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Role of Encapsulated Nano Curcumin in Induced Sh-Sy-5y Cell Line

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Abstract:-

- ***Background:***

The distinguishing features of neurodegenerative disorders include the death of neurons in the brain or spinal cord that, over time, results in the loss of a particular neuronal subtype or indiscriminate loss of neuronal populations, consequently Alzheimer's and Huntington's diseases are said to cause the loss of neurons, while Parkinson's disease specifically and insufficiently damages dopaminergic neurons in the substantia nigra..

- ***Aims:***

In this study, inducers are used inside in vitro examinations of neuroblastoma cells as well as a fresh, contemporary nanobiotechnological approach to disease therapy.

- ***Method:***

The materials' cytotoxicity was evaluated using the MTT colorimetric test technique with minor modifications. Each well was seeded with $2*10^5$ cells per well and incubated for 24 hrs at 37 with 5% CO2. The concentration ranges for the test samples were 160, 120, 80, 40, 200, µg/ml were added to a confluent monolayer plate containing the cell lines that had been cultured for 24 hours. The supernatant was collected after 72 hours of incubation at 37°C in a 5% CO2 incubator, and 25 µl of MTT reagent (2 mg/ml) was added to each well.

- ***Result:***

The cell lines SH-S5Y5. After 24 hours of treatment, cells were suspended and adherent, undifferentiated neuroblastoma cells, while cells after being induced with IC50 concentration of glyceraldehyde, Blebbing cells are a sign of early apoptosis. Cell after dopamine IC50 concentration induction. very few circularized cells. Cells were beginning to differentiate, Cell after treatment with IC50 concentrations of dopamine and Encapsulation Nano Curcumin. Absence of any circularized cell indicating all cells are adhered.

- ***Conclusion:***

This work highlighted the possibility that Encapsulated Nano Curcumin might be a potent therapeutic agent, especially for the treatment of neurodegenerative diseases like Parkinson's Disease. However, more pre-clinical and clinical research is still required.

Keywords:- *Encapsulated Nano Curcumi, Neurodegenerative diseases and Sh-Sy-5y Cell Line.*

I. INTRODUCTION

Neurodegenerative disorders are distinguished by the death of neurons in the brain or spinal cord, that over time causes the loss of a particular neuronal subtype or indiscriminate loss of neuronal populations. According to reports, neurons are lost in Alzheimer's and Huntington's diseases, while dopaminergic neurons in the substantia nigra are specifically and inadequately lost in Parkinson's disease, (Brain 202). Alzheimer's disease (AD) is characterized by behavioral changes, cognitive deficiencies in areas including language, spatiotemporal orientation, and executive function, all of which contribute to the eventual loss of personal autonomy. The actual cause of AD is still unknown, despite the fact that memory and cognition are significantly impacted. One of the most common hypotheses used to explain AD pathophysiology is the idea of amyloid plaque development and aggregation. Histopathology describes the two pathological characteristics of AD as intracellular deposition of abnormally phosphorylated Tau protein, which promotes the formation of neurofibrillary tangles (NFTs) in the cerebral cortex and subcortical gray matter, and extracellular aggregates of Amyloid-beta peptide (Aβ) fibrils in the form of neuritic plaques, (Guerrero etal., 20203). Parkinson disease (PD), the most prevalent neurodegenerative and movement disorder, is characterized by bradykinesia, akinesia, festinating gait, rigidity, postural problems, and hunched posture. These motor difficulties, which are a hallmark of Parkinson's disease (PD), have been primarily linked to the progressive death of neuromelanin-containing dopaminergic neurons in the substantia nigra (SN), which results in the loss of dopamine (DA) in the striatum. Environmental and genetic variables are both involved in the etiology of Parkinson's disease, which is still a mystery. A well-known cause of parkinsonism is the usage of agricultural pesticides, Kouli etal., 2018):(Armstrong 2020). In the field of neuroscience, the neuroblastoma cell line SH-SY5Y has been a popular and frequently used in vitro model, with a focus on nervous system disorders that impair neurodevelopment, such as Parkinson's disease. One of the few appropriate models without relying on a primary

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culture of neuronal cells is this cell type since it can differentiate in comparison to other neurobiology models .(Feles etal., 2022). ROS levels, MMP loss, Cyt-c release, pro-apoptotic marker expression, and anti-apoptotic marker expression all significantly increase with rotenone treatment, Rotenone easily crosses the blood-brain barrier, builds up inside the brain, impairs mitochondrial function, and ultimately results in neurodegeneration.. According to studies, Alzheimer's disease causes degenerating neurons to have elevated local Al concentrations. As a known neurotoxin, aluminum causes cognitive dysfunction and may be a factor in Alzheimer's disease. The main justification is that aluminum may accumulate inside the body and get deposited in the brain . Okadiac acid is used in studies on the potential of SH-SY5Y cells treated with protein phosphatase inhibitor to investigate the processes of tau aggregation and neurodegeneration. Dopamine has been shown to result in a dose- and time-dependent (10–40 M) loss of cell viability that is accompanied by mitochondrial dysfunction and an elevated level of α-synuclein intracellular accumulation. (Ganguly etal., 2019):):(Del etal., 2019):(Abdul-Latif etal 2021);(Angelo etal., 2021): (ELBini etal 2021)(Cetin etal., 2022):(Guerrero etal., 2023):(Chainoglou etal.,2020)

II. ALZHEIMER'S DISEASE'S

Alzheimer's disease (AD) is a neurological disorder that accounts for 60 to 80% of dementia cases worldwide. According to estimates, 50 million people worldwide currently experience some form of dementia; however, due to rising life expectancy rates, it is anticipated that by 2050, 139 million people will experience dementia, having a significant negative socioeconomic and health system impact, (Guerrero etal., 20203). The leading cause of dementia is (AD), which is a neurodegenerative disorder. Despite the significant financial burden on the world economy and the effect on the patient's immediate family, there is no cure for the condition, there is a need for enhanced therapeutic approaches. It is a proteinopathy (amyloid and tau) is the most prevalent type of dementia in the world. which include loss of synapses and neurons, neuroinflammation, and reactive astrogliosis together with vascular amyloid deposits (cerebral amyloid angiopathy). Other aging-related conditions include Lewy and TDP-43 pathologies, hippocampal sclerosis, argyrophilic grain disease, cerebrovascular lesions, and others that have been linked to AD, (Ellinger, 2020). AD is characterized by behavioral changes, cognitive deficiencies in areas including language, spatiotemporal orientation, and executive function, all of which contribute to the eventual loss of personal autonomy. The actual cause of AD is still unknown, despite the fact that memory and cognition are significantly impacted. One of the most common hypotheses used to explain AD pathophysiology is the idea of amyloid plaque development and aggregation. Histopathology identifies two pathological features of AD: extracellular amyloid-beta peptide (Aβ) fibril aggregates in the form of neuritic plaques and intracellular deposition of abnormally phosphorylated Tau protein, which promotes the development of neurofibrillary tangles (NFTs) in the cerebral cortex and subcortical gray matter. (Guerrero etal., 20203). It is found that through exposing SH-SY5Y neuroblastoma cells to glyceraldehyde (GA), in research on investigating the association between nervous system disease and the etiology of AD. It has been claimed that toxic advanced glycation end products (toxic AGEs) generated from GA cause AD-like changes, including intracellular tau phosphorylation.

III. PARKINSON DISEASE (PD)

Parkinson disease (PD), the most prevalent neurodegenerative and movement disorder, is characterized by bradykinesia, akinesia, festinating gait, rigidity, postural problems, and hunched posture. These motor difficulties, which are a hallmark of Parkinson's disease (PD), have been primarily linked to the progressive death of neuromelanin-containing dopaminergic neurons in the substantia nigra (SN), which results in the loss of dopamine (DA) in the striatum. Environmental and genetic variables are both involved in the etiology of Parkinson's disease, which is still a mystery. A well-known cause of parkinsonism is the usage of agricultural pesticides. Genetic and environmental factors both contribute to the complex nature of PD. The main risk factor for PD is age, with a median onset age of 60 years old. The disease's prevalence increases with age, reaching 93.1 cases per 100,000 person-years in the age range of 70 to 79. Cross-cultural differences exist as well, with higher prevalence observed in Europe, North America, and South America in comparison to African, Asian, and Arabic countries, (Kouli etal., 2018). One of these is the lipophilic neurotoxic rotenone, which rapidly crosses cellular membranes and the blood-brain barrier. Studies conducted in vitro and in vivo have shown that exposure to rotenone mimics a number of the clinical characteristics of Parkinson's disease (PD), including dopaminergic neuronal loss, mitochondrial dysfunction, oxidative stress, inflammation, and behavioral abnormalities. Rotenone causes the death of neurons in SN by suppressing mitochondrial complex, I activity, generating reactive oxygen species (ROS), inhibiting adenosine triphosphate (ATP) synthesis, and depolarizing the mitochondrial membrane. Changes in the permeability of the mitochondrial membrane brought on by ROS produced by dysfunctional mitochondria activate apoptotic caspases. Plants and their based bioactive substances are crucial in the development of medication for an array of illnesses and disorders, and in our study, we used Amorphous calcium phosphate nanoparticles that are drug-loaded with curcumin, (Balraj etal 2020): (Chia etal., 2022).

IV. SH-SY-5Y CELL LINE

With a focus on nervous system disorders that impair neurodevelopment, such as Parkinson's disease, the neuroblastoma cell line SH-SY5Y has been a well-known and often used in vitro model. Therefore one of the few appropriate models without relying on a primary culture of neuronal cells is this cell type since it can differentiate in comparison to other neurobiology models .(Feles etal., 2022):(Ganguly etal.,2019):(Boban etal., 2019);(Octavian

etal., 2019) This experimental model can be used to more thoroughly understand the pathophysiological causes of Parkinson's disease and develop brand-new pharmaceutical treatments, the two primary pathologic changes of Parkinson's disease (PD) must be replicated in in vitro models: the loss of dopaminergic neurons and the intraneuronal deposition of alpha-synuclein. Given its accessibility, affordability, and general use, this in vitro model might serve as the initial stage in the evaluation of disease-related pathomechanisms or treatments. Additionally, the cell culture might either be transfected with the pathogenic protein or subjected to one of the many neurotoxins that cause cellular PD-specific changes. However, given that the cell line originates from a neuroblastoma, it has certain drawbacks, including the potential to develop an epithelial phenotype and the existence of genetic instability,(Ioghen etal., 2023).

V. ROTENONE

With rotenone treatment, ROS levels, MMP loss, Cyt-c release, pro-apoptotic marker expression, and anti-apoptotic marker expression all significantly rise. Rotenone also affects mitochondrial activity and eventually leads to neurodegeneration because it easily crosses the blood-brain barrier. Additionally, Rotenone inhibits HO-1 expression, autophagic flow, and the buildup of autophagic vacuole formations, all of which cause dopaminergic neurons to malfunction, (Lin etal., 2014). Thus, oxidative stress and mitochondrial dysfunction, which are expected to play a significant role in the etiology of PD, have been detected in the brains of individuals with PD together with other biochemical abnormalities. According to recent research, mitochondrial failure, increased oxidative stress, excitotoxicity, inflammation, and malfunctioning of the ubiquitin-proteasome system may be the root causes of alpha-synuclein aggregation, the emergence of Lewy bodies, and dementia. A number of genes linked to Parkinson's disease (PD) are associated with defective mitochondria-specific autophagy, which leads to mitochondrial malfunction and may directly cause neuronal dysfunction and neurodegeneration. From our research Encapsulated nano curcumin dramatically decreased the amount of rotenone-induced cell death in SH-SY5Y cells after pretreatment in a dose-dependent manner, demonstrating the substance's effective neuroprotective properties,(Lin etal 2014):(Ramkumar etal 2017).

VI. ALUMINIUM

The third most prevalent element in the crust of the earth is aluminum (Al), and its compounds are used to make household items, medications, antiperspirants, and other products. A rising number of research points to Al+3 ions' potential role in a range of neurodegenerative diseases, including Alzheimer's. According to studies, Alzheimer's disease causes degenerating neurons to have elevated local Al concentrations. As a known neurotoxin, aluminum causes cognitive dysfunction and may be a factor in Alzheimer's disease. The main justification is that aluminum may accumulate inside the body and get deposited in the brain. There are three ways that aluminum can get into the brain, which include the bloodstream or the absorption site.

Aluminum enters the brain via the blood-brain barrier (BBB), choroid plexuses, and the nasal cavity. Citric acid, parathyroid hormone (PTH), and vitamin D all contribute to the blood-brain barrier becoming more permeable, which makes it easier for aluminum to enter the brain. However, aluminum can stay in the brain for a very long time since it is slowly dispersed from there. Senile plaques and neurofibrillary tangles have been discovered to have elevated aluminum concentrations in the brains of individuals with Alzheimer's disease (Wang, 2018).

VII. OKADIAC ACID

Research on potential of SH-SY5Y cells treated with protein phosphatase inhibitor uses okadiac acid as a model to examine the processes of tau aggregation and neurodegeneration. The effects of okadiac acid (OA) treatment on the generation of high molecular weight tau are discovered. the development of a high molecular weight phospho-protein species immunoreactive to tau antibodies against phosphorylated Ser202 and phosphorylated Ser396 is caused by the incubation of SH-SY5Y cells with OA. Results suggest that OA-treated SH-SY5Y cells are a potentially useful cell culture model for investigating tau oligomerization in the presence of downregulated protein phosphatase, as well as tau aggregation inhibitors and neuroprotective substances, (Boban etal 2019).

VIII. GLYCERALDEHYDES'

In studies examining the relationship between nervous system disorders and the etiology of AD, glyceraldehyde (GA) was exposed to SH-SY5Y neuroblastoma cells. It has been asserted that alterations resembling AD, such as intracellular tau phosphorylation, are brought on by toxic advanced glycation end products (toxic AGEs) produced from GA. The glycolysis inhibitor glyceraldehyde (GA) was administered to human neuroblastoma cells SH SY5Y. In addition to increasing tau phosphorylation and decreasing the medium concentrations of amyloid β 1-42 (Aβ42),, GA also caused cell death, glycolytic inhibition, and the generation of GA-derived advanced glycation end-products (GA-AGEs). Additionally, it is recently reported that GA intracellularly caused TAGE formation and neurotoxicity in SH-SY5Y human neuroblastoma cells within 24 hours, as well as induced AD-like changes like tau phosphorylation, which is a common occurrence in neurofibrillary tangles (NFTs) of AD, (Koriyama etal., 2015): (Nasu etal., 2020).

IX. DOPAMINE

According to studies, the cytotoxicity of dopamine on neural-derived cultured cells has been utilized as a technique to investigate the mechanisms underlying Parkinson's disease's dopaminergic neurodegeneration. In cultured SH-SY5Y cells, it had been demonstrated that dopamine causes a dose-dependent (10–40 M) and time-dependent (up to 96 h) loss of cell viability associated with mitochondrial

malfunction and increased intracellular accumulation of α-synuclein, (Ganguly etal., 2019).

X. SY-SY-5Y CELL LINE

The SY-SY-5Y neuroblastoma cell is a neural Cell lines were procured from NCCS, Pune. Cells were sub cultured in DMEM media. It is used as a simple and inexpensive in vitro experimental model for neurodegenerative research, which helps researchers understand the pathophysiological mechanisms behind neurological disorders and serves as an initial foundation for developing new drugs for treating dementia.

- *Subculture of Cell Lines*

Examined cultures under an inverted microscope to determine confluency and the absence of bacterial and fungal contamination. Remove the used media. PBS without $Ca2^{+}/Mg2^{+}$ washed the cell monolayer. If the cells are known to attach aggressively, repeat this wash step. Pipette trypsin/EDTA onto the cleaned cell monolayer, 1 ml per 25 cm2 of surface area. To cover the monolayer with trypsin, rotate the flask. Remove any excess trypsin. Return the flask to the incubator for 2-10 minutes. Using an inverted microscope, inspect the cells to check that they are all unattached and floating. To free any remaining adhered cells, lightly touch the edge of the flasks. To inactivate the trypsin, resuspend the cells in a tiny volume of new serum-containing media. Remove 100-200 µl and count the cells. Transfer the necessary number of cells to a fresh labelled flask with pre-warmed media (refer to the appropriate ECACC Cell Line Data Sheet for the required seeding density). Incubate the cell line as directed. Repeat this technique as needed to accommodate the cell line's growth characteristics.

Cells were sub-cultured in 20 ml DMEM media with 10% FBS and incubated in CO2 incubator with 5% CO2 and 95% humidity at temperature of 35^{0}. Cell were trypsinized after 70-80% confluency and proceeded for MTT assay.

XI. CELL PREPARATION

Transfer the media in centrifuge tube to prevent loss of suspension cells. Wash the adhered cell with 1XPBS 2X10 ml with 10-15 min incubation. Decant the PBS. Added 1 ml of Trypsin/EDTA or the required volume to cover the T-flask surface. Incubated for 10-15 min with occasionally tapping. Observed under microscope to observe complete detachment of adhered cells. Collect all in centrifuge tube. Centrifuge it at 10,000 rpm for 10 min. Discard the media and dissolve the pellet in 1 or 2 ml of prewarmed DMEM media. Count the cells under normal microscope using Trypan blue. Add 20 ml of DMEM media and count the cells.

XII. MTT ASSAY IC50 ESTIMATION FOR THE SAMPLES

The materials' cytotoxicity was evaluated using the MTT colorimetric test technique with minor modifications. Each well was seeded with $2*10^{5}$ cells per well and incubated for 24 hrs at 37 with 5% CO2. The concentration ranges for the test samples were 160, 120, 80, 40, 200, µg/ml were added to a confluent monolayer plate containing the cell lines that had been cultured for 24 hours. The supernatant was collected after 72 hours of incubation at 37°C in a 5% CO2 incubator, and 25 µl of MTT reagent (2 mg/ml) was added to each well. After a 4-hour incubation period at 37°C, 100 µl of dimethyl sulphoxide was added to each well to solubilize the formazan precipitate, and the wells were agitated for another 15 minutes. An ELISA reader was used to determine the absorbance at a wavelength of 490 nm. The medium without the tested chemical was given to the control wells. The percent inhibitory activity of the tested sample was computed and represented as the IC50 value of cellular growth inhibition (concentration of the tested sample to inhibit 50 percent growth of the cells).

$$\% \text{ inhibition} = (100*[A0-A1])$$

Where;

A0 is the absorbance of the control and
A1 is the absorbance of the extracts.
IC50 Concentrations Graphs and Inducing Process

Table 1 Exhibiting the IC50 Concentrations Graphs and Inducing Process

Samples	Concentration(µg/ml)	O.DI	%cytotoxicity	IC50 (µg/ml)
cell+curcumin	40	0.713	27.9	
	80	0.692	30	127.8305
	120	0.434	55.8	
	160	0.434	55.8	
cell+C.P	40	0.57	42.2	
	80	0.469	52.3	98.46698
	120	0.466	52.6	
	160	0.458	53.4	
Cell+NP	40	0.856	13.6	
	80	0.734	25.8	
	120	0.623	36.9	195.2101
	160	0.598	39.4	

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Cell+ocadaic acid	40	0.636	35.6	
	80	0.63	36.2	
	120	0.47	52.2	119.5099
	160	0.401	59.1	
	80	0.52	47.2	
	120	0.462	53	108.3871
	160	0.464	52.8	
Cell+Glyceraldehyde	40	0.627	36.5	
	80	0.53	46.2	92.56164
	120	0.383	60.9	
	160	0.357	63.5	
Cell+Rotenone	40	0.85	14.2	
	80	0.75	24.2	167.5161
	120	0.683	30.9	
	160	0.48	51.2	

- *Cell Images Under the Effect of Inducers and Inducer and Encapsulated (ACP) NaNO Curcumin*

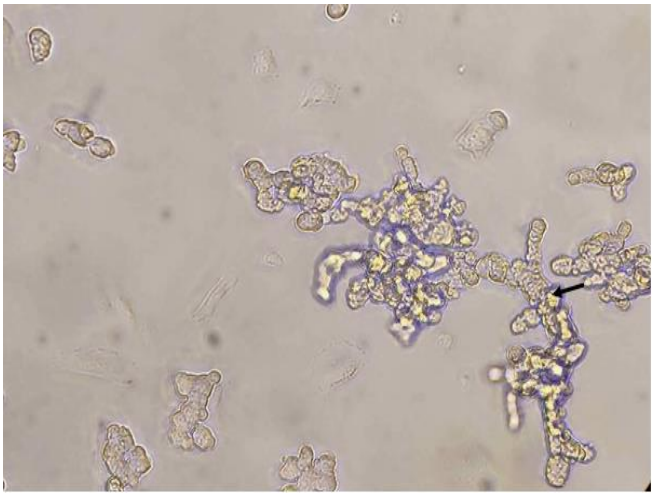

Fig 1 CTRL SH-S5Y5 cell lines Cells were in suspension and adhered also. Undifferentiated neuroblastoma cells after 24 hrs of incubation

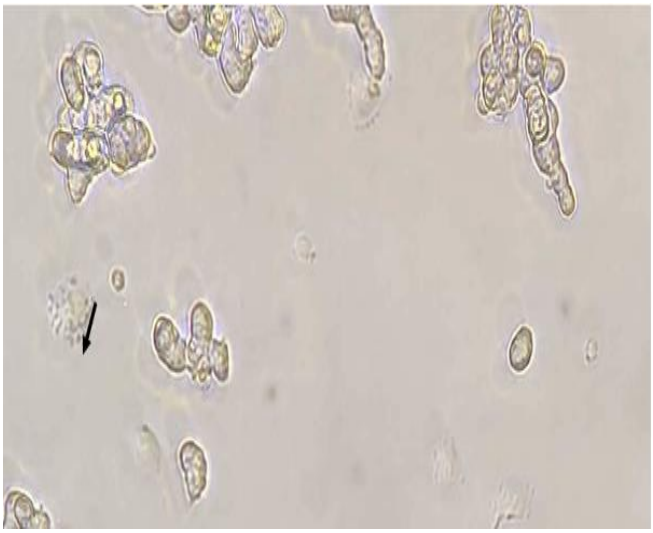

Fig 2 Cell after induced with IC50 concentration of Glyceraldehyde. Cell blebbing, indication of early apoptotic cells Cells circularized and no initiation of differentiation of cells.

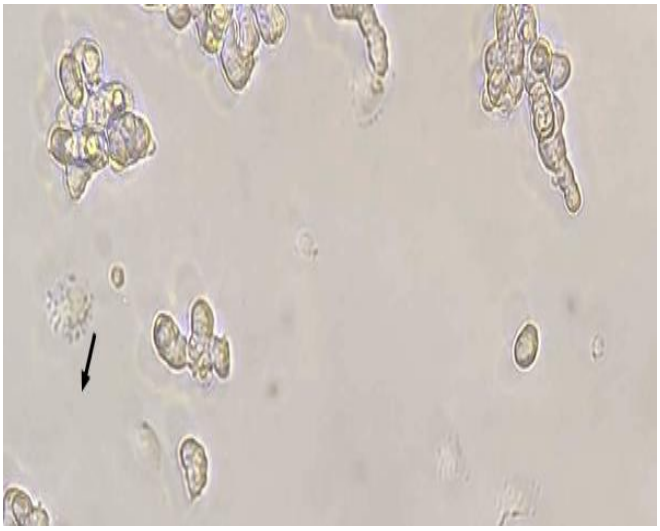

Fig 3 Cell after induced with IC50 concentration of Glyceraldehyde. Cell blebbing, indication of early apoptotic cells. Cells circularized and no initiation of differentiation of cells.

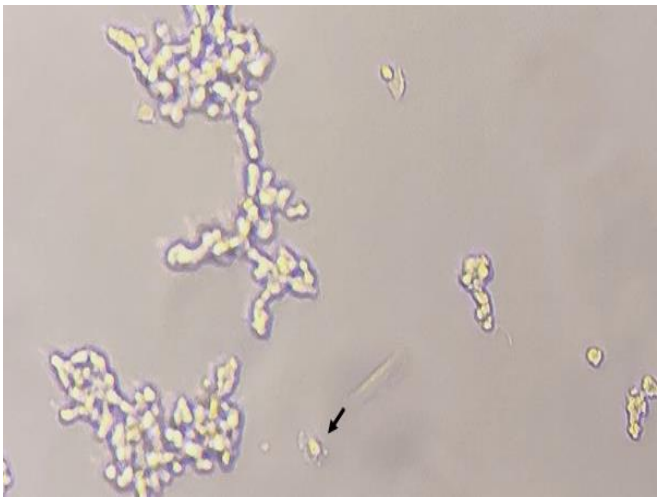

Fig 4 Cell after treatment with IC50 concentrations of glyceraldehyde and Enapsulated (ACP) Nano Curcumin. More differentiated cells after treatment of Enapsulated (ACP) Nano curcumin. Some early apoptotic cells observed

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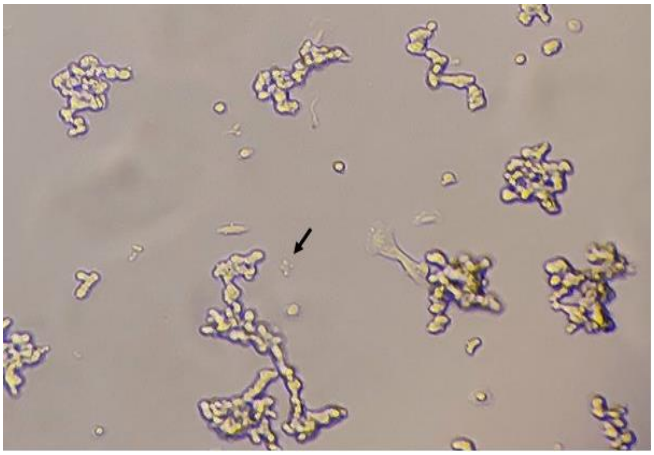

Fig 5 Cell after induced with IC50 concentration of rotenone. Cell blebbing indicating late apoptotic cells, more circularized cells indicating more cells in suspension and lost properties of adhering.

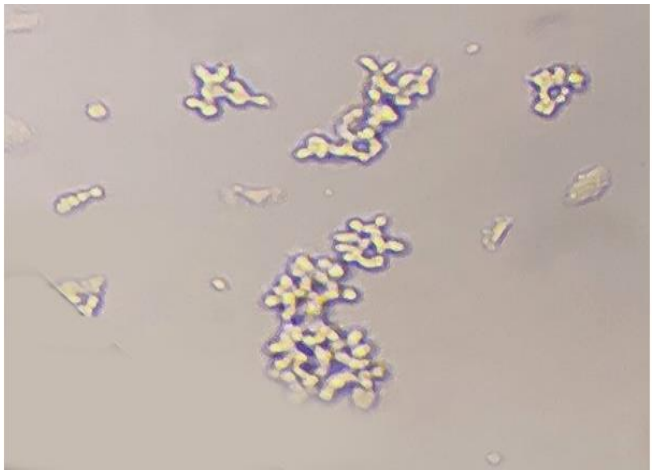

Fig 6 Cell after treatment with IC50 concentrations of rotenone and Enapsulated (ACP) Nano Curcumin.

Increased circularization of cells implies a greater presence of cells in a suspended state, resulting in a loss of their adhesive characteristics. The impact of Enapsulated (ACP) Nano Curcumin. curcumin on rotenone induced morphological changes in cells were found to be relatively insignificant.

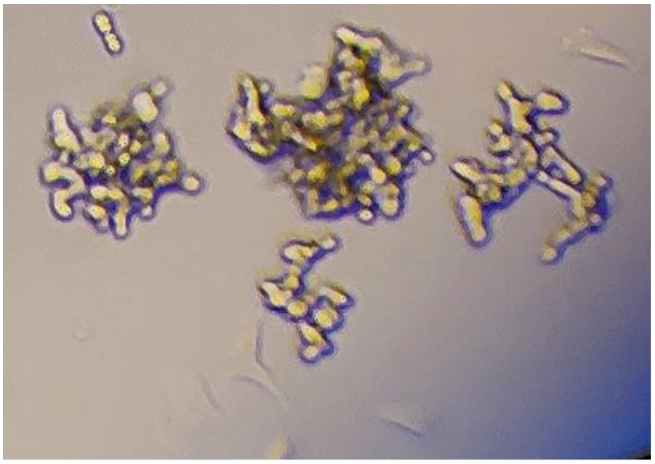

Fig 7 Cell after treatment with IC50 concentration of curcumin only. No differentiation of neuroblastoma cells

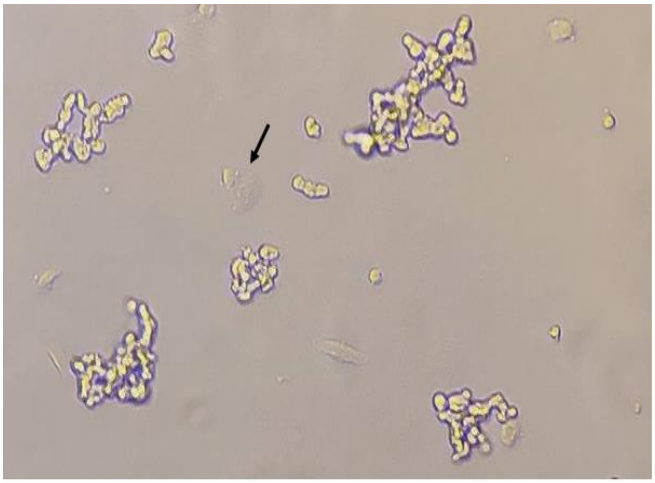

Fig 8 Cell after induced with IC50 concentration of AlCl3. Cell blebbing indicating late apoptotic

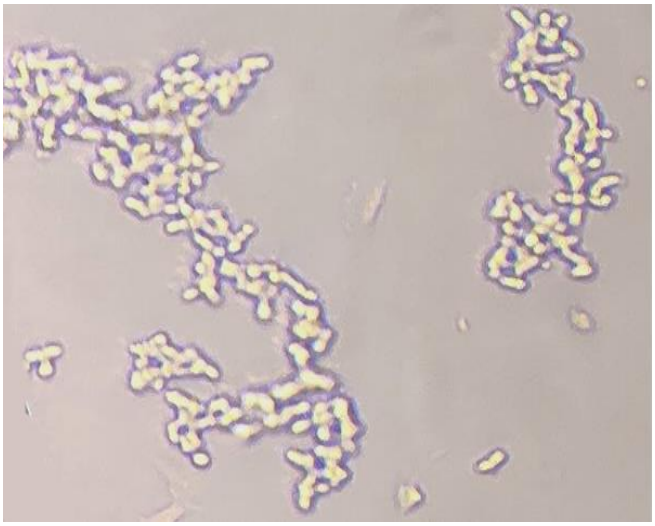

Fig 9 Cell after treatment with IC50 concentrations of AlCl3 and Enapsulated Nano Curcumin. Cell revival by Enapsulated (ACP) Nano curcumin addition in AlCl3 toxicity induced cells. No cell blebbing, induction of cell differentiation and regaining of cell.

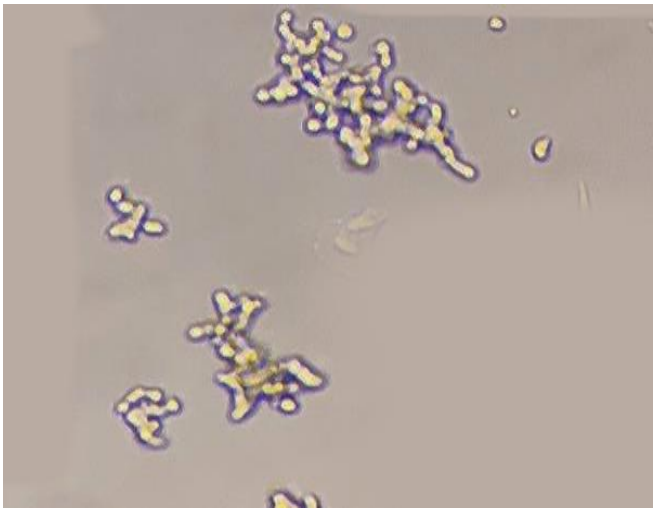

Fig 10 Cell after induced with IC50 concentration of Dopamine. Few numbers of circularized cells. Cells were in initial differentiation

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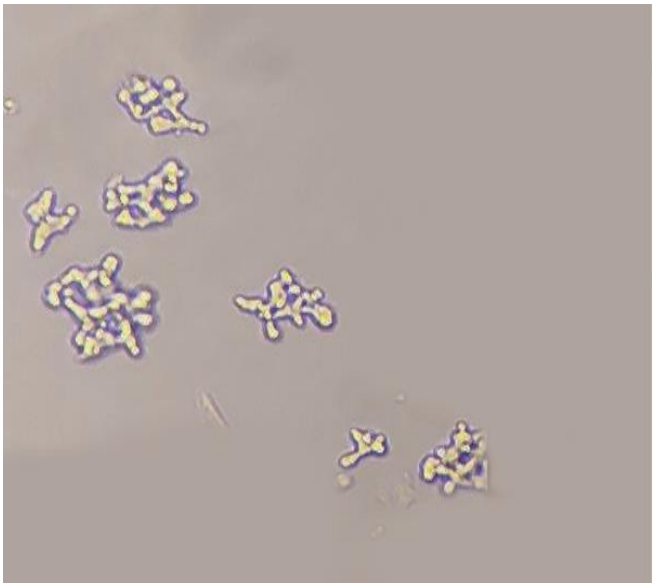

Fig 11 Cell after treatment with IC50 concentrations of dopamine and Encapsulation Nano Curcumin. Absence of any circularized cell indicating all cells are adhered.

XIII. CONCLUSION

According to reports, neurons are lost in Alzheimer's and Huntington's diseases, while dopaminergic neurons in the substantia nigra are specifically and inadequately lost in Parkinson's disease, Encapsulated nano -curcumin emerges as a potential therapeutic agent, exhibiting modulatory effects on gene expression changes induced by different compounds. Which provide insights into potential avenues for developing treatments that target the genetic aspects of Alzheimer's and Parkinson's diseases. Additional pre-clinical and clinical research is still needed. . Hence future research might explore an in vivo mouse model, using encapsulated amorphous calcium phosphate nanocurcumin which may be able to break through the blood-brain barrier, that is necessary for treating neurological disorders including Parkinson's and Alzheimer's diseases.

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