

Finding effective combinations of compounds to prevent Alzheimer's disease

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Alzheimer's disease (AD) remains one of the significant causes of death and morbidity in the older population (2021). The cause of AD remains unclear despite there being numerous hypotheses. Perhaps the most widely accepted hypothesis is the amyloid cascade hypothesis which is based on the accumulation of amyloid beta ($A\beta$) in neurons (Dhakal et al., 2019). $A\beta$ is produced from amyloid precursor protein (APP) after its processing via beta-secretase and gamma-secretase enzymes, while the processing of APP by alpha-secretase instead of β -secretase forms a non-amyloidogenic amyloid- α protein. The increased processing of APP via β -secretase and not by the α -secretase resulting in $A\beta$ formation is an important aspect of AD. Recent studies suggest iron overloading as one of the major contributors of APP processing by β -secretase, proposing it as a major cause (Gleason and Bush, 2021). Insights from genomic studies in Iceland revealed mutations on amyloid precursor protein restricting $A\beta$ formation protect people from AD (Jonsson et al., 2012). It has been reported that people having the Icelandic variant are at least five times less likely to develop AD as compared to the people without it. Considering this evidence, $A\beta$'s cruciality in AD progression has been undisputed. The $A\beta$ protein comprises two major isoforms: 40 amino acids ($A\beta_{40}$) and 42 amino acids ($A\beta_{42}$) (Nair et al., 2014). While $A\beta_{40}$ is soluble and readily cleared from cells, $A\beta_{42}$ is toxic and aggregate-prone that can cause proteotoxic stress. In young individuals, the amyloid aggregates are efficiently cleared from neurons. But with aging the ability of the cells to clear these aggregates is reduced and soon develops to become part of the amyloid plaques (Dhakal et al., 2019). Although $A\beta_{42}$ aggregates to form fibrils and plaques in affected brains, only the soluble oligomeric forms of $A\beta_{42}$ are reported to cause oxidative damage and mitochondrial dysfunction aiding in the cascade of pathological events ultimately progressing towards AD pathology. Although it is still unclear what causes the initial $A\beta$ accumulation and impairment in cellular defense systems, the combined effect of aging, mitochondrial dysfunction, impairment of mitochondrial turnover, iron overloading, loss of cellular ability to clear aggregated proteins, and accumulation of oxidative damage are some of the most important aspects of AD pathogenesis (Dhakal and Macreadie, 2020).

Anti-AD compounds: Despite considerable research, there are essentially no reliable treatments specifically prescribed for AD. Previously, the FDA has approved two classes of drugs: one that targets the NMDAR receptor and the second type that inhibits the acetylcholine esterase, both of which are considered as palliative care rather than treatment (Dhakal et al., 2019). Recently, the FDA has approved a monoclonal antibody, Aducanumab, that acts against amyloid beta (Liu and Howard, 2021). Again, the drug cannot rescue the irreversible neuronal damage that has happened prior to the treatment. It can be very late by the time the disease is diagnosed and treated, since current diagnostic methods depend on the detection of amyloid plaques and neurofibrillary tau tangles. Recent discoveries of blood biomarkers could possibly be beneficial in determining an effective treatment or preventative strategy, but as of now the strategy to cure and/or prevent AD remains unresolved (Olsson et al., 2016).

Several investigations have been conducted to find therapeutic agents against AD. The cholesterol-lowering drug simvastatin has been shown to lower the incidence of AD (and Parkinson's disease) by 50%, giving hope that effective chemo preventatives can be found (Wolozin et al., 2007). Studies on simvastatin provide the clue that its actions deal with at least one aspect of AD, the reduced proteostasis associated with aging. Proteostasis refers to the balance of proteins that is needed for a cell to function properly. The current problem with simvastatin is that it is currently only prescribed for hypercholesterolemia. It is desirable to find other chemo preventatives that are more acceptable to the entire population, such as compounds like polyphenols that are naturally occurring and safe for regular consumption. Polyphenols are reported to have multifactorial benefits against AD with several anti-AD properties (Dhakal et al., 2019). Some of the beneficial anti-AD properties of polyphenols include their senolytic, antioxidant, anti-amyloidogenic, anti-inflammatory, and antiproliferative properties. It has been reported that polyphenols rescue cells with defective mitochondria, enhance lifespan, restore lipid/protein homeostasis, and ameliorate cognition as well as synapses (**Figure 1**). These properties of polyphenols make them excellent therapeutic agents against AD. We previously compiled a comprehensive

analysis of 18 polyphenols that provide benefits for neuronal health (Dhakal et al., 2019). The details of 362 studies and the doses used provide an indication of the huge efforts in studying such compounds and the reality of their use in humans. For example, for resveratrol to be used as an AD chemo-preventative, daily intake would need to be around 290 mg/d for a 60 kg adult (Dhakal et al., 2019). This high level is unlikely to be sustainable and is not practical. Moreover, not all polyphenols have a wide range of anti-AD effects, and some may still have very few specific benefits. Hence, it is important to find the compounds that have high efficacy and broad anti-AD effects or to combine them to achieve multifactorial activity against AD. In addition, these compounds, when ingested, are unlikely to reach an effective dose in the brain due to several layers of metabolic hurdles in the human body. Hence, it is crucial to devise strategies to increase the brain bioavailability of these compounds possibly via a different route of administration or in a protected carrier. The requirement to enhance the efficacy and anti-AD target range of polyphenol-based therapies include finding synergistic combinations of these compounds.

Combination studies and yeast models: Studies with chemotherapy have demonstrated the phenomenon of the synergistic actions of compounds. For example, some of the oldest therapeutics, the antifolate drugs comprising sulfa drugs that inhibit dihydropteroate synthase (involved in folate synthesis), and inhibitors of dihydrofolate reductase (involved in folate utilization) can work together in powerful synergy to inhibit bacterial, fungal, and parasitic infections (Ding et al., 2013). Such depiction of compound synergy instigates several questions in AD research. Can bioactive compounds be used together to achieve a synergistic outcome in AD treatment? Which bioactive compounds can be combined, and which should be avoided? Can such compound combinations be able to target multiple factors involved in AD pathogenesis? The answers to these questions are yet to be discovered. One of the most important reasons behind such a lag in AD research is the inaccessibility of living human brains for such studies. To address issues of AD, numerous disease models have been developed from yeasts to humans (Dhakal and Macreadie, 2020). Conservation of the fundamental eukaryotic processes in yeast makes it a fascinating model for AD research. Today, there is no disease model, apart from yeast, that can survive with defective mitochondria allowing us to embark on ideas that involve investigations on mitochondrial health. Most importantly, the enormous understanding of yeast genetics, facile modification of the yeast genome, availability of gene deletion libraries, and numerous platforms to study

yeast cells to a single cell level provides an unprecedented power in AD research (Khurana and Lindquist, 2010).

Considering these positive aspects of yeasts, a microbial model was developed for assessing compounds that aid in reducing levels of amyloid beta. It involved engineering yeast to produce $A\beta_{42}$ fused to green fluorescent protein (GFP). The model has allowed the observation that young yeast cells degrade $A\beta_{42}$ as well as $A\beta_{42}$ fused to GFP, while the older yeast exhibit reduced proteostasis, so the protein accumulated (Nair et al., 2014; Dhakal et al., 2021). The older yeast comprise about 25% of the population and are readily observed by their green fluorescence, so this assay was established for compounds that affect proteostasis (or aging). In the assay, simvastatin as well as many polyphenols reduced levels of $A\beta_{42}$ fused to GFP as judged by rapid assays of the population by flow cytometry (Dhakal and Macreadie, 2020; Dhakal et al., 2021). They also reduce levels of $A\beta_{42}$ as evaluated by mass spectrometry. Because of the relative speed and simplicity of the assay, it is also possible to assay for synergistic effects.

Previously, the model was used to evaluate the ability of some phenolic compounds to reduce GFP- $A\beta$. Following the study, a similar approach was used to screen a different set of compounds with some common compounds. In the latter study, baicalein and *trans*-chalcone reduced GFP- $A\beta$ levels dramatically and the responses were dose-dependent (Dhakal et al., 2021). But as the concentration of compounds that significantly reduced $A\beta_{42}$ was too high for human usage, the compounds were tested for any underlying mutual relationship, with a hope to find a synergistic combination. The combination of baicalein and *trans*-chalcone was found to have a synergistic ability to reduce GFP- $A\beta$ expressed in yeast hinting towards its potential to restore the proteostasis. This study depicted how a combination of lower concentration of baicalein and *trans*-chalcone was more effective in combination justifying the need to a search for such combinations (Figure 2). The combination was also found to act against the native $A\beta_{42}$ expressed in yeast. This study has set an example for how yeast can rapidly screen compounds that can act synergistically against $A\beta_{42}$ and improve reduced proteostasis that occurs with aging. Certainly, these compounds are to be tested further in humans to validate the observed benefits of these compounds can be translated in humans. In addition to the abovementioned benefits, the microbial models have also been used in studies involving a search for compounds that may increase $A\beta_{42}$ toxicity.

Despite the positive aspects of yeast as a model for studying AD, the unicellular model

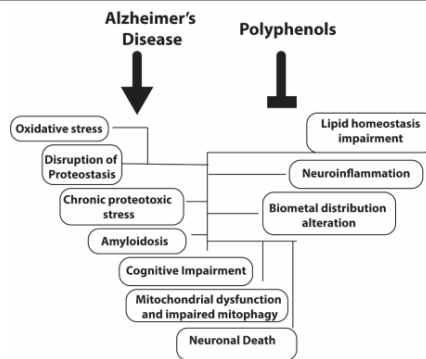


Figure 1 | Some important anti-Alzheimer's disease (AD) beneficial properties of different polyphenols showing their multifactorial benefits.

The forward arrow coming from AD to different features represents that the features are common to AD and the inverted T-arrow originating from polyphenols shows they have a multifactorial effect against AD.

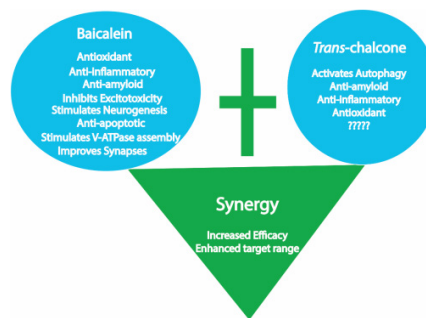


Figure 2 | Anti-Alzheimer's disease (AD) effects of baicalein and *trans*-chalcone depicting benefits of their synergistic combination.

The light blue circles represent features of baicalein and *trans*-chalcone against AD. The inverted green triangle depicts the benefits of synergy between the two compounds.

is limited to intracellular events that are evolutionarily conserved. But yeast studies may not be sufficient to understand the role of cell to cell interactions and different physiological systems including the nervous, immune, circulatory, and endocrine systems (Khurana and Lindquist, 2010). However, facile manipulation of the yeast genome and modifiable culture conditions may provide ways to mimic the environment required for the study of these physiological systems.

In summary, our focus here has been on studying the effects of $A\beta_{42}$ and reducing its levels in the elderly. We have demonstrated that yeast, because of its similarities to humans, can help in these studies in a way that is rapid, inexpensive, ethical, and relevant.

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