendrocytes, provide structural support, maintain homeostasis, regulate synaptic activity, and participate in neuroinflammatory responses (Akay, 2023). Effective communication between neurons and glial cells is essential for maintaining normal brain function (Verkhratsky et al., 2019). This communication occurs through neurotransmitters, cytokines, and extracellular vesicles, particularly exosomes. Exosomes are small extracellular vesicles released by cells that carry proteins, lipids, and nucleic acids to recipient cells, thereby facilitating intercellular communication (Di Bella, 2022). Under pathological conditions, the composition of exosomes changes, allowing them to transfer disease-associated molecules and influence the function of neighbouring cells. Exosomes have gained significant attention in neurodegenerative disorders because they can transport pathological proteins such as amyloid-β and tau between cells, thereby contributing to disease progression (Zhang et al., 2023). Tau is a microtubule-associated protein that normally maintains neuronal structure and function (Weingarten et al., 1975). However, abnormal phosphorylation of tau results in its aggregation, leading to a group of tauopathies. Alzheimer's disease is the most common secondary tauopathy, characterized by both amyloid-β deposition and tau aggregation (Devi, 2023). To study tau pathology, several experimental models have been developed, among which chemical models provide a rapid and reproducible approach. Okadaic acid is one of the most widely used chemical agents because it inhibits protein phosphatases PP1 and PP2A, leading to tau hyperphosphorylation (Medina et al., 2020). Oxidative stress is an important feature of tauopathies. Excessive production of reactive oxygen species (ROS) damages cellular components and further promotes tau hyperphosphorylation (Swerdlow, 2020).

Therefore, the study evaluated whether neuronal stress induced by okadaic acid may also alter the cargo of released exosomes, enabling them to transmit pathological signals to glial cells and trigger inflammatory responses such as increased interleukin-1β (IL-1β) expression.

2. AIM AND OBJECTIVE

2.1 Hypothesis

Exosomes released from Neuro2A cells, particularly under okadaic acid-induced stress conditions, can transmit pathological signals to LN229 cells, leading to enhanced intracellular Reactive Oxygen Species (ROS) generation and Interleukin-1Beta (IL-1β) associated inflammatory activation.

2.2 Aim

To investigate the effect of Neuro2A cell-derived exosomes, isolated under basal and okadaic acid treated conditions, on oxidative stress and inflammatory responses in LN229 cells.

2.3 Objectives

1. To isolate exosomes from untreated and okadaic acid-treated Neuro2A cells.
2. To evaluate intracellular Reactive Oxygen Species (ROS) generation in LN229 cells following treatment with Exo-Basal and Exo-OA using the DCFDA assay.
3. To assess Interleukin-1Beta (IL-1β) protein expression in LN229 cells after treatment with Neuro2A-derived exosomes by Western blot analysis.
4. To compare whether basal neuronal exosomes and okadaic acid-stress-derived neuronal exosomes modulate oxidative and inflammatory responses in LN229 cells.

3. REVIEW OF LITERATURE

3.1 Brain

The brain is the central organ of the nervous system and acts as the primary control centre for cognition, memory, emotion, sensory perception, and motor coordination (Purves et al., 2018). The mature human brain contains nearly 100 billion neurons and is considered one of the most complex biological systems (Maldonado et al., 2023). Structurally, it is divided into three major regions: the cerebrum, cerebellum, and brainstem (Figure 1).

The cerebrum is the largest part of the brain and is divided into left and right hemispheres. Its surface contains ridges called gyri and grooves called sulci, while deeper grooves are known as fissures. Each cerebral hemisphere consists of an outer gray matter layer, the cerebral cortex, and inner subcortical white matter. The cerebrum regulates sensory and motor processing, conscious and unconscious behaviour, emotion, intelligence, and memory (Chayer et al., 2001).

The cerebellum consists of the cerebellar cortex and deep cerebellar nuclei. The cerebellar cortex has three layers: molecular, Purkinje, and granular layers. It is connected to the brainstem through cerebellar peduncles and plays a major role in coordinating voluntary movement by integrating sensory information from the brain and spinal cord. The cerebellum also contributes to cognitive functions such as attention, language, pleasure response, and fear memory (Jimsheleishvili et al., 2023).

The brainstem is composed of the midbrain, pons and medulla and lies between the cerebrum and spinal cord, anterior to the cerebellum. It regulates vital autonomic functions, including respiration, heart rate, temperature control, sleep–wake cycles, digestion, coughing, sneezing, vomiting, and swallowing (Gil et al., 2010).

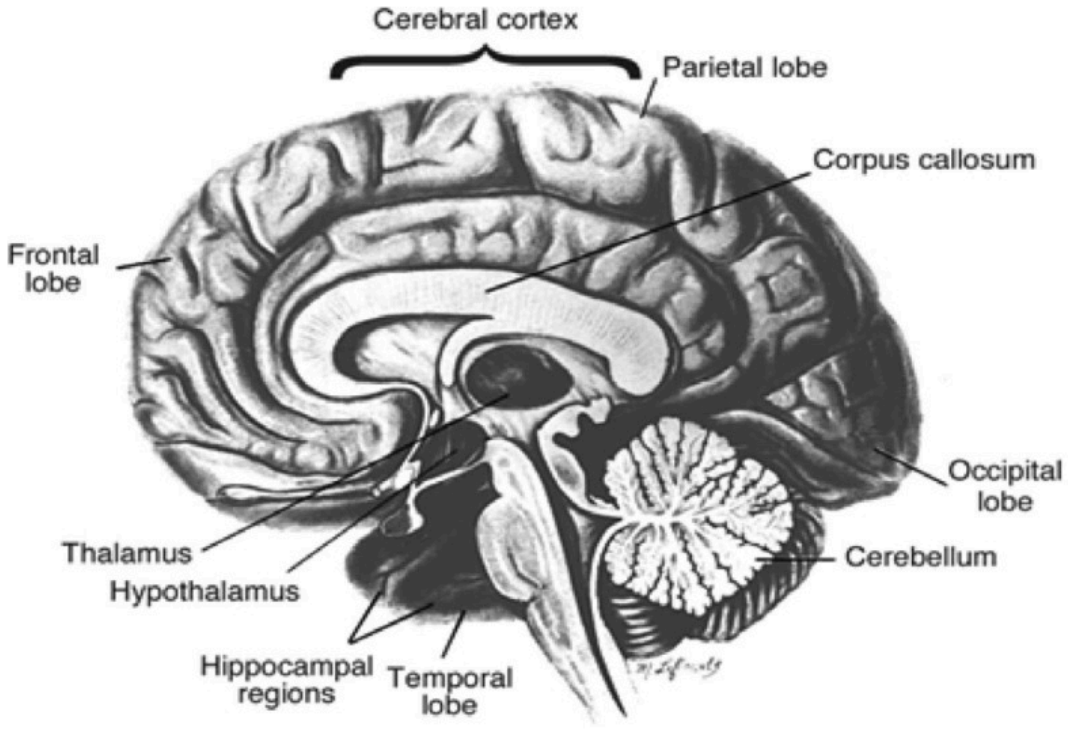


Figure 1: Schematic diagram of the human brain (Adapted from Oscar-Berman et al., 2003).

3.2. Cells of brain

The brain is composed primarily of two major categories of cells: neurons and glial cells, both of which are essential for maintaining normal nervous system function (Kandel et al., 2021).

3.2.1 Neurons

Neurons are the functional unit of nervous system. Through the use of electrical and

chemical impulses, these are in charge of transporting sensations throughout the human body and assisting in the coordination of all essential life functions (Akay, 2023). There are different types of neurons, such as multipolar, bipolar, pseudounipolar, and anaxonic, which vary mainly in the quantity and configuration of axons and dendrites. The soma, or cell body, an axon, and dendrites (Figure 2) make up a typical multipolar neuron. It is believed that the axon is the component that transmits efferent signals, whereas afferent signals from their environment are being received by the dendrites (Ludwig et al., 2023). The nucleus and other organelles required for neuronal function are found in the soma.

3.2.2 Glial Cells

Glial cells are non-neuronal cells that fall into four main categories: microglia, astrocytes, oligodendrocytes, and their progenitors, NG2-glia (Jakel et al., 2017). Nearly half of the brain mass is composed of these cells (Allen et al., 2018).

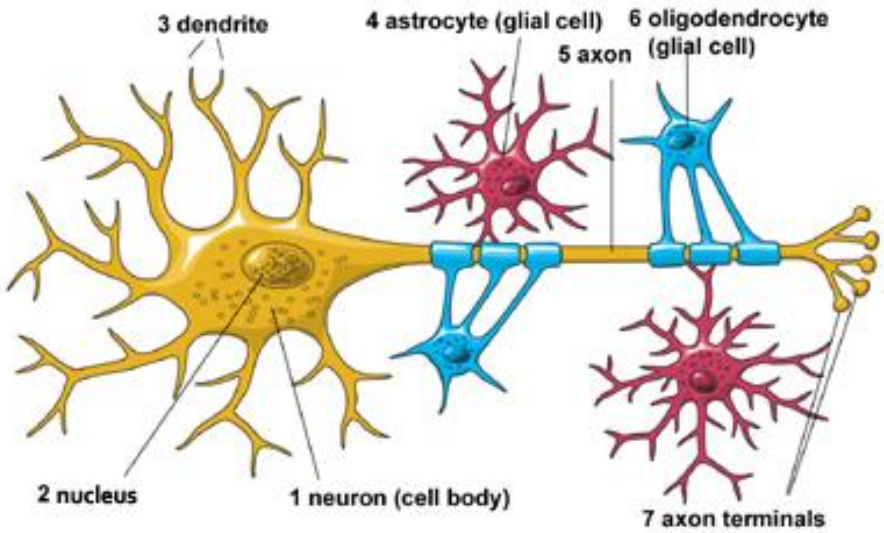


Figure 2: Structure of a neuron (yellow) supported by astrocytes (red) and oligodendrocytes (blue) (Adapted from Taylor et al., 2023)

3.2.2.1 Astrocytes

Astrocytes are a population of central nervous system (CNS) cells with distinctive morphological and functional characteristics that differ within specific areas of the brain and are widely distributed throughout the CNS(Karthik et al., 2021) There are

mainly two types of astrocytes, protoplasmic and fibrous, which differ in morphologic appearance and location. Astrocytes are important cells of the CNS that not only provide structural support, but also modulate synaptic activity, regulate neuroinflammatory responses, maintain the blood–brain barrier, and supply energy to neurons (Chalmers et al., 2024).

3.2.2.2 Microglia

Microglia is known as the resident macrophages of the central nervous systems (CNS) linked to a variety of processes that are critical to the health and development of the adult and developing brain. According to Kierdorf et al., (2013), microglia are typically produced from erythromyeloid progenitors in the yolk sac in a colony-stimulating factor 1 receptor (CSF1R)-dependent way, depending on the transcription factors PU.1 and interferon (IFN) regulatory factor 8 (IRF8) signaling. In healthy settings, microglia can undergo fast clonal proliferation (Jordão et al., 2019), but in homeostasis, the microglia population is long-lived and maintained by modest, local proliferation without input from the periphery (Prinz et al., 2021).

3.2.2.3 Oligodendrocytes

The central nervous systems (CNS) myelinating cells are called oligodendrocytes. According to Bradl et al., (2009), these are produced from oligodendrocyte progenitor cells by carefully planned processes of migration, proliferation, and differentiation. According to Nave (2010), oligodendrocytes are essential for myelin development in the developing central nervous system and for myelin regeneration after injury, notably in multiple sclerosis (MS), the most prevalent demyelinating illness.

3.3 Neuro to glial communication

Neuron–glia communication is essential for normal central nervous system function. It allows neurons and glial cells to coordinate synaptic transmission, neural plasticity, and overall network homeostasis. Neuronal activity can influence glial responses, while glial cells in turn regulate neuronal excitability, synaptic strength, and circuit organization (Verkhratsky et al., 2019).

This bidirectional communication occurs through several mechanisms. Neurons release neurotransmitters that activate calcium signalling in glial cells, and glial cells release gliotransmitters that modulate synaptic activity. These interactions support learning, memory, and brain homeostasis during increased neuronal activity (Khakh and Sofroniew, 2015; Araque et al., 2014). Disruption of neuron–glia communication has been linked to neuroinflammation, synaptic dysfunction, and neurodegenerative disorders (DeMeulenaere et al., 2026).

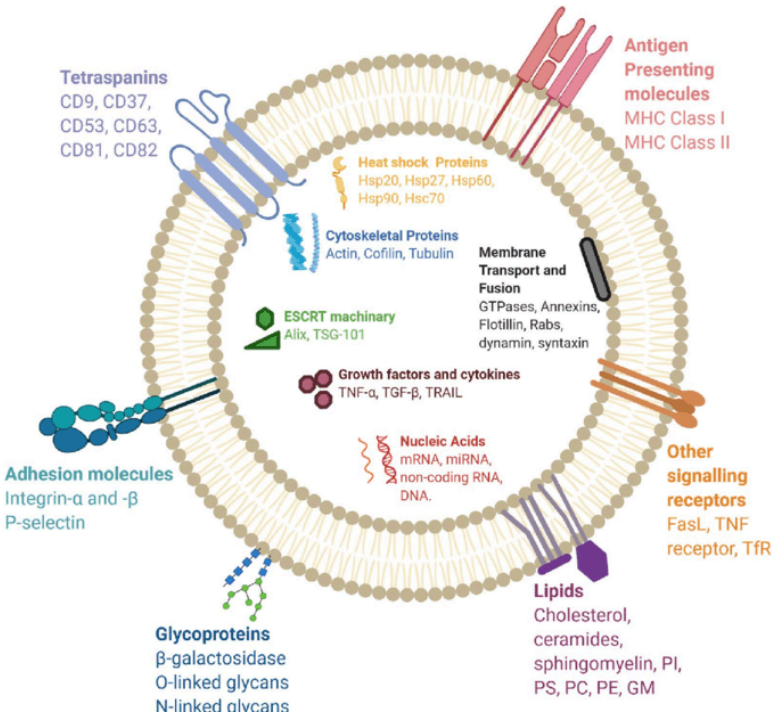
Recent studies also show that neuron–glia signalling contributes to network plasticity by regulating the strength and stability of synaptic connections. Extracellular vesicles and cytokines may also mediate long-range communication between neurons and glial cells (Feng et al., 2025). Therefore, understanding neuron–glia communication is important for studying normal brain function and identifying therapeutic targets in neurological disorders.

3.4 Exosomes

Exosomes are small extracellular vesicles ranging from approximately 30 to 150 nanometers in size. These are formed inside multivesicular bodies and released into body fluids such as blood, urine, and cell culture media when these bodies fuse with the plasma membrane (Di Bella, 2022).

Initially, exosomes were considered cellular waste products; however, current research has established that these play a crucial role in cell-to-cell communication (Malgundkar and Tamimi, 2023). The structure appears cup-shaped under transmission electron microscopy due to sample preparation methods such as dehydration. These have a characteristic density range of about 1.13–1.19 g/mL (Kumari and Anji, 2022).

The membrane of exosomes are rich in lipids including cholesterol, ceramides, phosphatidylserine, and sphingomyelin. These also contain specific proteins such as CD9, CD63, CD81, Alix, TSG101, and heat shock proteins (Hsp70/90), along with cytoskeletal components(Figure 3). In addition, exosomes carry genetic material such as mRNA, miRNA, lncRNA, and rRNA, which reflect the physiological state of their



parent cells (Hánelová et al., 2024; Ortega et al., 2020).

Figure 3: Exosomes and its composition of exosomes (Adapted from Gurung et al., 2021)

Functionally, exosomes influence gene expression in recipient cells and regulate various biological processes, including immune responses, tissue repair, and the maintenance of neuronal communication and synaptic stability (Fan et al., 2022). In pathological conditions, exosomes are linked to tumor progression and the spread of harmful proteins associated with neurodegenerative diseases (Zhang et al., 2023). Due to their nanoscale size, stability, and ability to cross biological barriers, these are increasingly being studied as potential tools for drug delivery and disease diagnosis (Ren et al., 2024). Exosome formation begins in the endosomal system, where early endosomes mature into multivesicular bodies. Inside these structures, intraluminal vesicles are created through inward budding of the membrane, capturing cellular proteins and genetic material (Figure 4). When multivesicular bodies fuse with the plasma membrane,

these vesicles are released into the extracellular space as exosomes (Gurung et al., 2021; Aheget et al., 2020)

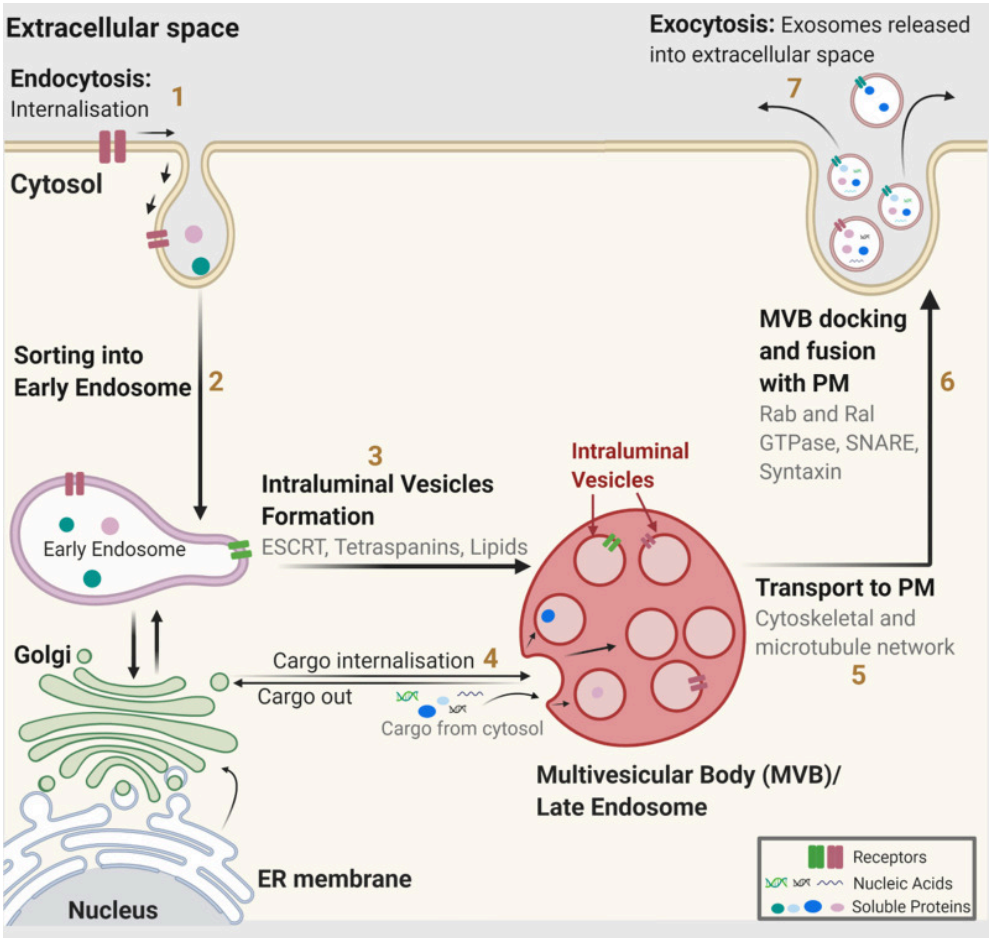
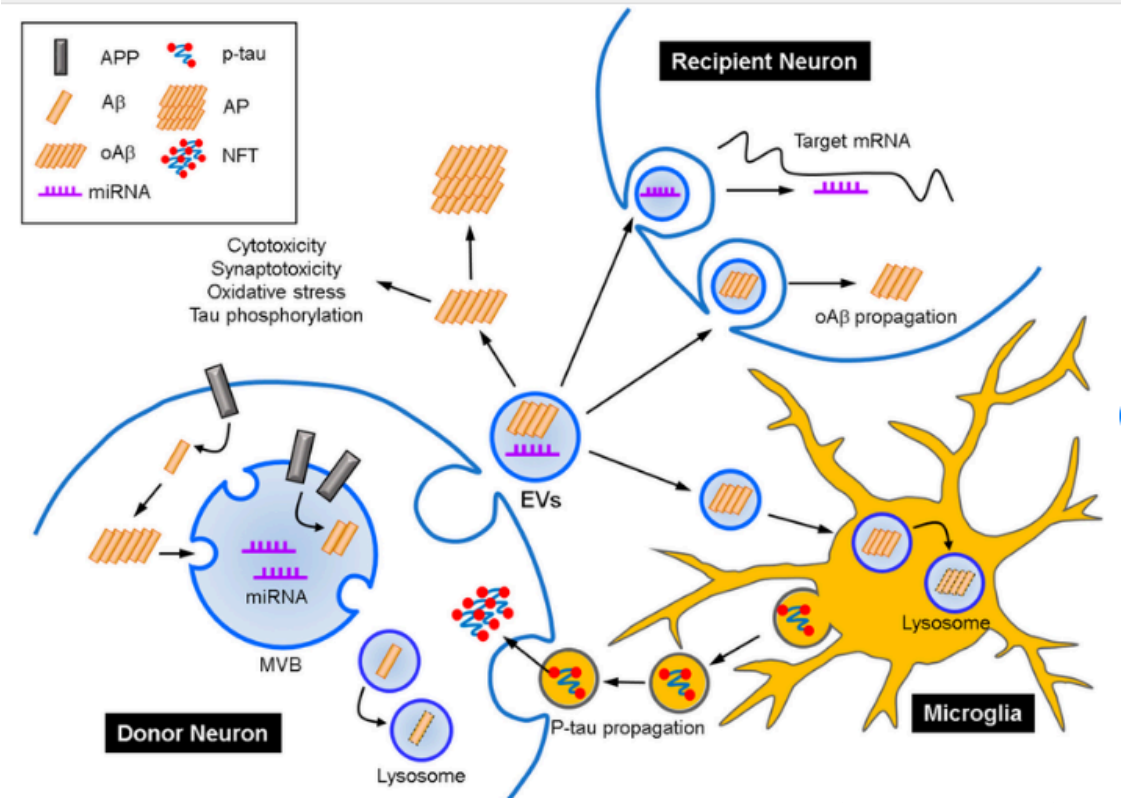


Figure 4: Exosome Biogenesis (Adapted from Gurung et al., 2021).

Exosomes participate in intercellular communication and significantly contribute to the progression of Alzheimer's disease (Figure 5). These facilitate the aggregation and transmission of misfolded amyloid-β (Aβ) and tau proteins (Soliman et al., 2021). During cellular stress or neuronal injury, neuronal exosomes enhance Aβ fibril formation, where early oligomers act as toxic seeds that precede plaque development and impair synaptic communication (Ameer et al., 2024). Exosomes also promote the spread of pathological tau between neurons, mirroring the progression of AD from the entorhinal cortex to the hippocampus and neocortex (Daksh et al., 2025). Neurons secrete tau-containing exosomes in response to synaptic activity, while microglia release tau-rich exosomes after engulfing tau-associated debris, a mechanism linked to ceramide biosynthesis (Liu et al., 2024). Proteomic studies have demonstrated that exosomes transport tau, Aβ, and other neurodegenerative proteins across cells. Increased level of exosomal tau in cerebrospinal fluid strongly correlate with disease advancement,



emphasizing the important role of exosomes in AD pathology (Mu et al., 2023).

Figure 5: Schematic representation of the role of exosomes in Alzheimer's disease pathology (Adapted from Lee et al., 2019)

3.5 Tauopathy

Tau is a microtubule-associated protein that is abundant in the nervous system. It was originally identified as a protein that promotes microtubule assembly and stability (Weingarten et al., 1975). Under normal physiological conditions, tau supports axonal transport, cytoskeletal organization, and neuronal structure. In addition to its role in microtubule regulation, tau is also involved in synaptic function, signal transduction, and protection of nucleic acids such as DNA and RNA (Catarina Silva and Haggarty, 2020).

Tauopathies are a group of neurodegenerative disorders characterized by abnormal aggregation of tau protein in neurons, glial cells, or both. These include several dementias, among which Alzheimer's disease (AD) is the most common. Although tau aggregation is a shared pathological feature, tauopathies differ in their clinical presentation, affected brain regions, major tau isoforms, aggregate morphology, and the cell types involved (Chung et al., 2021). Even within the same cell type, tau inclusions show different morphologies, suggesting that distinct mechanisms may operate in different tauopathies (Beach et al., 2023).

Pure tauopathies are relatively uncommon because tau pathology often coexists with other neurodegenerative changes. For example, argyrophilic grain disease, an underdiagnosed tauopathy, has been reported in a proportion of AD, progressive supranuclear palsy, and corticobasal degeneration cases (Yokota et al., 2023). Similarly, many post-mortem cases meeting neuropathological criteria for AD also show additional pathologies such as infarcts or other protein deposits (Karanth et al., 2021).

Tauopathies may be broadly classified into primary and secondary tauopathies. In primary tauopathies, abnormal tau aggregation is considered the major pathological driver and may occur in neurons, glial cells, or both (Devi, 2023). In secondary tauopathies, tau pathology develops along with or in response to other primary pathological events. Alzheimer's disease is considered a classic example of a secondary tauopathy, where amyloid-β pathology occurs along with tau aggregation.

Environmental tauopathies, such as chronic traumatic encephalopathy, are also recognized within the broader spectrum of tau-related disorders (Devi, 2023).

Tauopathies are commonly divided into primary and secondary tauopathies. Primary tauopathies are disorders in which tau misfolding and aggregation are considered central to disease pathogenesis. These changes are often associated with post-translational modifications of tau, particularly hyperphosphorylation (Devi, 2023). Most tauopathies are sporadic, but genetic forms also occur. One example is frontotemporal lobar degeneration linked to chromosome 17, which is caused by mutations in the MAPT gene. In secondary tauopathies, tau pathology is thought to arise in association with other pathogenic processes rather than being the primary initiating event. In such cases, there may be no direct pathogenic mutation in the MAPT gene, and the exact contribution of tau to disease progression may vary depending on the disorder (Bensch et al., 2021). Alzheimer's disease is the most common example, where both amyloid-β and tau contribute to disease pathophysiology. Oligomeric amyloid-β, tau mislocalization, tau phosphorylation, and tau aggregation have been modelled in both mouse systems and human neuronal models (Gonzalez et al., 2018). Other disorders associated with secondary tau pathology include myotonic dystrophy, Niemann–Pick disease type C, Parkinson's disease, Lewy body dementia, Huntington's disease, and Down syndrome (Sexton et al., 2022).

Chronic traumatic encephalopathy (CTE) is a tauopathy associated with repetitive traumatic brain injury, including repeated concussions and subconcussive impacts. It has been reported in individuals with a history of contact sports and other repeated head trauma exposures. Pathologically, CTE is characterized by abnormal tau deposition in a distinct distribution pattern, particularly around small blood vessels and at the depths of cortical sulci (Gonzalez et al., 2018).

Alzheimer's disease is the most common form of dementia and is considered a secondary tauopathy because both amyloid-β deposition and tau aggregation are required for its neuropathological diagnosis. Most cases are sporadic and occur in older adults, usually from the sixth decade onward. Rare familial forms account for less than

5% of cases and may present much earlier in life (Devi, 2023). Memory impairment is the most common early clinical feature, although other cognitive domains such as language, executive function, visuospatial ability, and praxis may also be affected as the disease progresses.

3.6 Chemical models of Tauopathy

Understanding tau pathology is important for developing targeted therapeutic strategies for neurodegenerative disorders. While transgenic and genetic models are useful for studying progressive tau accumulation, chemical models provide a faster and more controllable approach to induce tau-related changes in both in vitro and in vivo systems.

Chemical models use pharmacological agents to alter tau phosphorylation, aggregation, and microtubule stability. Okadaic acid (OA) is one of the most commonly used chemical agents for inducing tau pathology. It inhibits protein phosphatases PP1 and PP2A, leading to increased tau hyperphosphorylation in neuronal cultures and rodent models (Medina et al., 2020; Gong and Iqbal, 2008). OA-induced tau pathology reproduces important biochemical features of Alzheimer's disease, including neurofibrillary tangle-like changes, and is widely used to evaluate tau-targeted therapeutic interventions.

Other chemical agents are also used to model tauopathy. Colchicine disrupts microtubule dynamics and mimics cytoskeletal instability observed in tau-related disorders, often producing cognitive deficits in rodent models (Mahdi et al., 2019). Staurosporine, a broad-spectrum kinase inhibitor, can induce tau phosphorylation and neuronal apoptosis and is therefore useful for studying tau-associated cytotoxicity (Yadikar et al., 2020). Oxidative stressors such as hydrogen peroxide and metal ions, including copper and iron, have also been reported to promote tau hyperphosphorylation and aggregation, reflecting the contribution of oxidative stress and environmental factors to tau pathology (Hernández et al., 2021).

Chemical models have several advantages because these are rapid, reproducible, and suitable for mechanistic studies and drug screening. However, these usually produce acute tau-related changes and may not fully reproduce the slow and progressive nature of human tauopathies. In addition, non-specific toxicity and off-target effects may influence the results, making dose optimization and proper validation essential (Silva et al., 2020). Therefore, chemical models are often used along with transgenic, viral, or genetic tau models to obtain a more complete understanding of tauopathy mechanisms (Gong and Iqbal, 2008).

3.7 Tauopathy and reactive oxygen species

Reactive oxygen species (ROS), which include superoxide anions, hydrogen peroxide, and hydroxyl radicals, are generated as byproducts of mitochondrial metabolism and cellular enzymatic reactions. Under physiological conditions, ROS levels are tightly regulated by endogenous antioxidant systems; however, dysregulation leads to oxidative stress, damaging lipids, proteins, and nucleic acids (Swerdlow, 2020). In tauopathies, oxidative stress has been shown to exacerbate tau pathology through multiple mechanisms.

Reactive oxygen species (ROS) can directly promote tau hyperphosphorylation by activating stress-related kinases, such as glycogen synthase kinase-3β (GSK-3β) and c-Jun N-terminal kinase (JNK) (Olesen et al., 2023). Additionally, oxidative modifications of tau including nitration and carbonylation, enhance its propensity to aggregate and form neurofibrillary tangles (Butterfield & Halliwell, 2019). Mitochondrial dysfunction, a hallmark of neurodegeneration, further amplifies ROS production, creating a vicious cycle where ROS promote tau pathology, which in turn exacerbates mitochondrial damage and oxidative stress (Roy et al., 2023).

Animal and cellular models support these mechanistic insights. Exposure of neurons to ROS-inducing agents or mitochondrial inhibitors accelerates tau hyperphosphorylation

and aggregation, while antioxidants such as N-acetylcysteine or mitochondrial-targeted compounds can mitigate tau pathology (Uddin, 2025). These findings underscore the therapeutic potential of targeting oxidative stress to slow or prevent tau-driven neurodegeneration.

3.8 Okadaic acid

Okadaic acid (OA) is a marine-derived polyether toxin mainly produced by dinoflagellates belonging to the Prorocentrum and Dinophysis genera (Giannelli et al., 2025). It is widely known for causing diarrhetic shellfish poisoning and for its strong inhibitory effect on serine/threonine protein phosphatases, particularly PP1 and PP2A (Kamat et al., 2025). Structurally, OA is a lipophilic molecule with the chemical formula $C_{44}H_{68}O_{13}$, characterized by a rigid polyether backbone, multiple ether rings, hydroxyl groups, and a terminal carboxyl group, all of which contribute to its cellular stability and uptake (Arriazu et al., 2020; Yadikar et al., 2019) (Figure 6).

The selective inhibition of phosphatases by OA interferes with phosphorylation-dependent signaling pathways, making it an important experimental tool in biomedical research for investigating cell cycle regulation, apoptosis, and neurodegenerative mechanisms (NazariSerenjeh et al., 2024). In addition, OA promotes tau-associated pathology, synaptic dysfunction, and oxidative stress, thereby increasing neuronal vulnerability (Shen et al., 2016). Extended exposure to OA has also been reported to induce neuroinflammation through the activation of microglia and astrocytes (Baluchnejadmojarad et al., 2024).

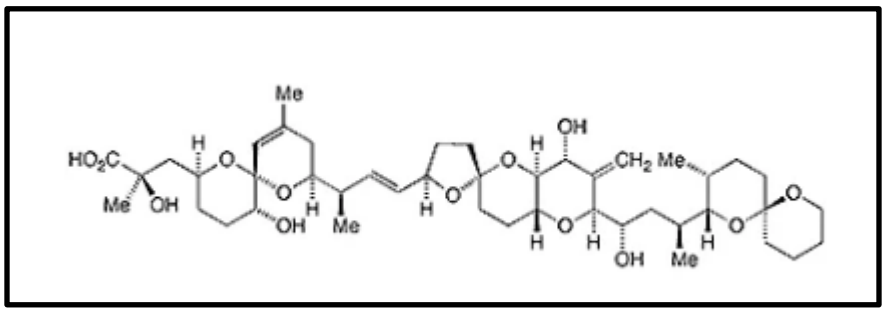


Figure 6: Chemical structure of Okadaic acid (Adapted from Arriazu et al., 2020)

3.8.1 Okadaic Acid and Tau Hyperphosphorylation:

By suppressing Protein phosphatase 2A (PP2A) activity, OA disrupts the normal dephosphorylation of tau protein, resulting in tau hyperphosphorylation (Metin et al., 2018) (Figure 7). Hyperphosphorylated tau dissociates from microtubules, leading to microtubule destabilization and accumulation of abnormal tau within the central nervous system. This accumulation facilitates the formation of neurofibrillary tangles (NFTs), which are highly neurotoxic and considered a hallmark pathological feature of Alzheimer's disease (Song et al., 2025; Kamat et al., 2025; Foidl et al., 2018).

Reduced expression and activity of PP2A have been strongly associated with the progression of Alzheimer's disease, highlighting the importance of PP2A-mediated tau dephosphorylation in maintaining microtubule integrity and preventing NFT formation (Zhoa et al., 2014; Ma Xia et al., 2018; Qian et al., 2010). Furthermore, OA challenge has been shown to enhance the expression of glycogen synthase kinase-3β (GSK3β), an important kinase involved in tau phosphorylation, thereby contributing to tau pathology, amyloid-β accumulation, neurodegeneration, and cognitive impairment (Kamat et al., 2025;).

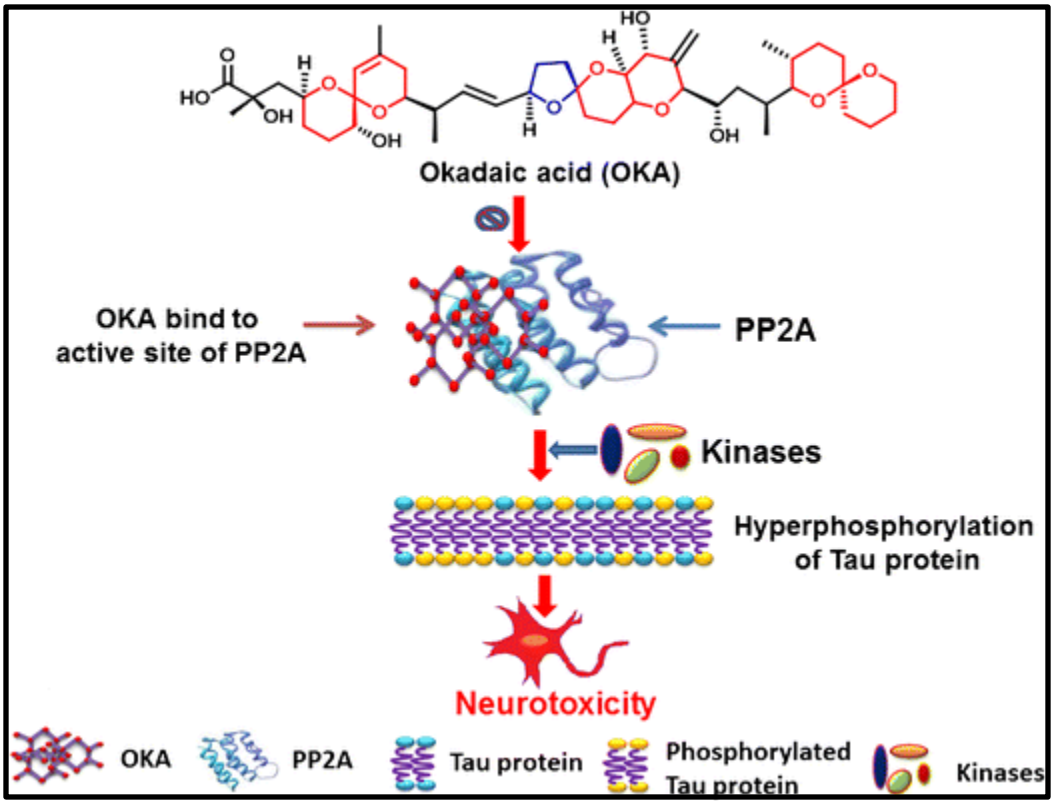


Figure 7: Representation of okadaic acid induced hyperphosphorylation of tau protein (Adapted from Kamat et al., 2014).

4. MATERIALS AND METHODS

4.1 Chemicals, Biochemicals, Kits and Antibodies

- Okadaic acid (Cat no: 5934) used in this study was obtained from Cell Signaling Technology, Danvers, Massachusetts, USA.
- 2',7'-dichlorodihydrofluorescein diacetate (DCFDA) Cat no: D399 was obtained from Thermo fisher scientific Invitrogen, Carlsbad, California, United States.
- Other chemicals, reagents, consumables used are listed in Table 1 and Table 2.

Table 1: List of chemicals, reagents and consumables used in this study

Name of reagent	Catalogue number	Company name
Dulbecco's modified eagle medium	12800017	Gibco, NY, USA
10% Fetal bovine serum	10270-106	Gibco, NY, USA
1% penicillin-streptomycin	A001	Himedia, Maharashtra, India
2 mM alanine-glutamine	G8541	Sigma-Aldrich, MA, USA
Total exosome isolation reagent	4478359	Thermo Fisher Scientific Invitrogen, United States.
Bradford reagent	B6916	Sigma-Aldrich, MA,USA
RIPA lysis buffer	20-188	EMD Millipore Corp., MA, USA
Qubit™ Protein and Protein Broad Range (BR) Assay kit	Q33211	Invitrogen, Thermo Fisher Scientific, California, US
Non- Reducing Lane Marker Sample Buffer	39001	Thermo Scientific, California, US
Tween-20	P9416	Sigma-Aldrich, MA, USA

SuperSignal™ West Femto Maximum Sensitivity Substrate	34094	Thermo Fisher Scientific, California, US
Restore™ Western Blot Stripping Buffer.	21059	Thermo Fisher Scientific, California, US
60 mm dishes	TCP007	Himedia, Maharashtra, India
Protease phosphatase inhibitor	PPC1010	Sigma Aldrich, MA, USA
Qubit 4 Flurometer	A5068	Invitrogen, Thermo Fischer Scientific, California, US
30% Acrylamide Bis-acrylamide	ML037-100ML	Himedia, Maharashtra, India
10%Ammonium Persulphate	A378-25G	Sigma Aldrich, MA, USA
Eppendorf Centrifuge	5425R	Eppendorf, Hamburg, Germany
Poly-L-Lysine coated glass coverslip	#72292-01	Electron Microscpy Sciences, Pennsylvania, US

Table 2: List of antibodies used in the study

Name of Antibody	**Catalogue number**	**Company name**	**Dilution**	**Temperature for incubation**
B- actin	#AC026	ABClonal (BioAb), New Taipel City, Taiwan	1:20,000	4°C
IL-1Beta	#12242	Cell Signaling Technologies, Danvers, Massachusetts, USA	1:1000	4°C
Goat anti-	#96714	Cell Signaling Technologies,	1:2000	Room

mouse IgG1 HRP Conjugate		Danvers, Massachusetts, USA		temperature

4.2 Cell Line

The Neuro2A (N2A) neuroblastoma cell line, originally derived from *Mus musculus* (mouse), was purchased from NCCS, Pune. The cells were maintained in T25 culture flasks containing Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, and 2 mM alanine-glutamine. Cultures were maintained at 37°C in an incubator (Eppendorf CellXpert C170) with 95% humidity and 5% CO_2 in a CO_2 incubator.

Human glioblastoma LN229 cell line was purchased from NCCS, Pune and grown in T25 culture flasks containing DMEM supplemented with 10% FBS, 1% penicillin-streptomycin and 2 mM alanine-glutamine. Cultures were maintained at 37°C in an incubator (Eppendorf CellXpert C170) with 95% humidity and 5% CO_2 in a CO_2 incubator.

4.3 Experimental design

Neuro2A cells were cultured in 60 mm dishes. Upon reaching approximately 80% confluency, the cells were transferred to a serum free medium and and incubated for 48 hours at 37°C in a 5% CO_2 incubator. It is followed by treatment with okadaic acid (100 nM) and incubated for 4 hours. The cells were then divided into two experimental groups as follows:

Table 3: Experimental groups of Neuro2A cells treatment with Okadaic acid

Groups	Group name	Treatment
Group 1	Basal	Untreated
Group 2	Okadaic acid	Cells were exposed to Okadaic acid (100 nM) for 4 hours

4.3.1 Exosome isolation

After 4 hours of incubation, 1 ml media is collected and centrifuged at 2000*g* for 20 minutes at 4°C. After centrifugation the supernatant is collected and 500 ml of total exosome isolation reagent is added. This was mixed well and incubated overnight at 4°C. It is then centrifuged at 10,000*g* for 1 hour at 4°C using an Eppendorf 5425 R Centrifuge. Supernatant is discarded and the pellet is resuspended in 50 µl of 1X PBS and stored in -20°C.Exossomes identification was done by dot blot assay using antibodies against Alix and CD9 (Data not shown).

4.3.2 Exosome quantification using Bradford assay

Protein estimation was performed in 96-well plates using BSA standards ranging from 0–4 mg/ml. Briefly, 300 µl of Bradford's reagent was added to each well and incubated at room temperature in the dark for 10 minutes. Absorbance readings were recorded at 595 nm using an Epoch microplate reader (BioTek). Protein concentrations were calculated from the standard calibration curve using Microsoft Excel 2010.

4.3.3 Exosome exposure to LN229 Cells.

LN229 cells were cultured in 60 mm dishes. Upon reaching approximately 80% confluency, the cells were transferred to a serum free medium. Followed by the cells were exposed with 10 µg/ml of Neuro2A derived exosomes for 4h at 37°C in a humidified incubator containing 5% CO_2. Following treatment, cells were collected for DCFDA staining and western blot analysis was done. A PBS control was taken by adding syringe filtered PBS (pH 7.4).

4.3.4 DCFDA staining for detection of intracellular reactive oxygen species.

Cells were grown in Poly-L-Lysine treated coverslips. After 4 hours of incubation, the culture medium was replaced with 2 ml of serum-free medium for 30 minutes. The cells were then exposed with exosomes isolated from Neuro2A cells under different experimental conditions for 4 hours. Experimetal groups for DCFDA staininig are given in Table 4.

Table 4: Experimental groups for DCFDA staining

Groups	Group name	Treatment
Group 1	PBS	Cells were exposed to syringe filtered PBS (10 µl/ml)
Group 2	H_2O_2	Cells were exposed to H_2O_2 (100 µM)
Group 3	Exo Basal	Cells were exposed to exosomes derived from basal cells (10 µg/ml)
Group 4	Exo OA	Cells were exposed to exosomes derived from Okadaic acid challenged cells (10 µg/ml)

Following treatment, the cells were washed once with 1 ml phenol red-free culture medium. Subsequently, 2 ml of phenol red-free medium containing DCFDA (10µM) was added, and the cells were incubated for 30 minutes at 37°C in the dark. After incubation, the staining solution was removed, and the cells were washed with phenol red-free medium to eliminate excess dye. Fresh phenol red-free medium was then added, and the stained cells were immediately observed under a fluorescence microscope. Intracellular ROS production was visualized as green fluorescence resulting from the oxidation of DCFDA to fluorescent dichlorofluorescein (DCF).

4.3.4.1 Image analysis

Fluorescence images were captured using Evos FLoid cell imaging station fluorescent microscope. The fluorescence intensity was quantified using Image J (NIH) software. Images were converted to grayscale, and the mean fluorescence intensity (MFI) was measured from randomly selected fields after background subtraction. The average fluorescence intensity obtained from each treatment group was normalized to the control group and expressed as relative fluorescence intensity.

4.3.5 Protein isolation

Following 4 hours of treatment of LN229 cells with exosomes derived from Okadaic acid challenged Neuro2A cells, the culture medium was removed and the cells were placed on ice. The cells were rinsed with 1 ml of ice-cold PBS (pH 7.4). After removing

the PBS, 200 µl of RIPA lysis buffer containing protease inhibitors was added to each culture dishes. The cells were maintained on ice for 5 minutes and then harvested using a cell scraper. The resulting lysate was transferred into fresh 1.5 ml centrifuge tubes and centrifuged at 4°C at 21,130*g* for 15 minutes using an Eppendorf 5425 R centrifuge. The supernatant was carefully collected into new tubes, and protein concentration was determined by the Qubit assay

4.3.6 Protein Estimation by Qubit BR (Broad Range) Assay

Protein concentration was quantified using Qubit™ Protein and Protein Broad Range (BR) Assay kit as per the manufactures' instruction using Qubit 4 Fluorometer (A5068, Invitrogen, Thermo Fisher Scientific).

- Standards were prepared by adding 10 µl of each Qubit standard (Standard 1 and Standard 2) and 160 µl of Qubit Protein BR Assay Buffer to separate designated tubes. The solutions were mixed thoroughly by pipetting up and down.
- For sample preparation, 10 µl of each protein lysate was transferred into the assigned sample tubes. To each tube, 160 µl of Qubit protein BR assay buffer was added, bringing the final volume to 170 µl. The samples were thoroughly mixed.
- Sample buffer blank was prepared by first adding 10 µl of RIPA buffer to a tube, then adding 160 µl of Qubit protein BR assay buffer to bring the total volume to 170 µl. The mixture was then mixed by pipetting.
- Lastly, 30 µl of Qubit protein BR assay reagent was added to each sample tube, standard tube, and the sample buffer blank.
- All solutions were promptly mixed by pipetting and vortexing for 5–7 seconds, resulting in a final volume of 200 µl in each tube.
- After incubating at room temperature for 10 minutes, protein concentration was measured using the Qubit 4 Fluorometer.

4.3.7 SDS-PAGE

4.3.7.1 Reagents

- 30% Acrylamide/Bis-acrylamide solution
- 10% SDS: Prepared by dissolving 5 g SDS in 45 ml distilled water and adjusting the final volume to 50 ml.
- 0.5 M Tris-HCl Buffer (pH 6.8): Prepared by dissolving 19.7 g Tris-HCl in 150 ml distilled water, adjusting the pH to 6.8 with 15 N NaOH, and making up the volume to 250 ml.
- 1.5 M Tris-HCl Buffer (pH 8.8): Prepared by dissolving 118.2 g Tris-HCl in 250 ml distilled water, adjusting the pH to 8.8 using 15 N NaOH, and bringing the final volume to 500 ml.
- 10% Ammonium Persulfate (APS): Freshly prepared by dissolving 0.1 g APS in 1 ml distilled water.
- Running Buffer (pH 8.3): Prepared by dissolving 30.30 g Tris base, 144.10 g glycine, and 10 g SDS in 1000 ml distilled water.
- TEMED (N,N,N',N'-Tetramethylethylenediamine)

4.3.7.2 Preparation of 8% polyacrylamide gel

The gel was prepared by combining 4.69 ml of distilled water, 2.5 ml of Tris-HCl buffer (pH 8.8), 100 µl of 10% SDS and 2.64 ml of 30% bisacrylamide. Subsequently, 50 µl of 10% APS and 20 µl of TEMED were added, and the mixture was thoroughly mixed. The prepared solution was poured between the glass plates of the vertical gel apparatus and allowed to polymerize.

4.3.7.3 Preparation of 4% polyacrylamide gel

The gel was formulated using 3 ml of distilled water, 1.26 ml of Tris-HCl buffer (pH 6.8), 50 µl of 10% SDS and 0.66 ml Bisacrylamide. To this mixture, 25 µl of 10% APS and 20 µl of TEMED were added and mixed well. The solution was then poured on top of polymerized 8% gel between the glass plates of the vertical gel assembly and left undisturbed until polymerization was complete.

4.3.7.4 Sample Preparation

Protein samples were mixed with a non-reducing sample loading buffer and incubated at 90°C for 10 minutes before being loaded into the wells of the gel.

4.3.7.5 Electrophoresis

Electrophoretic separation was performed using a BIORAD PowerPac™ HC. Initially, the gel was run at 80 V for 20 minutes, after which the voltage was increased to 120 V and maintained until the tracking dye reached the bottom of the gel.

4.3.8 Immunoblotting

4.3.8.1 Reagents

a) 10X Tris-Buffered Saline (TBS)

A 10X TBS stock solution was prepared by dissolving 24 g of Tris-HCl and 88 g of NaCl in 800 mL of deionized water. The pH was adjusted to 7.6 using 15 N NaOH, and the final volume was made up to 1000 ml. Prior to use, the stock solution was diluted to obtain a 1X working solution.

b) 25X Tris-Glycine Transfer Buffer (pH 8.3)

The transfer buffer stock was prepared by dissolving 9.1 g of Tris base and 45 g of glycine in distilled water and adjusting the volume to 500 ml. Before use, the stock solution was diluted to a final concentration of 1X.

c) 1X Tris-Buffered Saline with Tween-20 (TBST)

TBST was prepared by adding 1 ml of Tween-20 and 100 ml of 10X TBS to distilled water, with the final volume adjusted to 1000 ml. This solution was used as the washing buffer during immunoblotting procedures.

4.3.8.2 Procedure

Following electrophoresis, proteins were transferred onto a nitrocellulose membrane

having pore size 0.45 µm using a Bio-Rad transfer system operated at 100 V for 2 hours at 4°C. The membrane was then blocked with 5% BSA prepared in TBS for 1 hour and 30 minutes at room temperature. The membrane was then incubated overnight at 4°C with the respective primary antibodies. Following incubation, the membrane was washed thrice with TBST (Tris-buffered saline containing 0.1% Tween-20), with each wash lasting 10 minutes. The secondary antibody was then incubated with the membrane for 2 hours. Subsequently, the membrane was washed thrice in TBST for 10 minutes each. Protein bands were visualized using a Bio-Rad XRS+ ChemiDoc imaging system and SuperSignal™ West Femto Maximum Sensitivity Substrate. Blots were analyzed using Image J (NIH) software.

4.3.9 Stripping

The membrane was stripped using Restore™ Western Blot Stripping Buffer. Prior to use, the stripping buffer was warmed to 40°C. The membrane was incubated with the warmed buffer for 20 minutes at room temperature, followed by an additional 10 minutes with gentle agitation. After removing the stripping solution, the membrane was washed thrice with TBST, each wash lasting 10 minutes. The membrane was subsequently blocked for 1 hour at room temperature using 5% BSA prepared in TBS.

4.3.10 Image Analysis

Densitometric analysis of Western blot bands was performed using Image J (NIH) software. The acquired images were converted to grayscale, and individual protein bands were selected for analysis. Background intensity was subtracted, and the resulting values were normalized against the loading control, β-actin. The normalized data were used to assess changes in protein expression following exposure to okadaic acid challenged Neuro2A cells derived exosomes.

4.3.11 Statistical Analysis

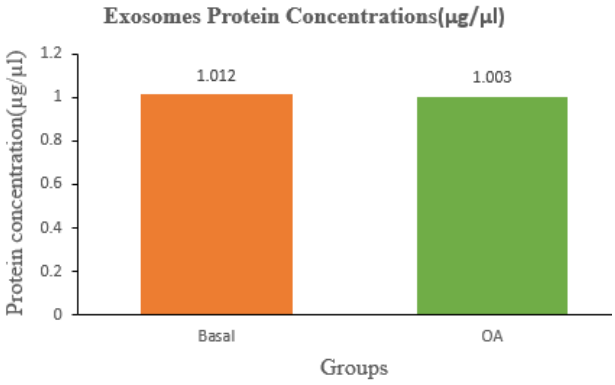
Statistical evaluation of the data was carried out using GraphPad Prism 5. Comparison between experimental groups were analyzed using paired t-test and the statistical significance was represented in the graph as $p<0.05$.

5.RESULT

5.1 Exosomes quantification using Bradford Assay

Table 5: Exosomes protein concentrations

Groups	Protein concentration(µg/µl)
Basal	1.012
OA	1.003



Protein concentration of exosomes were determined prior to downstream analysis using Bradford Assay

5.2 Effect of okadaic acid exposed Neuro2A derived Exosomes on intracellular Reactive Oxygen Species (ROS) Generation in LN229 Assessed by DCFDA Staining

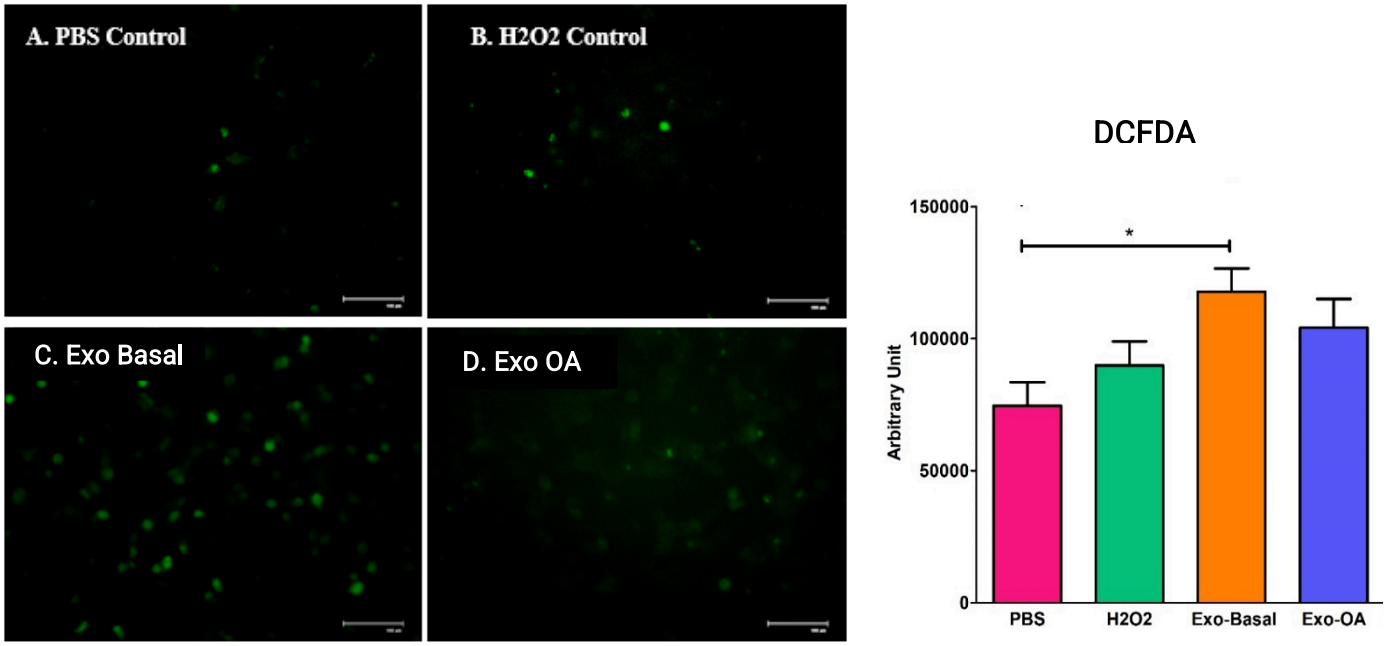


Figure 8. Representative DCFDA fluorescence images showing intracellular ROS production in LN229 cells following exposure with Okadaic acid induced Neuro2A cell derived exosomes. Data are presented as mean ± SEM indicates statistical significance with PBS at $p < 0.05$.

Intracellular reactive oxygen species (ROS) generation in LN229 was evaluated using the DCFDA fluorescence assay following treatment with exosomes derived from Neuro2A cells.PBS control cells showed low basal DCFDA fluorescence (Figure 8A) representing basal intracellular ROS levels, whereas H_2O_2 exposed cells exhibited enhanced fluorescence intensity (Figure 8B) confirming increased ROS generation under oxidative stress conditions.Exposure to exosomes isolated from untreated Neuro2A cells (Exo-Basal) significantly increased intracellular ROS levels (Figure 8C) compared with the PBS control ($p < 0.05$). Exosomes derived from okadaic acid challenged Neuro2A cells (Exo-OA) induced an increase in DCFDA fluorescence (Figure 8D) but the intensity of staining was lower than that induced by Exo-Basal. These observations suggest that Neuro2A cell-derived exosomes, particularly those obtained under basal and okadaic acid-stressed conditions, can promote oxidative stress responses in recipient LN229 cells.

5.3 Changes in IL-1Beta expression of Okadaic acid induced N2A cells –derived exosomes in LN229 cells.

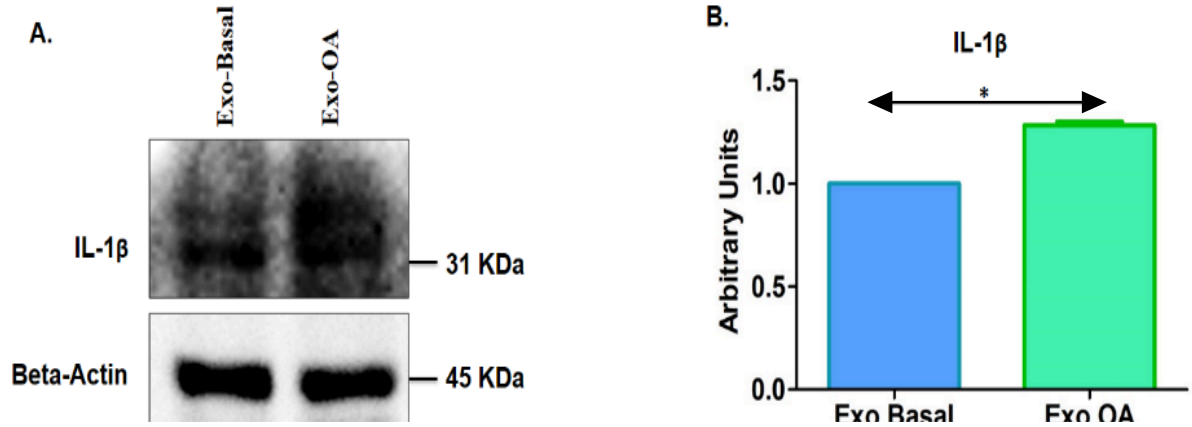


Figure 9. (A) Representative western blots images showing changes in protein expression in LN229 cells. β-Actin was used as the loading control. (B) The protein densities were analyzed using Image J (NIH)1.53t software.
* Statistical significance between Exo Basal and Exo OA (p value = 0.0017) for IL-1β was done using GraphPad Prism 5.

Western blot analysis showed basal expression of IL-1β in LN229 cells treated with exosomes from challenged Neuro2A cells (Exo Basal). Exposure of LN229 cells with

exosomes derived from okadaic acid challenged Neuro2A cells (Exo OA) resulted in an increase in IL-1β protein expression compared with the Exo Basal group, as indicated by the increased band intensity and densitometric analysis (Figure 9).

5.4 Comparative effect of Exo-Basal and Exo-OA on Oxidative stress and Inflammatory Responses in LN229 cells

Comparison of the effect of basal neuronal exosomes (Exo-Basal) and okadaic acid stressed neuronal exosomes (Exo-OA) demonstrated distinct modulation of Oxidative and Inflammatory responses in LN229 cells. Exo-Basal treatment induced a greater increase in intracellular Reactive Oxygen Species generation (ROS), as evidenced by higher DCFDA fluorescence, whereas Exo-OA exposure produced comparatively lower ROS levels but markedly enhanced IL-1β protein expression. These findings indicate that although both exosome populations are biologically active, exosomes derived from okadaic acid challenged Neuro2A cells preferentially promote inflammatory signaling, while basal neuronal exosomes predominantly induce oxidative stress in recipient LN229 cells.

6. DISCUSSION

Treatment of LN229 cells with Neuro2A-derived exosomes showed that neuronal exosomal cargo can modulate oxidative and inflammatory responses in recipient glial cells. Both exosomes derived from untreated Neuro2A cells (ExoBasal) and from okadaic acid-challenged Neuro2A cells (ExoOA) increased intracellular ROS generation, as evidenced by enhanced DCFDA fluorescence compared with PBS-treated controls. This indicates that neuronal exosomes, even under basal conditions, are biologically active and capable of altering the redox status of recipient LN229 cells. The higher DCFDA fluorescence observed in Exo-Basal-treated cells suggests that the process of exosome uptake or the transfer of basal neuronal exosomal cargo, have the capacity to impose metabolic or oxidative stress on recipient glial cells.

Interestingly, the pattern of IL-1β expression did not strictly parallel the DCFDA fluorescence profile. Although Exo-Basal induced prominent ROS generation, IL-1β protein expression was comparatively lower than that observed in cells treated with exosomes derived from okadaic acid-treated Neuro2A cells. Exo-OA treatment resulted in a marked increase in IL-1β expression compared with Exo-Basal, suggesting that okadaic acid-induced neuronal stress modifies the exosomal cargo in a manner that preferentially promotes inflammatory signaling in recipient LN229 cells. Thus, while Exo -Basal may primarily increase intracellular oxidative burden, Exo-OA appears to transmit a stronger pro-inflammatory signal.

This dissociation between ROS intensity and IL-1β expression is biologically relevant, as DCFDA fluorescence reflects general intracellular oxidant accumulation and may not directly represent activation of inflammatory pathways. Exosomes released from okadaic acid-stressed neuronal cells contain stress-associated proteins and damage-associated molecular signals that can activate inflammatory cascades in recipient glial cells (Ph.D thesis of Aadithye R Nair). This work is a continuation of that research and indicates that the increase in IL-1β following Exo-OA treatment may be activation of glial inflammatory responses secondary to neuronal stress. Since okadaic acid is known to induce tau-associated and phosphatase-inhibition-mediated cellular stress (Zang, 2010),

the exosomes released under this condition may carry pathological signals capable of propagating neuroinflammatory changes.

Together, these findings suggest that exosomes act as intercellular mediators between stressed neuronal cells and glial-like recipient cells. Basal neuronal exosomes are sufficient to induce oxidative changes in LN229 cells, whereas exosomes derived from okadaic acid-challenged neuronal cells appear to more effectively enhance IL-1β-associated inflammatory responses. This supports the possibility that neuronal stress can reprogram exosomal cargo, allowing the transfer of pathological signals to neighbouring glial cells. The findings also indicate that exosome-mediated communication may contribute to the coupling of oxidative stress and neuroinflammation under tauopathy-like conditions (Dyndal, 2026).

However, the present work at this stage has limitations. DCFDA is a broad indicator of intracellular ROS and does not specify the source or type of reactive species generated. Similarly, increased IL-1β protein expression alone does not confirm complete inflammasome activation or cytokine secretion. Future studies assessing NF-κB activation, NLRP3 inflammasome components, caspase-1 activation, mature IL-1β release, and exosomal cargo composition would be carried out to clarify the precise mechanism by which okadaic acid-modified neuronal exosomes induce inflammatory responses in recipient glial cells.

7. SUMMARY AND CONCLUSION

The present study investigated whether exosomes derived from untreated and okadaic acid-treated Neuro2A cells can induce oxidative stress and inflammatory responses in LN229 glial cells. Exosomes isolated from untreated Neuro2A cells were designated as Exo-Basal, while those from okadaic acid-treated cells were designated as Exo-OA. LN229 cells were treated with these exosomes, and intracellular ROS generation was assessed using the DCFDA assay. IL-1β protein expression was evaluated by Western blotting.

Both Exo-Basal and Exo-OA increased intracellular ROS levels compared with PBS-treated control cells, indicating that neuronal exosomes can influence the redox status of recipient glial cells. Exo-Basal induced a stronger ROS response, whereas Exo-OA produced a comparatively lower increase in ROS. In contrast, IL-1β expression was higher in Exo-OA-treated LN229 cells than in Exo-Basal-treated cells, suggesting that exosomes released from okadaic acid-stressed neuronal cells promote inflammatory signaling.

Overall, the findings suggest that neuronal exosomes are biologically active mediators of neuron-glia communication and may differentially regulate oxidative stress and inflammation depending on the stress status of the donor neuronal cells.

Exosomes derived from Neuro2A cells modulate oxidative and inflammatory responses in LN229 glial cells. While Exo-Basal predominantly increased intracellular ROS generation, Exo-OA enhanced IL-1β expression, indicating stronger inflammatory activation. These results suggest that okadaic acid-induced neuronal stress may alter exosomal signaling and promote glial inflammatory responses. The study provides preliminary evidence that neuronal exosomes may contribute to the propagation of oxidative stress and neuroinflammation under tauopathy-like conditions.

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