Effects of different medications used to treat Alzheimer's disease on *Caenorhabditis elegans*

Divyata Desai & Dr. Kundan Kumar Mishra

Natubhai V. Patel College of Pure & Applied sciences, Charutar Vidhya Mandal University, Vallabh Vidhyanagar - 388120

Abstract: -

The most prevalent kind of dementia is Alzheimer's disease, which develops when the brain no longer functions properly. The main causes of issues are with behaviour, thinking, and memory. A complex ailment called Alzheimer's is characterized by abnormal protein accumulation in the brain regions. Although the precise molecular causes of this disease are not yet known, it is well acknowledged that a protein homeostasis network exists. When they serve as an enzyme substrate or a signaling molecule to activate a biochemical pathway, metabolites have an impact on crucial steps in cellular pathways. It is relatively new to model Alzheimer's disease in a basic microscopic organism like *C. elegans*. Worms that have the human amyloid beta peptide genetically altered to be expressed in muscle cells build up immunoreactive deposits of amyloid beta 1-42 as well as insoluble beta amyloid, which is seen in senile plaques in AD brains. The impact of various medications can be studied in *C. elegans.*

Keywords :-

Alzheimer’s Disease, *Caenorhabditis elegans*, Amyloid beta, Amyloid Precursor Protein

1. Introduction

1.1 Alzheimer's disease

German psychiatric Alois Alzheimer noticed an amyloid plaque and loss of memory before the death of patient which was a serious medical condition known as Alzheimer's disease (Zeinab Breijyeh et al., 2020). In Emil kraeplien 8th edition of psychiatry handbook Alzheimer's disease as a medical condition mentioned (Gabriele et al., 2011). There are 50 million AD patients at present worldwide which can be double every 5 years (Yiannopoliz et al., 2020). Some treatments that are available only to improve the symptoms, no permanent cure is there (Livingstone G et al., 2020). In Alzheimer's disease two types of neuropathological changes occur:

I. Positive lesions which include senile plaque formation and neurofibrillary tangles.
II. Negative lesions which include synaptic loss (Zeinab Brijyeh et al., 2020)

At present, two types of hypotheses for Alzheimer's disease.

1) Cholinergic Hypothesis - Ach (Acetyl Choline) is secreted in brain and responsible of physiological activities like attention, memory, learning etc. In AD degeneration of cholinergic neurons was found which directly affect to memory loss (Ferreira veria et al., 2016)
2) Amyloid hypothesis – According to the amyloid hypothesis, ageing or pathological conditions slow down the degradation of Aβ, which is produced by secretase, which causes amyloid beta peptide (Aβ40 and Aβ42) to accumulate. An increase in the Aβ42/Aβ40 ratio causes the development of amyloid fibrils, which cause neurotoxicity, tau disease, and ultimately the death and degeneration of neuronal cells. Amyloid beta catabolism and anabolism were discovered to be affected by AD risk factors and mutations of multiple genes, including APP, PSEN1 and PSEN2, which quickly lead to an accumulation of and quick progression of neurodegeneration (Paroni et al., 2019, Kametani et al., 2018, Ricciarelli et al., 2017).

There are several risk factors for Alzheimer's disease.

Aging – Aging is a significant risk factor for AD. Hardly ever does this disease affect young people, and most cases of AD begin in persons over 65. (Guerrieo et al., 2015).

Genetics – A number of genetic factors are crucial in the development of AD. The majority of instances were linked to genetic variables such apolipoprotein E, presenilin 1 and 2, and amyloid precursor protein. (Van Cauwebreghe et al., 2016, Khanahmadi et al.; 2015). Environmental factors – AD is also impacted by environmental risk factors such as air pollution, nutrition, metals, oxidative stress, and inflammation. (Wainania et al., 2014, Grank et al., 2002). Medical factors – Elderly AD sufferers frequently have other health issues, such as diabetes, obesity, and cardiovascular disease (Stampfer 2006 , Santos et al., 2017).

1.2 *C. elegans* use as model organism

In 1963, Sydney Brenner developed the *C. elegans* which belongs to phylum Nematode of the animal kingdom. It is a transparent nematode and multicellular organism having a 1mm length (Brenner 1988; Brenner 2002). The organism is fully sequenced genome and the size of genome is 9.7×10^7 bp (White *et al.* 1986). *C. elegans* has 60-80% homology similar to humans. It has a simple nervous system having 302 nerve cells. *C. elegans* is widely used for genetic study and to research on human disease. *C. elegans* used as a model organism because they are small, easy and inexpensive to rear in the lab as well as it reproduce quickly (Sulston and Horvitz 1977; Kimble and Hirsh 1979; Sulston *et al.* 1983).

1.3 Usage of drugs for Alzheimer's disease

(Emeline Teo et al.,2019, Michele perni et al.,2021, M Obulesu et al.,2011)

Compounds	Mode of action
Mito Q	It is an orally active antioxidant that have ability to target mitochondrial dysfunction.
N- acetylcysteine	It is antioxidant and used in treatment of cancer.
Butylated Hydroxy-anisole	It is lab made chemical which is used for food preservative and treatment in genetic disorder.
Met formin	It is a metabolic modulator that improve stress resistance.

Alpha -ketoglutarate	It extends life span via ATP synthesis and mTOR inhibition.
Lithium	It is type of medicine known as mood stabilizer. It is a salt which is used as psychiatric medication, primarily for bipolar disorder. It is metabolic modulator and anti- aggregation class.
Rapamycin	It is also called sirolimus, which suppress the immune system which led to prevention of transplant rejection. It is produced by soil streptomyces hygroscopicus.
Thioflavin -T	It is a anti aggregation and reduces paralysis. It extends life span and involve in the gene of protein homeostasis. It is stain which is used for monitor invitro amyloid fibril formation
Curcumin	It is anti -aggregation, antioxidant and anti- inflammation drug. It is stress resistance and induces stress response gene
Carnosine	It is used for preventing aging and complication of diabetes such as nerve damage kidney problem.
Kynurenic acid	It acts as an ant excitotoxic and anticonvulsant. This acid possesses neuroactive activity

2. Effect of drugs on *C. elegans*

There are three different ways to target drugs: I first-order drug targeting to a specific organ or tissue, (ii) second-order drug targeting to particular cells, and (iii) third-order drug targeting to an intracellular compartment such the nucleus, endoplasmic reticulum, or mitochondria. (S.S Davis 1997, R. K Keservani et al., 2017).

2.1 Morphological study

2.1.1 Mito Q

Circular mitochondrial DNA serves as the self-replicating genome of mitochondria (mtDNA). One animal cell typically has 100–10,000 copies of mtDNA. Ribosomal RNA, mitochondrial transport RNA, and a few hydrophobic ETC proteins are all encoded by human mtDNA. Inherited mitochondrial illnesses are caused by mutations in either mtDNA or nuclear DNA genes that code for mitochondrial proteins. (D. C Wallace et al., 2010).

The amyloid cascade theory has eclipsed others to date, although the pathophysiology of AD is still not fully understood. Nevertheless, none of the medication candidates blocking amyloidβ -peptide buildup have even been able to postpone this condition. Consequently, it was believed that the fundamental factor causing amyloid -peptide (Aβ) deposition, synaptic degeneration, and the development of neurofibrillary tangles in the sporadic type of AD is mitochondrial malfunction. (R. H Swerdlow et al.,2010) This hypothesis is supported by the evidence of mitochondrial dysfunction in AD patients (I. G Onyango et al.,2016).and in animal models(J.W lustbader et al., 2004 , M. Manczak et al., 2006). This approach also identifies new therapeutic targets located in the mitochondria (P. I Mereia et al., 2010). A covalent bond between a ubiquinone molecule and the lipophilic decyltriphenylphosphonium (dTPP) cation is used to form the mitochondria-targeted ubiquinol known as mitoQ. (kelso et al., 2001). Due to the high mitochondrial membrane potential, MitoQ is intended to concentrate specifically several hundred times in the mitochondrial matrix. (J .M Macmanus et al.,2011, R.A smith et al., 1999). By using various assays, such as the paralysis assay and the stress tolerance assay, the Alzheimer's disease model

CL2006 demonstrated that mitoQ may represent a potential therapy against Aβ-induced toxicity and oxidative stress. (Li fang Ng et al., 2014).

2.1.2 N- acetylcysteine

In mucolytic therapy and to treat paracetamol overdose, N-acetylcysteine (NAC), a precursor to L-cysteine, is frequently utilised. (Giuseappe Tardiolo et al., 2018). NAC is regarded as a drug that exhibits pro-neurogenic and neuroprotective qualities. (R. S Bavarsad et al., 2014). Moreover, it is applied in treatments for mental and neurodegenerative illnesses. (L.s Ooi et al., 2018). NAC has undergone testing as a drug, serving as a precursor for the creation of GSH, and displaying potential actions that suggest an alternative as a potential future drug. (Y Hara et al.,2017). NAC's down-regulation suggests that it may contribute to the transcription of the APP gene. In actuality, neuroblastoma cells don't have any detectable APP mRNA levels. Such activity is associated with decreased NF-kB binding activity, which is boosted by oxidative stress and Aβ. (R. Studer et al., 2001). In *C. elegans* strain CF512, ASSNAC enhances glutathione concentration, enzyme activity, and the expression of the GST gene. This boosts the organisms' tolerance to oxidative stress and lengthens their life span. (Napthtali savior et al., 2018). Determined whether NAC can extend lifespan and affect fertility, which is closely related to ageing, as well as the effect of NAC supplementation on resistance to various environmental stressors, including oxidative stress, heat shock, and ultraviolet (UV) irradiation in vivo on N2 wild type strain of *C. elegans*. (Seung II Oh et al., 2015).

2.1.3 Butylated Hydroxy-anisole

Three phenol model molecules are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and bisphenol-A (BPA). (Bashar Alhoch et al., 2019) Several prevalent human diseases, such as cancer, diabetes, inflammatory conditions, and various neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis, are characterised by a pathological stress response. (S. Fuida et al., 2010, R karam et al., 2015). BHA exhibits an antioxidant effect and is a food supplement that affects the gastrointestinal tract and extends life in N2 wildtype strain. (Sebastain schmeisser et al.,2013, M. Scheieber et al., 2014) and further mechanism is not studied yet.

2.1.4 Met formin

For managing hyperglycaemia in diabetics, metformin is a highly effective first-line medication. It is a desirable candidate for medication repurposing to treat age-related diseases that are reliant on and independent of glycaemic control. (Jie Zheg et al., 2022). Incident dementia is one of the main objectives in large trials like Targeting Aging with Metformin (TAME). (N Brazilai et al., 2018). Studies have shown a strong correlation between Type 2 diabetes mellitus (T2DM) and Alzheimer's disease (AD), pointing to possible shared pathophysiological processes between the two conditions. Glucose metabolism balance, reduced amyloid plaque formation, normalised tau protein phosphorylation, and increased autophagy are possible effects of metformin. (Ping Ping Ning et al., 2022). The lifetime extension effect of metformin is mediated by both v-ATPase-mediated TORC1 inhibition and v-ATPase-AXIN/LKB1-mediated AMPK activation in *Caenorhabditis elegans*. (Jie Chen et al., 2017). In the *C. elegans* Parkinson's disease model, met formin exhibits neuroprotective effects via α synuclein aggregation. (Nada Saewanee et al., 2019).

2.1.5 Alpha -ketoglutarate

They play a crucial role in the study of Alzheimer's and mitochondrial illness because they are a crucial Krebs cycle enzyme that is very susceptible to the damaging effects of ROS. (Dora Csaban et al., 2021). Astroctyes' ability to produce ATP is compromised by oxidative stress brought on by a variety of conditions, including aluminium poisoning, as a result of malfunctioning mitochondria. As a result, globular, glycolytic, lipogenic, and ATP-deficient astrocytes are produced, which are cerebral traits that are typical in AD patients. (S C Thomas et al., 2015). The development of transgenic AD strains (GRU102) in *C. elegans* that express human Aβ-peptides in various cell types, including neurons, muscle, or specific subsets of neurons, and under various promoters, including temperature-inducible or constitutive, has confirmed AD-like pathology with varying disease severity. (Emelyne Teo et al., 2019). Some medications that affect metabolism have positive effects on AD, but the underlying mechanisms are still poorly understood. Hence, significant metabolic abnormalities seen in GRU102 may partially be explained by aKGDH activity inhibition, as seen in this mutation. Inactivation of aKGDH may explain the general decrease in spare respiratory capacity and energy deficits in the

GRU102 animal in addition to the observed fall in levels of TCA cycle metabolites downstream of aKG (malate and fumarate). (Butterfield et al., 2006).

2.1.6 Lithium

In the treatment of Alzheimer's disease and its prodromal stages, lithium may be a therapeutic alternative. But more research on its medicinal effectiveness is necessary. Head-to-head comparisons with recognised dementia therapy alternatives should be a part of future research. Due to the therapeutic toxicity of lithium, careful patient selection and intensive therapeutic monitoring are required. (Robert Haussmann et al, 2021). For more than 60 years, bipolar disorder (BD) has been treated pharmacologically using lithium as a mood stabiliser. After oral treatment, lithium is quickly and completely absorbed from the digestive tract. Its concentration is initially highest in the serum before being clearly dispersed to other tissue compartments. (Jinhuawen et al., 2019). Lithium and metformin were studied in combination in transgenic animals using the GRU102 strain in an effort to prolong lifespan. (Emelyne Teo and Sheng Fong et al., 2020)

2.1.7 Rapamycin

Rapamycin is a macrocyclic lactone made by the bacterium Streptomyces hygroscopicus, which was identified from soil samples taken from Easter Island by Georges Nogrady in the late 1960s. Rapamycin is also marketed under the names sirolimus and rapamune. (Ramasamy Selvarani et al., 2021). Maf1 is a kinase that promotes ageing known as the mechanistic target of rapamycin (mTOR). Maf1's role in the lifetime extension brought on by mTOR inhibition, such as calorie restriction or dietary restriction, is yet unclear. Here, we demonstrate that deletion of maf1 disables DR or CR to lengthen lifespan in the budding yeast S. cerevisiae but not in *C. elegans* due to mafr-1. (Ying Cai and Yue Hua Wei ,2016).

2.1.8 Thioflavin -T

When natural chemical compounds were administered to transgenic mouse models of Alzheimer's disease, the anti-aggregating effects were determined by the thioflavin T assay and through behavioural, biochemical, and histological examination**.** (Raluca Stefanescus et al., 2020). Thioflavin T molecules are bound by beta-amyloid fibrils and oligomers, but not by beta-amyloid monomers, which have no chemical interactions. (M .Biancalana et al., 2010 , I Maezawa et al., 2008). The created a peptide called A3 that, by increasing the aggregation kinetics,

considerably improved the creation of amorphous aggregates of Aβ. Thioflavin T fluorescence tests showed faster A monomer aggregation along with diminished Aβ cytotoxicity. The paralysis caused by the buildup of Aβ oligomers in transgenic *Caenorhabditis elegans* over-expressing amyloid precursor protein might be reduced by giving the worms A3 peptide. These data imply that the Aβ-aggregation-promotion effect may be helpful for creating Aβ-toxicity reduction techniques. (Aihua Yang et al., 2017).

2.1.9 Curcumin

Turmeric comes from the plant Curcuma longa, often known as Haldi, and is used in curries and other hot cuisines from India, Asia, and the Middle East. (Shrikant Mishra and Kalpana Pulanivelu 2008). From ancient times, it has been widely utilised in Ayurveda (Indian system of medicine) as a painkilling and anti-inflammatory substance to treat pain and inflammation in the muscles and skin. It has also demonstrated anti-cancer qualities. (S Shishodia et al., 2005, HP Ammon et al., 1991). The right diet and calorie restriction are essential for good ageing. It has been demonstrated that curcumin, a polyphenolic molecule extracted from the Curcuma longa, exhibits anti-aging properties. Studies on curcumin and age-related disease in model organisms have recently shown that curcumin and its metabolites increase the average lifetime of some ageing model organisms like *C. elegans*. (Aliabbas Zia et al., 2020).

2.1.10 Carnosine

Natural endogenous chemical carnosine has been the subject of intense research in recent years because of its potential to have positive impacts on human health. (Cristina Solana Manrique et al., 2022). Carnosine (β-alanyl-L-histidine) is a histidine-containing dipeptide (HCD) that, together with its analogs homocarnosine, anserine and ophidine/balenine, is widely distributed in mammalian tissues (F. Bellia et al., 2014, G. Caruso et al., 2019). The majority of this dipeptide is found in the skeletal and cardiac muscles, although it is also found in the brain. Identified carnosine (-alanyl histidine) as a potential treatment for vascular dementia after searching numerous agricultural products for compounds that shield neurons from Zn2+-induced neurotoxicity. (Masahiro Kawahara et al., 2020).

2.1.11 Kynurenic acid

Tryptophan's metabolite kynurenic acid (KYNA), which is produced by the kynurenine pathway, has neuroprotective properties both in vitro and in vivo. As new KYNA targets were found, the compound's unique biological function came to light. In Huntington's, Parkinson's, and Alzheimer's disorders, absolute or relative deficit of KYNA in comparison to neurotoxic kynurenines suggests that increasing brain KYNA levels may have therapeutic significance. (Aleksandra ostapiuk and Ewa M. Urbanska 2022). Analogs of the naturally occurring chemical kynurenic acid (KYNA) are being created, and their pharmacological effects are being studied, in hopes of developing multifaceted treatments for Alzheimer's disease (AD). In transgenic *Caenorhabditis elegans* strain GMC101 expressing full-length Aβ42, the effects of synthesised KYNA analogues on NMDA receptor binding, mGluR5 binding and function, acetylcholinesterase (AChE) inhibition, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, interference with the amyloid peptide (Aβ) fibrillation process (Girdhar Singh Deora et al., 2017). They studied the functions of carnosine and kynurenic acid, two metabolites, in the removal of protein aggregates. These two metabolites cause a cytosolic unfolded protein response mediated by HSF-1 and elevated levels of the DNJ-12 and DNJ-19 J-proteins, which prevent Aβ42 aggregation in a *C. elegans* model of AD. (Priyanka Joshi et al., 2021).

3. Molecular study

The Alzheimer's disease hypothesis states that certain proteins and genes cause AD. Hence, some molecular methods for protein profiling and gene expression were investigated. These techniques were used to examine the effects of various medicines in *Caenorhabditis elegans*, including metformin, N-acetylcysteine, and kynurenic acid.

3.1 Gene expression by Real time polymerase chain reaction

A method for tracking the PCR reaction in real time is real-time quantitative PCR. Based on its intended use, RT-qPCR can be roughly divided into two types: absolute

and relative quantification. While relative quantification is used in the fields of genomics and functional transcriptomics to perform gene expression analysis in biological experiments, absolute quantification is used in a wide range of fields, including microbiology, food technology, and biotechnology to quantify the microbiological load, adulterants in a commodity, and copy numbers, respectively. (Ravikumar Harshita and Durai pandian Rex Arunraj 2021).

3.2 Protein Profiling

3.2.1 SDS PAGE

When a protein is complexed with the powerful cationic detergent sodium dodecyl sulphate (SDS) and separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis, its migration distance can be used to calculate its apparent molecular weight (Hiroyuki Matsumato et al., 2019).

3.2.2 Western blotting

Biologists frequently employ the well-known molecular biology technique known as Western blotting (WB), also referred to as immunoblotting, to explore a variety of protein properties, from basic protein analysis to disease detection. WB is a straightforward, distinctive, speedy, routine instrument with simple interpretation and clear outcomes. (Habeebunnisa Begum et al.,2022)

Conclusion:-

Only a few drugs are now available due to the Alzheimer's disease's unclear pathological mechanism, which limits the creation of novel treatments. Several medications have a favorable morphological impact on *C. elegans*. However, certain aspects, such as genetic factors presenilin 1 & 2, and protein hypothesis, will determine if the molecular study will be a novel technique. The basic nervous system of *C. elegans* can be utilized as a model organism. A model of Alzheimer's disease that has been genetically altered can be utilized to profile protein and gene expression. The effect of a medicine on a certain gene or protein is understood, which will help with further clinical trials to determine which drug is more effective for AD, an inherited

neurological degenerative illness. Using bioinformatics tool like docking can be used to assess the impact of medication on amyloid beta protein.

4. References

1. Breijyeh, Zeinab, and Rafik Karaman. "Comprehensive review on Alzheimer's disease: causes and treatment." *Molecules* 25, no. 24 (2020): 5789.
2. Cipriani, Gabriele, Cristina Dolciotti, Lucia Picchi, and Ubaldo Bonuccelli. "Alzheimer and his disease: a brief history." *Neurological Sciences* 32 (2011): 275-279.
3. Atri, Alireza. "Current and future treatments in Alzheimer's disease." In *Seminars in neurology*, vol. 39, no. 02, pp. 227-240. Thieme Medical Publishers, 2019.
4. Livingston, Gill, Jonathan Huntley, Andrew Sommerlad, David Ames, Clive Ballard, Sube Banerjee, Carol Brayne et al. "Dementia prevention, intervention, and care: 2020 report of the Lancet Commission." *The Lancet* 396, no. 10248 (2020): 413-446.
5. H Ferreira-Vieira, Talita, Isabella M Guimaraes, Flavia R Silva, and Fabiola M Ribeiro. "Alzheimer's disease: targeting the cholinergic system." *Current neuropharmacology* 14, no. 1 (2016): 101-115.
6. Paroni, Giulia, Paola Bisceglia, and Davide Seripa. "Understanding the amyloid hypothesis in Alzheimer's disease." *Journal of Alzheimer's Disease* 68, no. 2 (2019): 493-510.
7. Kametani, Fuyuki, and Masato Hasegawa. "Reconsideration of amyloid hypothesis and tau hypothesis in Alzheimer's disease." *Frontiers in neuroscience* (2018): 25.
8. Ricciarelli, Roberta, and Ernesto Fedele. "The amyloid cascade hypothesis in Alzheimer's disease: it's time to change our mind." *Current neuropharmacology* 15, no. 6 (2017): 926-935.
9. Guerreiro, Rita, and Jose Bras. "The age factor in Alzheimer's disease." *Genome medicine* 7, no. 1 (2015): 1-3.
10. Van Cauwenberghe, Caroline, Christine Van Broeckhoven, and Kristel Sleegers. "The genetic landscape of Alzheimer disease: clinical implications and perspectives." *Genetics in Medicine* 18, no. 5 (2016): 421-430.
11. Khanahmadi, Mohammad, Dariush D. Farhud, and Maryam Malmir. "Genetic of Alzheimer's disease: A narrative review article." *Iranian journal of public health* 44, no. 7 (2015): 892.
12. Wainaina, Moses N., Zhichun Chen, and Chunjiu Zhong. "Environmental factors in the development and progression of late-onset Alzheimer's disease." *Neuroscience bulletin* 30 (2014): 253-270.
13. Grant, William B., Arezoo Campbell, Ruth F. Itzhaki, and John Savory. "The significance of environmental factors in the etiology of Alzheimer's disease." *Journal of Alzheimer's disease* 4, no. 3 (2002): 179-189.
14. Stampfer, M. J. "Cardiovascular disease and Alzheimer's disease: common links." *Journal of internal medicine* 260, no. 3 (2006): 211-223.
15. Santos, Cláudia Y., Peter J. Snyder, Wen-Chih Wu, Mia Zhang, Ana Echeverria, and Jessica Alber. "Pathophysiologic relationship between Alzheimer's disease, cerebrovascular disease, and cardiovascular risk: a review and synthesis." *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring* 7 (2017): 69-87.
16. Girard, Lisa R., Tristan J. Fiedler, Todd W. Harris, Felicia Carvalho, Igor Antoshechkin, Michael Han, Paul W. Sternberg, Lincoln D. Stein, and Martin Chalfie. "WormBook: the online review of Caenorhabditis elegans biology." *Nucleic acids research* 35, no. suppl_1 (2007): D472-D475.
17. Teo, Emelyne, Soon Yew John Lim, Sheng Fong, Anis Larbi, Graham D. Wright, Nicholas Tolwinski, and Jan Gruber. "A high throughput drug screening paradigm using transgenic

Caenorhabditis elegans model of Alzheimer's disease." *Translational Medicine of Aging* 4 (2020): 11-21.
18. Obulesu, M., and Dowlathabad Muralidhara Rao. "Effect of plant extracts on Alzheimer's disease: An insight into therapeutic avenues." *Journal of neurosciences in rural practice* 2, no. 01 (2011): 056-061.
19. Perni, Michele, Annemieke Van der Goot, Ryan Limbocker, Tjakko J. Van Ham, Francesco A. Aprile, Catherine K. Xu, Patrick Flagmeier et al. "Comparative Studies in the A30P and A53T α-Synuclein C. elegans Strains to Investigate the Molecular Origins of Parkinson's Disease." *Frontiers in Cell and Developmental Biology* 9 (2021): 552549.
20. Girard, Lisa R., Tristan J. Fiedler, Todd W. Harris, Felicia Carvalho, Igor Antoshechkin, Michael Han, Paul W. Sternberg, Lincoln D. Stein, and Martin Chalfie. "WormBook: the online review of Caenorhabditis elegans biology." *Nucleic acids research* 35, no. suppl_1 (2007): D472-D475.
21. Stiernagle, Theresa. "Maintenance of C. elegans." (1999): 51-67.
22. Ng, Li Fang, Jan Gruber, Irwin K. Cheah, Chong Kiat Goo, Wei Fun Cheong, Guanghou Shui, Kim Ping Sit, Markus R. Wenk, and Barry Halliwell. "The mitochondria-targeted antioxidant MitoQ extends lifespan and improves healthspan of a transgenic Caenorhabditis elegans model of Alzheimer disease." *Free Radical Biology and Medicine* 71 (2014): 390-401.
23. Zinovkin, Roman A., and Andrey A. Zamyatnin. "Mitochondria-targeted drugs." *Curr Mol Pharmacol* 12, no. 3 (2019): 202-214.
24. Davis, S. S. "Biomedical applications of nanotechnology—implications for drug targeting and gene therapy." *Trends in biotechnology* 15, no. 6 (1997): 217-224.
25. Keservani, Raj K., Anil K. Sharma, and Rajesh K. Kesharwani, eds. *Drug Delivery Approaches and Nanosystems, Volume 1: Novel Drug Carriers*. CRC Press, 2017.
26. Wallace, Douglas C. "Mitochondrial genetic medicine." *Nature genetics* 50, no. 12 (2018): 1642-1649.
27. Swerdlow, Russell H., Jeffrey M. Burns, and Shaharyar M. Khan. "The Alzheimer's disease mitochondrial cascade hypothesis." *Journal of Alzheimer's Disease* 20, no. s2 (2010): S265-S279.
28. Onyango, Isaac G., Jameel Dennis, and Shaharyah M. Khan. "Mitochondrial dysfunction in Alzheimer's disease and the rationale for bioenergetics based therapies." *Aging and disease* 7, no. 2 (2016): 201.
29. Caspersen, Casper, Ning Wang, Jun Yao, Alexander Sosunov, Xi Chen, Joyce W. Lustbader, Hong Wei Xu, David Stern, Guy McKhann, and Shi Du Yan. "Mitochondrial Aβ: a potential focal point for neuronal metabolic dysfunction in Alzheimer's disease." *The FASEB Journal* 19, no. 14 (2005): 2040-2041.
30. Manczak, Maria, Thimmappa S. Anekonda, Edward Henson, Byung S. Park, Joseph Quinn, and P. Hemachandra Reddy. "Mitochondria are a direct site of Aβ accumulation in Alzheimer's disease neurons: implications for free radical generation and oxidative damage in disease progression." *Human molecular genetics* 15, no. 9 (2006): 1437-1449.
31. Moreira, Paula I., Xiongwei Zhu, Xinglong Wang, Hyoung-gon Lee, Akihiko Nunomura, Robert B. Petersen, George Perry, and Mark A. Smith. "Mitochondria: a therapeutic target in neurodegeneration." *Biochimica et Biophysica Acta (BBA)-molecular basis of Disease* 1802, no. 1 (2010): 212-220.
32. Kelso, Geoffrey F., Carolyn M. Porteous, Carolyn V. Coulter, Gillian Hughes, William K. Porteous, Elizabeth C. Ledgerwood, Robin AJ Smith, and Michael P. Murphy. "Selective targeting of a redox-active ubiquinone to mitochondria within cells: antioxidant and antiapoptotic properties." *Journal of Biological Chemistry* 276, no. 7 (2001): 4588-4596.

33. McManus, Meagan J., Michael P. Murphy, and James L. Franklin. "The mitochondria-targeted antioxidant MitoQ prevents loss of spatial memory retention and early neuropathology in a transgenic mouse model of Alzheimer's disease." *Journal of Neuroscience* 31, no. 44 (2011): 15703-15715.
34. Smith, Robin AJ, Carolyn M. Porteous, Carolyn V. Coulter, and Michael P. Murphy. "Selective targeting of an antioxidant to mitochondria." *European Journal of Biochemistry* 263, no. 3 (1999): 709-716.
35. Tardiolo, Giuseppe, Placido Bramanti, and Emanuela Mazzon. "Overview on the effects of N-acetylcysteine in neurodegenerative diseases." *Molecules* 23, no. 12 (2018): 3305.
36. Bavarsad Shahripour, Reza, Mark R. Harrigan, and Andrei V. Alexandrov. "N-acetylcysteine (NAC) in neurological disorders: mechanisms of action and therapeutic opportunities." *Brain and behavior* 4, no. 2 (2014): 108-122.
37. Ooi, Soo Liang, Ruth Green, and Sok Cheon Pak. "N-Acetylcysteine for the treatment of psychiatric disorders: a review of current evidence." *BioMed Research International* 2018 (2018).
38. Studer, Rolf, Ginette Baysang, and Christine Brack*. "N-Acetyl-L-Cystein downregulates β-amyloid precursor protein gene transcription in human neuroblastoma cells." *Biogerontology* 2 (2001): 55-60.
39. Hara, Y., N. McKeehan, P. A. Dacks, and H. M. Fillit. "Evaluation of the neuroprotective potential of N-acetylcysteine for prevention and treatment of cognitive aging and dementia." *J Prev Alzheimers Dis* (2017).
40. Savion, Naphtali, Amir Levine, Shlomo Kotev-Emeth, Ulrike Bening Abu-Shach, and Limor Broday. "S-allylmercapto-N-acetylcysteine protects against oxidative stress and extends lifespan in Caenorhabditis elegans." *Plos one* 13, no. 3 (2018): e0194780.
41. Oh, Seung-Il, Jin-Kook Park, and Sang-Kyu Park. "Lifespan extension and increased resistance to environmental stressors by N-acetyl-L-cysteine in Caenorhabditis elegans." *Clinics* 70 (2015): 380-386.
42. Alhoch, Bashar, Alan Chen, Elaine Chan, Asmaa Elkabti, Sasha Fariña, Catherine Gilbert, Jean Kang et al. "Comparative genomic screen in two yeasts reveals conserved pathways in the response network to phenol stress." *G3: Genes, Genomes, Genetics* 9, no. 3 (2019): 639-650.
43. Fulda, Simone, Adrienne M. Gorman, Osamu Hori, and Afshin Samali. "Cellular stress responses: cell survival and cell death." *International journal of cell biology* 2010 (2010).
44. Karam, Rachid, Chih-Hong Lou, Heike Kroeger, Lulu Huang, Jonathan H. Lin, and Miles F. Wilkinson. "The unfolded protein response is shaped by the NMD pathway." *EMBO reports* 16, no. 5 (2015): 599-609.
45. Schmeisser, Sebastian, Steffen Priebe, Marco Groth, Shamci Monajembashi, Peter Hemmerich, Reinhard Guthke, Matthias Platzer, and Michael Ristow. "Neuronal ROS signaling rather than AMPK/sirtuin-mediated energy sensing links dietary restriction to lifespan extension." *Molecular metabolism* 2, no. 2 (2013): 92-102.
46. Schieber, Michael, and Navdeep S. Chandel. "TOR signaling couples oxygen sensing to lifespan in C. elegans." *Cell reports* 9, no. 1 (2014): 9-15.
47. Zheng, Jie, Min Xu, Venexia Walker, Jinqiu Yuan, Roxanna Korologou-Linden, Jamie RobBarzilai, Nir, Ana Maria Cuervoinson, Peiyuan Huang et al. "Evaluating the efficacy and mechanism of metformin targets on reducing Alzheimer's disease risk in the general population: a Mendelian randomisation study." *Diabetologia* 65, no. 10 (2022): 1664-1675.
48. Barzilai, Nir, Ana Maria Cuervo, and Steve Austad. "Aging as a biological target for prevention and therapy." *Jama* 320, no. 13 (2018): 1321-1322.
49. Ning, Pingping, Anling Luo, Xin Mu, Yanming Xu, and Tian Li. "Exploring the dual character of metformin in Alzheimer's disease." *Neuropharmacology* (2022): 108966.

50. Chen, Jie, Yuhui Ou, Yi Li, Shumei Hu, Li-Wa Shao, and Ying Liu. "Metformin extends C. elegans lifespan through lysosomal pathway." *Elife* 6 (2017): e31268.
51. Saewanee, Nada, Theethawat Praputpittaya, Nawaphat Malaiwong, Pawanrat Chalorak, and Krai Meemon. "Neuroprotective effect of metformin on dopaminergic neurodegeneration and α-synuclein aggregation in C. elegans model of Parkinson's disease." *Neuroscience research* 162 (2021): 13-21.
52. Csaban, Dora, Klara Pentelenyi, Renata Toth-Bencsik, Anett Illes, Zoltan Grosz, Andras Gezsi, and Maria Judit Molnar. "The role of the rare variants in the genes encoding the alpha-ketoglutarate dehydrogenase in Alzheimer's disease." *Life* 11, no. 4 (2021): 321.
53. Thomas, S. C., A. Alhasawi, V. P. Appanna, C. Auger, and Vasu D. Appanna. "Brain metabolism and Alzheimer's disease: the prospect of a metabolite-based therapy." *The journal of nutrition, health & aging* 19 (2015): 58-63.
54. Teo, Emelyne, Sudharshan Ravi, Diogo Barardo, Hyung-Seok Kim, Sheng Fong, Amaury Cazenave-Gassiot, Tsze Yin Tan et al. "Metabolic stress is a primary pathogenic event in transgenic Caenorhabditis elegans expressing pan-neuronal human amyloid beta." *Elife* 8 (2019): e50069.
55. Butterfield, D. Allan, and Barry Halliwell. "Oxidative stress, dysfunctional glucose metabolism and Alzheimer disease." *Nature Reviews Neuroscience* 20, no. 3 (2019): 148-160.
56. Haussmann, Robert, Felix Noppes, Moritz D. Brandt, Michael Bauer, and Markus Donix. "Minireview: Lithium: a therapeutic option in Alzheimer's disease and its prodromal stages?." *Neuroscience Letters* 760 (2021): 136044.
57. Wen, Jinhua, Darrell Sawmiller, Brendan Wheeldon, and Jun Tan. "A review for lithium: pharmacokinetics, drug design, and toxicity." *CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders)* 18, no. 10 (2019): 769-778.
58. Teo, Emelyne, Sheng Fong, Nicholas Tolwinski, and Jan Gruber. "Drug synergy as a strategy for compression of morbidity in a Caenorhabditis elegans model of Alzheimer's disease." *GeroScience* 42 (2020): 849-856.
59. Selvarani, Ramasamy, Sabira Mohammed, and Arlan Richardson. "Effect of rapamycin on aging and age-related diseases—past and future." *Geroscience* 43 (2021): 1135-1158.
60. Cai, Ying, and Yue-Hua Wei. "Stress resistance and lifespan are increased in C. elegans but decreased in S. cerevisiae by mafr-1/maf1 deletion." *Oncotarget* 7, no. 10 (2016): 10812.
61. Stefanescu, Raluca, Gabriela Dumitrița Stanciu, Andrei Luca, Luminita Paduraru, and Bogdan-Ionel Tamba. "Secondary metabolites from plants possessing inhibitory properties against beta-amyloid aggregation as revealed by thioflavin-T assay and correlations with investigations on transgenic mouse models of Alzheimer's disease." *Biomolecules* 10, no. 6 (2020): 870.
62. Biancalana, Matthew, and Shohei Koide. "Molecular mechanism of Thioflavin-T binding to amyloid fibrils." *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics* 1804, no. 7 (2010): 1405-1412.
63. Kung, Mei-Ping, Hank F. Kung, Kit S. Lam, Salvatore Oddo, Frank M. LaFerla, and Lee-Way Jin. "Congo red and thioflavin-T analogs detect Ab oligomers." *J. Neurochem* 104 (2008): 457-468.
64. Yang, Aihua, Chenxuan Wang, Baomin Song, Wendi Zhang, Yuanyuan Guo, Rong Yang, Guangjun Nie, Yanlian Yang, and Chen Wang. "Attenuation of β-amyloid toxicity in vitro and in vivo by accelerated aggregation." *Neuroscience Bulletin* 33 (2017): 405-412.
65. Mishra, Shrikant, and Kalpana Palanivelu. "The effect of curcumin (turmeric) on Alzheimer's disease: An overview." *Annals of Indian Academy of Neurology* 11, no. 1 (2008): 13.

66. Shishodia, Shishir, Gautam Sethi, and Bharat B. Aggarwal. "Curcumin: getting back to the roots." *Annals of the New York Academy of sciences* 1056, no. 1 (2005): 206-217.
67. Ammon, Hermann PT, and Martin A. Wahl. "Pharmacology of Curcuma longa." *Planta medica* 57, no. 01 (1991): 1-7.
68. Zia, Aliabbas, Tahereh Farkhondeh, Ali Mohammad Pourbagher-Shahri, and Saeed Samarghandian. "The role of curcumin in aging and senescence: Molecular mechanisms." *Biomedicine & Pharmacotherapy* 134 (2021): 111119.
69. Solana-Manrique, Cristina, Francisco José Sanz, Guillermo Martínez-Carrión, and Nuria Paricio. "Antioxidant and Neuroprotective Effects of Carnosine: Therapeutic Implications in Neurodegenerative Diseases." *Antioxidants* 11, no. 5 (2022): 848.
70. Bellia, Francesco, Graziella Vecchio, and Enrico Rizzarelli. "Carnosinases, their substrates and diseases." *Molecules* 19, no. 2 (2014): 2299-2329.
71. Caruso, Giuseppe, Filippo Caraci, and Renaud B. Jolivet. "Pivotal role of carnosine in the modulation of brain cells activity: Multimodal mechanism of action and therapeutic potential in neurodegenerative disorders." *Progress in neurobiology* 175 (2019): 35-53.
72. Kawahara, Masahiro, Yutaka Sadakane, Keiko Mizuno, Midori Kato-Negishi, and Ken-ichiro Tanaka. "Carnosine as a possible drug for zinc-induced neurotoxicity and vascular dementia." *International journal of molecular sciences* 21, no. 7 (2020): 2570.
73. Deora, Girdhar Singh, Srinivas Kantham, Stephen Chan, Satish N. Dighe, Suresh K. Veliyath, Gawain McColl, Marie-Odile Parat, Ross P. McGeary, and Benjamin P. Ross. "Multifunctional analogs of kynurenic acid for the treatment of Alzheimer's disease: synthesis, pharmacology, and molecular modeling studies." *ACS chemical neuroscience* 8, no. 12 (2017): 2667-2675.
74. Ostapiuk, Aleksandra, and Ewa M. Urbanska. "Kynurenic acid in neurodegenerative disorders—unique neuroprotection or double-edged sword?." *CNS Neuroscience & Therapeutics* 28, no. 1 (2022): 19-35.
75. Joshi, Priyanka, Michele Perni, Ryan Limbocker, Benedetta Mannini, Sam Casford, Sean Chia, Johnny Habchi, Johnathan Labbadia, Christopher M. Dobson, and Michele Vendruscolo. "Two human metabolites rescue a C. elegans model of Alzheimer's disease via a cytosolic unfolded protein response." *Communications biology* 4, no. 1 (2021): 843.
76. Harshitha, Ravikumar, and Duraipandian Rex Arunraj. "Real-time quantitative PCR: A tool for absolute and relative quantification." *Biochemistry and Molecular Biology Education* 49, no. 5 (2021): 800-812.
77. Matsumoto, Hiroyuki, Hisao Haniu, and Naoka Komori. "Determination of protein molecular weights on SDS-PAGE." *Electrophoretic Separation of Proteins: Methods and Protocols* (2019): 101-105.
78. Begum, Habeebunnisa, Periyasamy Murugesan, and Anjana Devi Tangutur. "Western blotting: a powerful staple in scientific and biomedical research." *BioTechniques* 73, no. 1 (2022): 58-69.