2

Review of Literature

Neuroprotection refers to the mechanisms and strategies employed to defend the central nervous system against injury due to both acute (e.g. trauma or stroke) and chronic neurodegenerative disorders like dementia, Parkinson's disease, Alzheimer's disease, epilepsy and so on (Ahmad Bhat *et al.,* 2017). Herbal medicine and nutraceuticals represent an important and valuable source in prevention rather than treatment of neurological disorders (Dadhania *et al.,* 2016). In various experimental models of neurological diseases, phytoconstituents were reportedly shown to have modulatory effects on the nervous system (Kumar and Khanum, 2012).

Based on the scientific data validation, it was observed that there is a lack of sufficient scientific data on treatment of neurological diseases. Hence the present study '**Neuroprotective effect of synthesized zinc oxide nanoparticle-capped catechin'**. Hence the present study on was designed to evaluate the neuroprotective activity of synthesized zinc oxide nanoparticle-capped catechin.

The Review of Literature pertaining to the topic of work '**Neuroprotective effect of synthesized zinc oxide nanoparticle-capped catechin'** was collected and discussed under the following headings:

2.1. *Camellia sinensis* (Green tea)

- 2.1.1. Cultivation and processing of *Camellia sinensis* (Green tea)
- 2.1.2. Different types of tea
  - 2.1.2.1. Oolong tea
  - 2.1.2.2. White tea
  - 2.1.2.3. Black tea
  - 2.1.2.4. Green tea
- 2.1.3. Significance of green tea

2.2. Neurological disorders

- 2.2.1. Types of neurological disorders

2.1. *Camellia sinensis* (Green tea)

The tea plant, *Camellia sinensis* (L.) Kuntze, belongs to the Theaceae family and is grown in over 30 countries across the world (Wang *et al.*, 2022; Liao *et al.*, 2022). It flourishes in tropical and subtropical climates with sufficient rainfall, well-drained soil and mild acidity (Rahman *et al.,* 2019). Around the world, tea is drunk as either black or green tea. But of all of them, drinking green tea has been shown to have the greatest beneficial impact on human health (Pervin *et al.,* 2018). China, Japan and Taiwan are major producers of *Camellia sinensis* var. *sinensis* (China tea), although south and southeast Asia, particularly Malaysia and more recently, Australia are the main producers of *Camellia sinensis* var. *assamica* (Assam tea). Tea is the most highly consumed manufactured drink in the world (Filippini *et al.,* 2020). Tea leaves are steamed at a high temperature after harvesting to inactivate the polyphenol oxidizing enzymes which protect the majority of vitamins present

in the tea. Thus, green tea possesses high levels of antioxidants and is used for its anti-ageing and neuroprotective effects, alongside treating or preventing several diseases such as cancer, cardiovascular conditions, obesity and so forth (Prasanth *et al.*, 2019). Green tea contains a number of chemical compounds, including green tea catechins, caffeine and theanine, which may affect brain function. In a comprehensive review article, (Chen *et al.*, 2019) discussed the neuroprotective effects and mechanism of action of tea components, including tea catechins, theanine, caffeine and theaflavins. They suggested that these bioactive tea components might be useful for neuronal degeneration treatment in the future.

2.1.1. Cultivation and processing of *Camellia sinensis* (Green tea)

Highland areas are common places to grow tea. It is grown at elevations of upto 2000 metres above sea level in Sri Lanka and India. Tea is grown in plantations at a density of 5000–10,000 plants per hectare and it is regularly pruned during harvest to keep the plants as low shrubs that grow to a height of 1.0–1.5 metres. The best tea is produced by hand-picking the terminal bud and the two youngest leaves however, machine harvesting is economically necessary in some places due to high labour costs (Seyis *et al.*, 2019).

Tea is made mainly by two methods: Orthodox method - Crush, Tear and Curl (CTC) tea which employs a proper maceration device and the Traditional method which entails rolling by hand or with a roller. Although orthodox teas are whole leaf teas, the crush, tear and curl process produces granular leaf particles by chopping the tea leaves into small, homogeneous pieces. Rupturing the tea leaf cells to reveal the cell sap is the primary goal of both rolling and maceration. Enzymes and chemical components react chemically in the presence of atmospheric oxygen during the process. This stage determines if the tea is crush, tear and curl or orthodox. During the biochemical, enzymatic process of fermentation, oxygen is absorbed when the enzymes in the leaves come into contact with the oxygen in the air. As soon as the rolling or maceration process commences, fermentation takes place. The quality of both conventional and crush, tear and curl black teas is largely determined by the fermentation technique used in tea processing. Thus, in order to create tea of the finest quality, it is essential to comprehend and keep an eye on the fermenting process (Pou *et al.*, 2019).

In order to prepare green tea, the leaves are heated, which inactivates the peroxidase and polyphenol oxidative enzymes that are often found in tea leaves (Balentine *et al*., 2019).

After that, the tea leaves are rolled or shaped, which covers the leaf surface and releases fluids from the plant cells. The leaves are then dried to lower the moisture content and produce a dry tea product (Wong *et al.*, 2022). For Japanese-style green tea, namely, Sencha (type of Japanese green tea that is made from tea leaves that are steamed, rolled and dried) the heating stage is often accomplished by steaming at around 100°C, while for Chinese-style green tea, it is typically accomplished by roasting or pan-firing at about 300–350°C (Hilal, 2017). The rolling stage of green teas is another area where Chinese and Japanese methods vary. Japanese green teas are rolled bidirectionally, while Chinese green teas are usually rolled unidirectionally (Wong *et al.*, 2022).

2.1.2. Different types of tea

Tea can be categorised as black, green, white, or oolong tea depending on the level of fermentation. Black tea is fully fermented or oxidised, whereas, green and white teas are unfermented or unoxidized and oolong tea is semi-fermented. Shorter fermenting time is the only difference between the processes used for making black and oolong tea (Chen *et al.*, 2010).

The production journey for green, oolong and black tea is as follows (Figure 1)

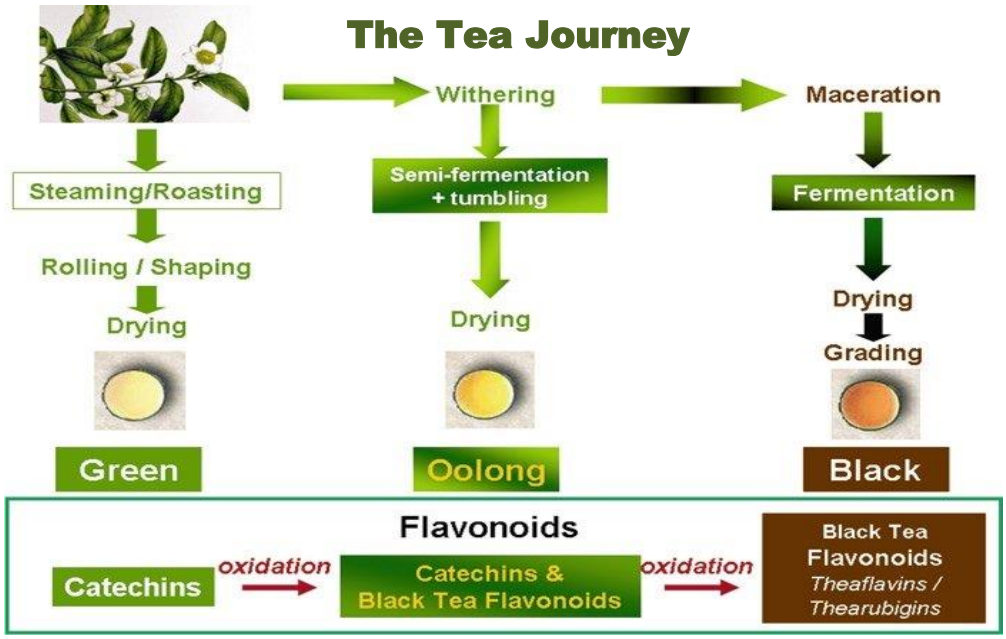

Figure1. The tea journey: a diagram summarizing the various stages of processing tea leaves, the types of tea produced and the impact on its key components, the flavonoids (Gibson and Rycroft, 2011)

2.1.2.1. Oolong tea

Dried oolong tea leaves can have a variety of colours, including bright and dark green, with some even seeming brown. A blend of flavones, theaflavins, thearubigins and catechins produce Oolong tea infusion that is often golden in colour. Furthermore, the flavour of Oolong tea infusion is related to a combination of various compounds, such as catechins (bitterness), amino acids (freshness), soluble sugar (sweetness), theaflavins (briskness) and thearubigin (mellowness) (Chen *et al.*,2010; Wang *et al.*,2010). In addition, the infusion tastes ripe, fruity, sweet and smooth and has a distinct floral scent (Wang *et al.,* 2010; Xu *et al.,* 2018). According to Sheibani *et al.,* (2016), the volatile component concentration of oolong tea determines its scent characteristics, while a mix of sugars, theanine, other amino acids, flavonoids and catechins determines its flavour and taste. Processing oolong tea usually includes rolling, fire, fermenting, panning and sunbathing or withering. Tea leaves are allowed to dry out in the sun by lowering their moisture content, which makes it easier for catechins to transfer from cell vacuoles to the cytoplasm of the leaf (Lin *et al.,* 2016; Kanwar, 2023). According to (Ho *et al*., 2018; Ng *et al*., 2018) the level of fermentation might vary from 10% to 80% (Chen *et al.,* 2010).

2.1.2.2. White tea

The white hairs that cover the buds when they are picked is the main reason for the tea's name (Hilal, 2017). Like certain green teas, white tea infusion has a light yellow colour. It smells fresh and green and tastes somewhat sweet and umami. Measurements of 29 metabolomics showed there were distinct differences among the different types of white teas such as silver needle white tea, white peony white tea, ShouMei white tea (Shoumei is a white tea from Fujian province, China that is also referred to as ‘Long noble life eyebrow’) and compressed brick white tea (Tan *et al.,* 2017). Low quantities of catechins and other polyphenols which minimise the intensity of astringency and bitterness and high levels of peptides, amino acids and soluble sugar, which offer a moderate umami and sweet flavour are the causes of these taste qualities (Chen *et al.,* 2020). White tea is made by comparatively little processing of tea leaves. For the purpose of making white tea, the buds or the initial leaves are plucked. In general, the selected buds and leaves are dried right away without being heated in order to make tea (Tan *et al.,* 2017). The withering process is the primary source for the change in chemicals in white tea. Cell membranes gradually break

throughout the withering process, enabling catechin to interact with peroxidase and polyphenol oxidase (Chen *et al.* 2020).

2.1.2.3. Black tea

Green fresh tea leaves turn into deep dark dried tea leaves during processing (Hilal, 2017). A deep reddish-brown infusion develops when dried black tea leaves are brewed (Ho *et al.*, 2018). The theaflavins impart color, brightness and astringency to black tea liquors. Thearubigins are red in colour and are responsible for much of the staining effect of tea (Das *et al*., 2020). The golden colour and sharp, astringent flavour are attributed to theaflavins, while the brown-red colour and rich mouthfeel belong to thearubigins (Farrell *et al.,* 2023). Withering tea leaves at room temperature reduces the moisture content and is the standard method of processing black tea (Lee *et al.,* 2019). Tea leaves become flexible as they wither, allowing for easy twisting and rolling without breaking (Deb and JolvisPou, 2016). The plant cell structures are then broken by rolling or macerating the tea leaves, which makes it easier for the next step fermentation where physical interaction between the catechins, polyphenol oxidase and peroxidase is made possible (Aaqil *et al.*, 2023). Following that, there is fermentation during which the majority of the catechins in tea leaves oxidise to become theaflavins and thearubigins (Koch *et al.,* 2018). Drying, which produces the final dried black tea product by inhibiting enzyme activity with heat ends the fermentation process (Wong *et al.*, 2022).

2.1.2.4. Green tea

Green tea leaf infusions are bitter and astringent with a distinctive pale yellow or green colour (Ye *et al.,* 2022). It has also been seen that some taste properties, such as umami and sweetness after tasting are associated with the amino acid L-theanine (Ye *et al.*, 2018; Zhang *et al.*, 2020). Green tea has a high amount of L-theanine since there is no fermentation throughout the manufacturing process (Boros *et al.,* 2016). One of the key characteristics thought to add to green tea's sensory uniqueness is its astringency (Zhang *et al.,* 2020). Green tea is processed to retain the catechins in the fresh leaves because astringency is determined by the content of catechins, especially gallated catechins. Low catechin concentration can cause the flavour of green tea to be lost (Ye *et al.*, 2018).

2.1.3. Significance of green tea

Camellia sinensis or green tea, is one of the most popular drinks worldwide. Because of its advantageous biological benefits, green tea has been examined in great detail (Zhang *et al.,* 2019; Melo *et al.,* 2021). Green tea has been shown to have anticarcinogenic, anti-inflammatory, antimicrobial, neuroprotective and antioxidant properties and is beneficial in cardiovascular diseases, diabetes, obesity, neurological and oral health oxidative stress resulting from the damaging effects of reactive oxygen species (Reygaert, 2018; Saeed *et al.,* 2017). The antioxidant properties of green tea include the ability to limit the amount of free radicals by binding to reactive oxygen species, up regulating basal levels of antioxidant enzymes and increasing the activity of these antioxidant enzymes (Dehghan *et al.,* 2016; Doganoglu and Erbas, 2021). The components of green tea that are the most medically relevant are the polyphenols. The most pertinent polyphenols are the flavonoids and the most pertinent flavonoids are the catechins. The catechins comprise 80-90 % of the flavonoids and around 40 % of the water-soluble solids in green tea (Azad *et al.,* 2016). Green tea contains more catechins than the other teas, mainly because of the way it is processed after harvesting (Martins Gregorio *et al.,* 2016). The four main catechins found in green tea are (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin-3-gallate and (-)-epigallocatechin-3-gallate. The most abundant catechin is (-)-epigallocatechin-3-gallate (~60%) and the next most abundant is (-)-epigallocatechin (~20%), then (-)-epicatechin-3-gallate (~14%) and (-)-epicatechin (~6%). (-)-epigallocatechin-3-gallate is the most studied in association with health, but (-)-epigallocatechin and (-)-epicatechin-3-gallate have been studied as well. As mentioned above, there can be a wide variation in the amount of catechins in any particular green tea beverage, although standardized extracts are available for use as supplements (Jigisha *et al.,* 2012; Atomssa and Cholap, 2015).

2.2. Neurological disorders

Neuroscience is the scientific study of the structure and cognitive functions of the brain in processing data, making decisions and interacting with the environment. It combines different disciplines such as physiology, anatomy, molecular biology, cytology, psychology, physics, computer science, chemistry, medicine, statistics, mathematical modelling and so on. Neuroscientists not only focus on the study of the brain for cognitive functioning but also investigate the whole nervous system to get a comprehensive understanding of different

neurological, psychiatric and neuro developmental disorders (Surianarayanan *et al.,* 2023). New therapeutic approaches need to manage these neurodegenerative diseases because the current therapies are limited in many areas. The blood brain barrier, which is the main obstacle when treating diseases of the central nervous system is composed of endothelial cells forming tight junctions and separates blood from the extracellular fluid of the brain. Furthermore, the permeability of the blood brain barrier is selective for only certain substances such as nutrients and water. Thus, the barrier prevents the passage of certain drugs and therapeutic agents required for the treatment of disorders related to the central nervous system (Poovaiah *et al.,* 2018).

Neurological disorders have a considerable impact on worldwide health. Conditions such as Alzheimer's disease, Dementias, Parkinson's disease, Multiple sclerosis, Epilepsy and Headache disorders are all part of the 'Global burden of disease' research. Recent reports reveal that these illnesses account for 3 % of the total global burden of disease. These statistics demonstrate the significant contributions of neurological conditions to the increasing global burden. Neurological disorders develop when neurons in the spinal cord and brain begin to age. The main pathological change in most brain-affecting diseases is neurodegeneration. These cells undergo changes leading to abnormal function and cell death over time. Initially, patients may experience mild symptoms like balance or memory issues as their brain neurons deteriorate, which worsen with further neuron degeneration (Vellingiri, 2023).

In the United States, as many as 6.2 million people may have common neurodegenerative diseases, according to a report from the Alzheimer's Disease Association in 2024. Mental and neurological illnesses together encompass disorders of the brain. According to the latest estimates, disorders of brain account for 13 % of global disease exceeding cancer and cardiovascular diseases (Lima *et al.,* 2022). More than 6 million people die due to stroke, more than 50 million people have epilepsy while 47.5 million people suffer from dementia and 7.7 million cases are reported every year. Meanwhile worldwide prevalence of migraine accounts for more than 10% of adult population in westren countries (Bavarsad *et al.,* 2023). The United Kingdom population aged 65 years and beyond is expected to rise from 18.5 % in 2019 to 23.9 % by 2039, similar to many other countries. With such a rise in the elderly population, the socio-economic burden of age-related health

conditions will rise, necessitating effective preventive or therapeutic interventions (Naser *et al.,* 2022). The most prevalent are anxiety, headache, sleep disorders and somatoform disorders (Somatoform disorders are a set of psychological conditions where a person experiences bodily symptoms that cannot be accounted for by a medical or neurological diagnosis) and the most costly disorders are psychotic disorders, dementia, mood disorders, addiction and anxiety disorders (Fineberg *et al.,* 2013).

An oppressive concern for medical treatment, society and the economy is the rise in patients with age-associated neurodegenerative illnesses in the ageing society. It is unclear what causes the two most prevalent neurodegenerative diseases, Parkinson's disease and Alzheimer's disease. Nonetheless, many illnesses have been linked to common pathological features, such as the buildup of inclusion bodies unique to each disease and the loss of a unique neuronal population. A novel therapeutic approach is currently being put forth to address a number of pathogenic factors, such as inflammation, the buildup of modified proteins, oxidative stress, mitochondrial dysfunction, reduced energy homeostasis, transition metals (iron, copper), calcium and hormones (insulin, estrogen for women), as well as deficiencies in neurotrophic factors (Trigo *et al.,* 2023).

As a ‘disease-modifying’ treatment for depression, Parkinson's disease and Alzheimer's disease, neuroprotection aims to stop neuronal death, repair the neural network and improve brain dysfunction. Numerous substances have been found to be neuroprotective. These include anti-inflammatory agents, antioxidants (vitamins E, C, transient metal chelators), buioenergetic substances (coenzyme Q) and inhibitors of monoamine oxidase like selegiline and rasagiline (Selegiline and rasagiline are two selective monoamine oxidase B inhibitors used in the treatment of Parkinson’s disease). However, clinical studies have not been able to fully demonstrate the prevention of disease progression (Naoi *et al.,* 2019).

India is a large developing country with limited resources, yet caters to approximately 18 % of the total world population. One of the main causes of the burden of communicable and non-communicable diseases is neurological disorders, both deadly and non-fatal. Disability-adjusted life years are a crucial indicator of the worldwide prevalence of neurological illnesses and they are on the rise. In order to shed light on the prevalence, illness burden and risk factors connected to neurological disorders in the Indian population, a thorough and methodical data analysis is necessary. Such data will assist policy makers in allocating funds (Mehndiratta *et al.,* 2021).

Neurological disorders have a significant impact on global health. According to the most recent estimates, neurological disorders and other dementias account for 3 % of the global disease burden. Despite what appears to be a modest overall percentage, dementia, epilepsy, migraine and stroke are among the top 50 causes of disability-adjusted life years. Migraine and epilepsy account for one-third and one-fourth of this neurological burden, respectively, while dementia and Parkinson's disease are among the top 15 disorders with the greatest burden growth over the last decade. In 2010, neurological illnesses accounted for 5.5 percent of years of healthy life lost due to disability or 42.9 million years of healthy life lost due to disability Migraine, epilepsy and dementia were among the top 25 causes of years of healthy life lost due to disability. Migraine tops the list of neurological illnesses, accounting for more than half of the neurological years of healthy life lost due to disability or 2.9 percent of worldwide years of healthy life lost due to disability. Epilepsy accounts for 1.1 percent of global years of healthy life lost due to disability. The neurological illness burden in low and middle-income countries is anticipated to rise exponentially during the next decade. Despite the considerable effect of neurological illnesses on individuals and society, knowledge of their epidemiology, including variation in disease incidence across location and time, as well as comprehension of related risk factors and outcomes, remains poor, particularly in low and middle-income countries. Because of physical, cognitive and interpersonal impairments, patients with neurological illnesses frequently require extensive social and economic support. Despite the high incidence of disability, there is growing understanding that services and resources are disproportionately inadequate, particularly in low and middle-income countries (Das *et al.*, 2023).

With a high disability-adjusted life years, a measurement of the number of years of healthy life lost owing to illness. These diseases exhibit a significant impact on quality of life. Stroke is also the biggest cause of non-traumatic death in developed nations, accounting for the greatest number of neurodegenerative diseases-related deaths (Sharifi-Rad *et al.,* 2018). These are second only to headache disorders in terms of prevalence and as life expectancies rise and the population grows, so too will the incidence of neurodegenerative diseases also grow globally (Newman and Cragg, 2016). The main goal of the neuro developmental dealy treatments now in use is to provide momentary symptom alleviation. Consequently, there is a great need to find new treatments and neuroprotective substances to stop or slow the development of neurodegenerative diseases (Sharifi-Rad *et al.,* 2020).

Over the last thirty years, a majority of research conducted in India have indicated that some diseases, such as stroke, epilepsy, headaches, Parkinson's disease and dementia, have a high disease burden. Inconsistencies in health care access continue to exist according to socio-economic position, age, geography and gender, even with advancements in the urban Indian population (Nadig *et al.,* 2019). India's government policy is based on the notion of equity in health and health care, so the country has a lot of work to do in this area. The Indian government has implemented welfare initiatives, notably the Ayushmann Bharat-Pradhan Mantri Jan Arogya Yojna, to address a range of illnesses, including neurological conditions. In order for these programs to be implemented and yield significant results, sincere and focused efforts are required. These initiatives consist of hiring a skilled labour force and educating the public through awareness campaigns on radio, television and other social media channels. Apart from the existing inequalities, the COVID-19 pandemic has also hindered the progress towards attaining health and care equity. This is because the government of India has implemented a vaccine program and diverted funds for managing the problems of SARS-CoV-2 infection (Chirra *et al.,* 2019).

The process of neurodegeneration is connected to both brain aging and neuropathological disorders. Brain pathology, namely, neurodegenerative and cerebrovascular diseases, is recognized as a major global cause of death, accounting for 8% of all deaths and an incidence of roughly 2/1000. In the twenty-first century, cognitive dysfunction is a significant health issue. A number of neuropsychiatric and neurodegenerative disorders and others can have a severely debilitating effect on one's ability to function (Farooqui *et al.,* 2017). Furthermore, the absence of effective treatment alternatives makes stroke and dementia a major cause of misery for both the affected person and their family. The latter drives investigations into the causes of neuronal death and the development of novel drugs to regulate it (Kumar *et al.,* 2016; Velmurugan *et al.,* 2018).

2.2.1. Types of neurological disorders

The illnesses of the peripheral and central nervous systems are known as neurological disorders. Muscle weakness, paralysis, convulsions, discomfort, poor coordination and loss of consciousness are common symptoms. There are more than 600 illnesses that affect the neurological system, including brain tumors, Parkinson’s disease, Alzheimer’s disease, multiple sclerosis, epilepsy, dementia, headache disorders, neuro infections, stroke, or

traumatic brain injury. Neuropathological examinations of patients are widely used to identify aberrant or a typical neurological diseases. However, most people have abnormal neurological abnormalities that are not usually linked to a neurological illnesses (Raghavendra *et al.*, 2020; Lima *et al.*, 2022).

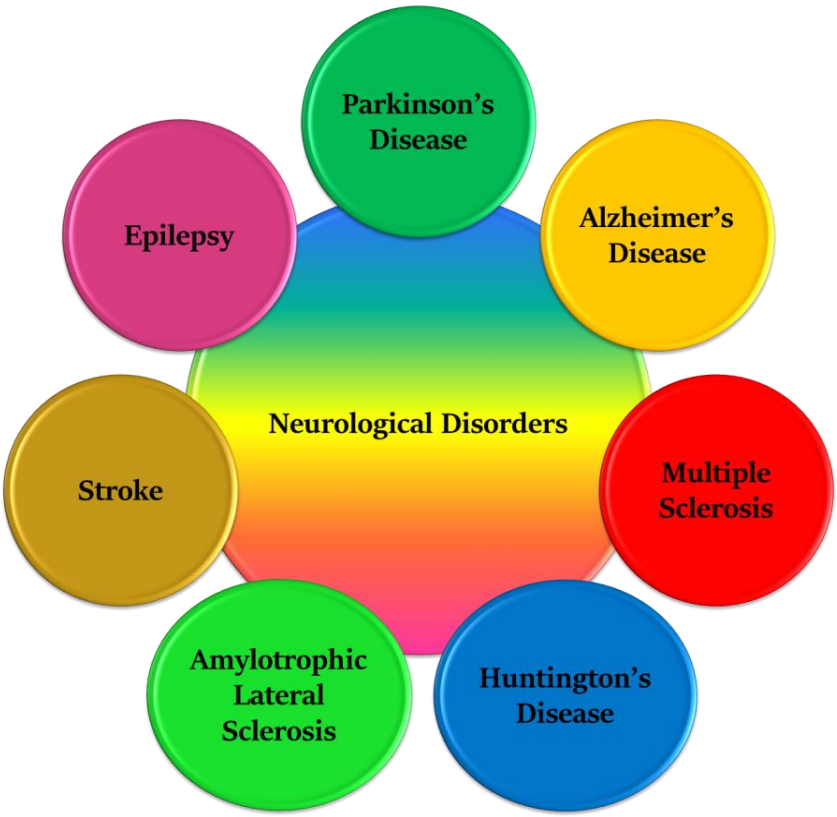

Figure 2. Classification of neurological disorders (Sindhu *et al.*, 2022).

2.2.2. Role of antioxidants in neurodegenerative diseases

Antioxidants are referred to as those substances which can reduce or prevent the cellular damage generally caused by free radicals (Grewal *et al.* 2021; Maurya *et al.*, 2021). Free radicals or oxidative stress may be a fundamental mechanism underlying several human neurological diseases. Therapy using free radical scavengers (antioxidants) has the potential to prevent, delay or ameliorate many neurological disorders. However, the biochemistry of oxidative pathobiology is complex and optimum antioxidant therapeutic options may vary and need to be tailored to individual diseases. *In vitro* and animal model studies support the potential beneficial role of various antioxidant compounds in neurological diseases. Antioxidants generally play an important role in reducing or preventing cell damage and other changes which occur in the cells like mitochondrial dysfunction, DNA mutations and

lipid peroxidation in the cell membrane (Sindhu *et al.*, 2022).The antioxidant defense system, including antioxidant enzymes and non-enzymatic antioxidant molecules, play a critical role in scavenging reactive oxygen species to maintain redox balance (Adwas *et al.*, 2019).

Oxidative stress-induced toxicity leads to the generation of reactive oxygen species, increased oxidative modification of proteins and lipids, macromolecular damage in mitochondria and also impairment of cellular calcium homeostasis which are mainly involved in structural and functional abnormalities in synapses including the activation of apoptotic cascade (Collin *et al.*, 2018).

Figure 3 illustrates the role of antioxidants in neurodegenerative diseases

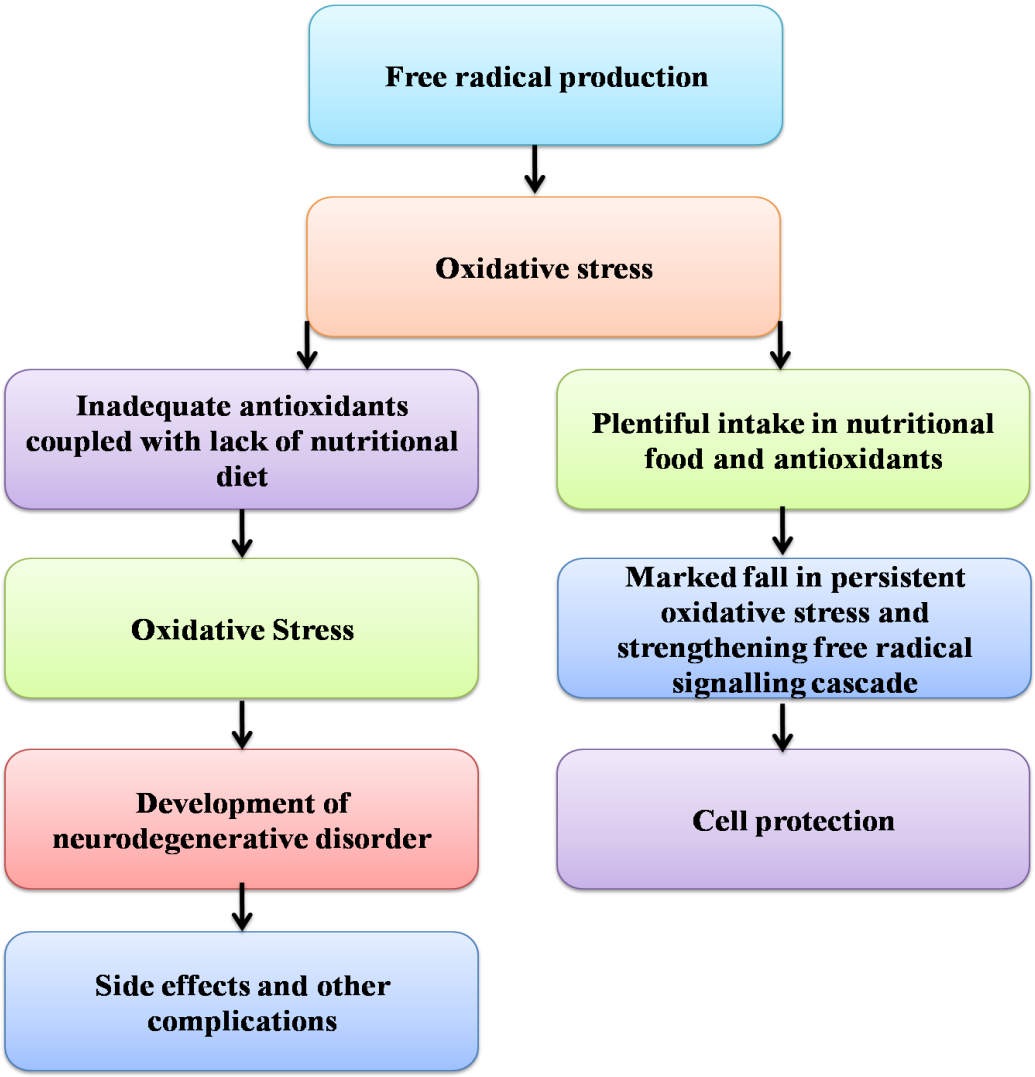

Figure 3. Role of antioxidants in neurodegenerative diseases (Adwas *et al.*, 2019; Sindhu *et al.*, 2022)

2.2.3. Impacts of neurological disorders

Neurodegenerative diseases are characterized by chronic progressive degeneration of the structure and functions of the nervous system which brings an enormous burden on patients, their families and society. It is difficult to make early diagnosis, resulting from the insidious onset and progressive development of the diseases. The drugs available in the market cannot cross the blood–brain barrier effectively, which leads to unfavourable prognosis and less effective treatments (Luo *et al.,* 2020). Challenges for the central nervous system drug delivery are serious threats to human health because the Blood Brain Barrier remains a major obstacle for treatment. Administered potential macromolecular drugs have proved unable to cross the capillary of brain endothelial cells and as a result the drug is prevented from reaching the central nervous system target. The Blood Brain Barrier consists of several kinds of cells (endothelial cells, astrocytes, pericytes and microglial cells) that restrict the exchange of substances between the brain tissues and blood. The tight junctions between the brain capillary endothelial cells are the chief components of the barrier that restrict the transportation of almost all drugs. Moreover, the presence of efflux transporter proteins such as multidrug resistant protein and breast cancer resistant protein or P glycoprotein is also greatly responsible for drug efflux out of the brain (Potschka and Luna-Munguia, 2014). A general transport pathway across the Blood Brain Barrier is illustrated in Figure 4.

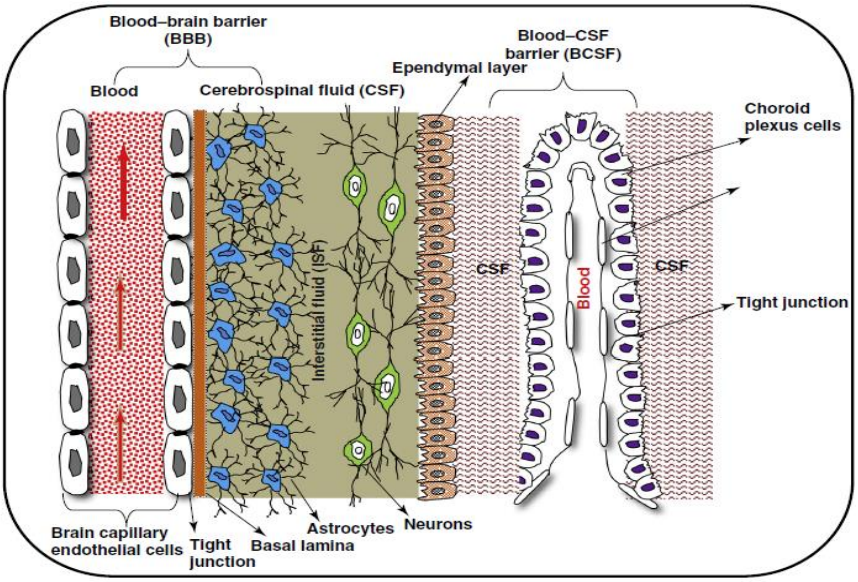

Figure 4. The model depicts two main barriers for drug delivery to the brain i.e. blood–brain barrier and blood–cerebrospinal-fluid barrier alongwith other cellular components of the brain (Shah *et al.*, 2013)

2.2.4. Treatments for neurological disorders

The cholinergic hypothesis of neurological disorders recommends that cholinergic systems in the basal forebrain are affected early in the disease process, resulting in memory loss and deterioration of other cognitive and non-cognitive functions such as neuropsychiatric symptoms. The conventional therapies for this (donepezil, rivastigmine, galantamine and memantine) are symptomatic and do not slow or stop the progression of the disease. However, these treatments show a modest but consistent benefit for cognition, global status and functional ability as shown in Table I (Herrmann *et al*., 2011).

Table I

Outline of approaching treatments for neurological disorders

(Lippincott *et al.*, 2010) and Food and Drug Administration

Drug name	Indication	Action	Dose	Adverse effects	Contraindications
Acetylcholinesterase inhibitors					
Brand Name: Aricept Donepezil (FDA-approved in 1996)	Mild to severe Alzheimer's disease	Prevents the breakdown of acetylcholine by inhibiting the action of acetylcholinesterase Treats cognitive symptoms of Alzheimer's disease	5 mg taken once daily Over time, may increase to 10 mg daily	CNS: headache, seizures, insomnia, fatigue, aggression CV: chest pain, hypertension, atrial fibrillation GI: nausea, vomiting, GI bleeding Metabolic: Weight loss, dehydration	Do not use in patients that are hypersensitive to the drug Use caution in patients with cardiovascular disease, asthma, COPD, ulcer disease or patients taking NSAID pain relievers
Brand Name: Razadyne Galantamine (FDA-approved in 2001)	Mild to moderate Alzheimer's disease	Prevents the breakdown of acetylcholine and stimulates receptors to release excess acetylcholine Treats cognitive symptoms of Alzheimer's disease	4 mg taken twice daily Over time, may increase to a maximum of 24 mg daily	CNS: depression, dizziness, fatigue, insomnia CV: bradycardia, AV block GI: diarrhea, nausea, anorexia, abdominal pain Hematologic:	Do not use in patients that are hypersensitive to the drug Use caution in patients that have cardiac conduction disorders, before procedures requiring anesthesia and in patients with ulcer

Drug name	Indication	Action	Dose	Adverse effects	Contraindications
				anemia	disease, seizures or asthma
Brand Name: Exelon Rivastigmine (FDA-approved in 2000)	Mild to moderate Alzheimer's disease Also used to treat dementia from Parkinson's Disease	Prevents the breakdown of acetylcholine by inhibiting the enzymes that degrade ACh Treats cognitive symptoms of Alzheimer's disease	1.5 mg taken twice daily Over time, may increase to a maximum of 12 mg daily	CNS: headache, dizziness, confusion, nervousness, paranoia, malaise CV: hypertension, chest pain, edema Musculoskeletal: back pain, bone fractures Respiratory: bronchitis, cough	Do not use in patients that are hypersensitive to the drug Use caution in patients with GI bleeding, cardiovascular disease, COPD or seizure disorders
NMDA receptor antagonist					
Brand Name: Namenda Memantine (FDA-approved in 2003)	Moderate to severe Alzheimer's disease	Blocks glutamatergic (NMDA) receptors and regulates the action of glutamate Treats cognitive symptoms of Alzheimer's disease	5 mg taken once daily Over time, may increase to a maximum of 10 mg daily	CNS: stroke, aggressiveness, agitation, fatigue, confusion, pain, syncope CV: heart failure, edema GI: anorexia, constipation, nausea, vomiting Skin: Rash	Do not use in patients that are allergic to the drug or its components Not recommended for mild Alzheimer's disease or in patients with renal impairment Use caution in patients with seizures or increased urine pH

CNS: central nervous system;

GI: Gastrointestinal;

CV: Cardiovascular;

AV: Atrioventricular;

COPD: Chronic obstructive pulmonary disease and

NSAID: Non-steroidal anti-inflammatory drugs

2.3. Neuroprotection

One could argue that the human brain is the body's most intricate organ. It is composed of neurons and neuroglia, the former of which are in charge of transmitting and receiving messages or nerve impulses. Astrocytes and microglia are necessary for maintaining the healthy operation of neurons. When neurons are harmed or under stress, they act quickly to restore functions. Pathological impairment of microglia or astrocytes could have disastrous effects on brain function, as they are sentinels of neuron well-being. It is thought that neural signals play a major role in regulating neuroglial activation. Neurons that have sustained acute damage send messages to neuroglia informing them of their condition. Neuroglia will either support injured neurons through regeneration or eliminate them if they are not viable, depending on the extent of the damage to the neurons. It is believed that these kinds of neuroglial responses are typical physiological and neuroprotective reactions. However, some chronic events cause neuroglia to become persistently activated, which ultimately results in a breakdown of their physiological ability to maintain homeostasis. This might have negative effects and cause neuroglial dysfunction, which could harm bystanders (Kumar *et al.,* 2012).

The techniques and corresponding mechanisms that protect the central nervous system from neuronal harm resulting from both acute and chronic neurodegenerative illnesses are referred to as neuroprotection (Velmmurugan *et al.*, 2018).

In addition, there has been a surge in interest in herbal remedies over the past 10 years due to the potential therapeutic or health-promoting effects of their phytochemical ingredients. On the other hand, a large number of medicinal plants have specific therapeutic effects without contributing nutritional value to the human diet and can be used either temporarily or permanently to treat a variety of health issues. Vegetables and fruits include phytochemicals that may lower the risk of a number of serious illnesses, including cancer, heart disease and neurological conditions. As a result, those who eat more fruits and vegetables may be less likely to develop certain illnesses brought on by malfunctioning neurons (Khan *et al.,* 2020; Guan *et al.,* 2021; Banerjee *et al.,* 2021)

Neural problems have traditionally been treated with herbal medication. Herbal medications exact mode of action are still unknown, although several of them

have been demonstrated to have antioxidant and/or anti-inflammatory effects in a range of peripheral systems. The central nervous system is now thought to be pathologically affected by neuroglia-derived chronic immune responses. Consequently, anti-inflammatory herbal therapy and its components are being shown to be effective neuroprotectors against a range of brain disorders. Because of their structural variety, medicinal herbs are a valuable source of new lead compounds to target therapeutic targets that have recently been identified by high-throughput screening, proteomics and genomics (Isgut *et al.,* 2018).

2.3.1. Neuroprotective herbs

Sale of herbal preparations have increased as a result of the 'Green' movement in Western civilization, which has altered public perceptions to the idea that naturally derived materials and extracts are intrinsically safer and more appealing than items made with synthetic chemicals (Winston, 2019; Prescott, 2019). Primitive healthcare for humans and animals is provided via phytomedicine to about 80 % of the population in poor nations (Houghton, 2021). Many herbs have been used in traditional medicine to treat cognitive disorders, including memory-related disorders and neurodegenerative diseases. Complicated combinations of organic compounds, such as fatty acids, sterols, alkaloids, flavonoids, glycosides, saponins, tannins, terpenes and so on, can be found in herbal products. In contrast to the individual chemicals of conventional medicines that are isolated by pharmacologists, proponents of herbal medicines claim that a plant's therapeutic value arises from the synergistic effects of its various components. As a result, traditional medicines are thought to be effective and to have few or no side effects. Approximately 25 % to 50 % of medications in the market today were first derived from plants (Pan *et al.,* 2014; Guan *et al.,* 2021).

2.3.1.1. Catechins

Catechins have many benefits including preventing or reducing skin damage. Catechins are important ingredients from tea leaves and have intensive antioxidant and representative physiological activities. They are members of the group of polyphenol compounds found in many medicinal plants. The major sources of catechins are *Camellia sinensis* and *Camellia assumica*. Green tea contains 75–80 % water and polyphenol compounds (flavanols, flavandiols, flavonoid and phenolic acid) and catechins account for more than 75 % of the polyphenol compounds in tea leaves (Bae *et al.,* 2020).

Figure 5 shows the structure of catechin.

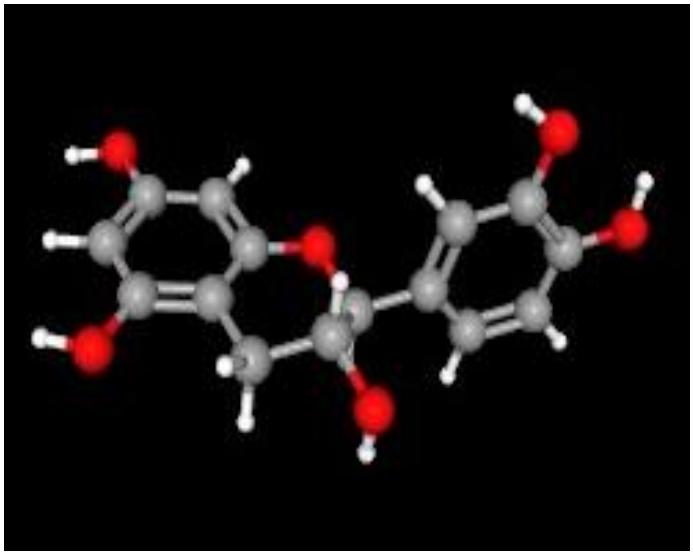

Figure 5. Structure of catechin

National Center for Biotechnology Information (2024).

2.3.2. Secondary metabolites for neuroprotection

Neurodegenerative diseases affect millions of people globally, with Alzheimer's disease being the most common. Researchers have focused on neurodegenerative diseases because of their social and economic impacts, as they are more common in countries with high average life expectancies (Abdulwanis Mohamed *et al.,* 2019). Neurodegenerative diseases are characterized by a progressive loss of nerve cell function and structure. While the exact cause of some of these diseases is still not clear, certain common factors contribute them, such as neuro-inflammation, β-amyloid, aggregation, neurofibrillary tangle formation, oxidative stress and impaired mitochondrial function. However, aging is thought to be the greatest risk factor for neurodegenerative diseases (Khan *et al.,* 2020). Given that many neurological problems are chronic and incurable, the most effective way to prevent and treat these disorders is through the isolation of bioactive components from medicinal plants. Because of their biological qualities, which include antioxidants, anti-inflammatory and neuroprotective effects, as well as their chemical properties, which include direct uptake of free radicals and modulation of enzymes linked to oxidative stress, phytochemicals have emerged as intriguing therapeutic candidates (Abdulwanis Mohamed *et al.,* 2019; Perez-Hernández *et al.,* 2016).

Plant metabolites that have been extracted and isolated have demonstrated neuroprotective activity. For example, polyphenols from yerba mate (*Ilex paraguariensis*) and green tea (*Camellia sinensis*) have been reported to have an antioxidant effect or to stimulate the expression of genes related to cell survival, respectively and to enhance memory in dementia patients (Abdulwanis Mohamed *et al.,* 2019). Alkaloids from *Corydalis ternate* (turkey corn) display anti-cholinesterase and anti-amnesic activity whereas, coumarins from the fruit of *Psoralea corylifolia* (Purple fleabane) have been demonstrated to alleviate scopolamine-induced amnesia in rats. The neuroprotective potential of secondary metabolites isolated from the *Melicopelunu-ankenda* (Chabang Tiga is a tree native to Singapore) plant, has not been researched much. To better understand the chemical diversity of bioactive substances in *Melicopelunu-ankenda* and their biological actions, more research is needed. Infections that affect the central and peripheral nerve systems are known as neurological disorders. Numerous conditions, including nervous system damage, ischemia, oxidative and endoplasmic reticulum cellular stress, inflammation, aberrant protein deposition in neural tissue, autoimmune-mediated neuronal loss and viral or prion infections, can result in neurodegenerative diseases. Deficits in motor function, behavioral abnormalities and cognitive decline may result from neuronal death, gliosis, or demyelination, depending on the affected region (Dugger *et al.,* 2017)

Transcranial magnetic stimulation has gained popularity recently as a non-invasive imaging method for assessing cortical function in stroke and non-disordered driving patients in order to better understand the neurological alterations caused and to implement individualized treatment (Patti *et al.,* 2019). Although its application are still in its early stages, transcranial magnetic stimulation has also demonstrated efficacy in aiding clinical recovery following stroke and neurodegenerative diseases, the latter of which includes vascular and post-stroke dementias (Sharifi-Rad *et al.,* 2017). A number of non-pharmacological interventions, such as music therapy, physical exercise and shiatsu (shiatsu is an ancient form of massage based on Chinese acupuncture theory that often includes the use of breathing and stretching), have also been shown to improve patients quality of life in cases of dementia (Sharifi-Rad *et al.,* 2017). It is important to develop alternative medicines with greater efficacy, bioavailability and fewer adverse effects because several pharmacological chemicals used in mainstream medicine have unpleasant side effects (Salehi *et al.,* 2018). In this sense, plants have the potential to be rich sources of

new chemicals that have potential as neurodegenerative disease therapies (Sharifi-Rad *et al.,* 2020).

2.3.3. Cell lines for neuroprotection

The homeostatic maintenance of most organs and tissues is ensured throughout the life of an organism by a controlled balance of cell division and cell death. In vertebrates, the nervous system's development involves both processes during the generation of functional circuitry. Once the mature nervous system is established, the threshold required to induce cell death becomes much higher and neuronal death is then limited to homeostasis maintenance. Indeed, uncontrolled cellular proliferation can result in the development of diseases such as cancer, whereas, an excessive level of cell death is a manifestation of diseases (Anselmi *et al.,* 2023). Today, studies of neuronal cell death are mainly performed *in vitro* using mammalian cell lines because of animal anatomical complexity. Reliable and simple animal models are very useful to *in vivo* studies on neuronal cell death and its role in the organisms as a whole. Tunicates are marine invertebrates considered the sister group of vertebrates (Delsuc *et al*., 2018).

Three major types of cell death, conserved throughout evolution, have been described on the basis of their morphological manifestations: apoptosis, autophagy and necrosis (Hollville *et al.,* 2019; Galluzzi *et al.,* 2018). Each of them is characterized by the expression of specific and common gene pathways. The term apoptosis describes a controlled (programmed) process of cell death marked by chromatin condensation, nuclear fragmentation and the initial maintenance of the plasma membrane, followed by cell fragmentation into small vesicles. During this process, several genes are selectively activated and participate in degenerative events. Phagocytes often remove apoptotic cells before they fragment, resulting in the containment of the dying cells within a tissue while reducing the risk of collateral damage to surrounding cells. The term necrosis is referred to as poorly controlled, unregulated process, induced by external injury, such as hypoxia or inflammation, resulting in the spilling of the cellular contents into surrounding tissue. A necrotic cell usually undergoes swelling, as it fails to maintain homoeostasis with its environment. However, it is now clear that there is also a genetically regulated necrosis, involving different molecular pathways (necroptosis, parthanatos, ferroptosis, pyroptosis, autolysis and mitochondrial permeability transition). Lastly, the term autophagy indicates a process where cellular

components are sequestered into lysosomes for degradation before recycling to form new cellular structures or further being processed and used as a source of energy. Autophagy can also result in destruction of the cell and in this way, it can be referred to as a form of cell death. Neurons can die through a dozen different modes, which include the three mentioned degenerative processes (Fricker *et al*., 2018; Anselmi *et al.,* 2023).

2.3.3.1. Mechanisms of cell death in neuronal cells

Although it is recognized that neurons die in neurodegenerative diseases, the mode of cell death is often unclear. There are a number of recognized ways in which neuronal cells can die, including apoptosis, necrosis, autophagic cell death and excitotoxicity. Other forms of cell death such as oncosis and paraptosis have not been studied much in neurons and a role for these modes of cell death in neurodegenerative diseases is not known, largely because of the lack of specific markers (Gorman, 2008; Bredesen *et* al., 2006; Fricker *et al.,* 2018).

Belonging to the Elaeagnaceae family, *Hippophae rhamnoides* (L.) is a rare and precious plant valued for its medicinal properties. Known by most as sea buckthorn, it is a prickly deciduous shrub that fixes nitrogen and is native to Europe and Asia. It grows well in cold, dry climates. Because of its nutritional and therapeutic qualities, it is currently domesticated in a number of countries throughout the world (Zuchowski, 2023; Mei *et al.,* 2023). *Hippophae rhamnoides* has pharmacological actions that are known to include anti-oxidant, immunomodulatory, anti-atherogenic, anti-stress, hepatoprotective, radioprotective and tissue regeneration properties. But not many studies have concentrated on exploiting it neuroprotective properties. Chronic disorders like atherosclerosis, cancer, diabetes, rheumatoid arthritis, cardiovascular diseases, inflammation, ageing and other degenerative diseases are caused by an excess and build-up of free radicals. One of the main factors causing cell death and damage, particularly in brain cells, is oxidative stress. Due to their high metabolic rate, neural cells are particularly vulnerable to oxidative stress-induced injury. The pathophysiology of ageing and neurodegenerative diseases has been linked to oxidative damage to DNA and biomolecules as well as an inadequate level of antioxidants.

Although biological systems possess an inherent defense mechanism to combat intracellular reactive oxygen species Zhang *et al.,* (2021), this eventually becomes ineffective due to over expression of reactive oxygen species. Therefore, finding a different supply of antioxidants is necessary to protect cells and delay the ageing and neurodegenerative

processes. The challenges of working with live human tissue samples led to the introduction of cell lines and numerous studies have shown that cell lines are among the best models for studying stress-induced cell damage and neurodegeneration (Slanzi *et al.,* 2020). Human neuronal cell line IMR32 may be the most suitable for researching how medications that effectively cure stress-induced neurodegeneration in people work. The IMR32 human neuroblastoma cell line have been used to assess the *in vitro* antioxidant and neuroprotective properties of *Hippophae rhamnoides* (Shivapriya *et al.,* 2015).

Multiple medication therapies will be necessary for canine cognitive dysfunction and neurodegenerative diseases to treat the various pathogenic elements, as multifactorial reasons have been identified. Developing a multifunctional molecule or extract that targets several brain regions is the new pharmacological approach, even if the strategy of combining medicines with distinct therapeutic targets is feasible. Therefore, the goal of the current study was to determine if treating the human neuroblastoma cell line SK-N-SH with a standardized extract of *Withania somnifera* could reduce reactive oxygen species levels, limit the activity of acetylcholinesterase and protect the cell line against toxicity generated by Aβ peptide and acrolein (Singh and Ramassamy, 2017).

2.4. Nanotechnology and Nanoparticles

Nanotechnology is an important branch in the major fields of biology, chemistry, physics and material sciences. The synthesis of nanoparticles with control over particle size, shape and crystalline nature has been one of the main objectives in chemistry that could be used for potential applications, such as medicine, electronics, therapeutics and as diagnostic agents bio-medical, biosensor, catalyst for bacterial biotoxin elimination and lower cost electrode. The nanoparticles having at least one dimension less than 100 nm such as nanosheets, nanotubes and nanowires have gained much attention because of their promising applications (Sharma *et al.,* 2016).

The nanomaterials can be synthesized by different methods including chemical, physical, irradiation and biological methods. In recent years, nanoparticles have been the subject of focused research due to their unique optical, electronic, mechanical, magnetic and chemical properties that are significantly different from those of bulk materials Khan and Hossain, 2022). Extracts from plant leaf, root, latex, seed and stem have also been used for the synthesis of nanoparticles as they act as stabilizing or reducing agents

(Yallappa *et al.,* 2015). Nanoparticles are solid, colloidal particles with size range from 10 nm to <1000 nm; however, for nanomedical application, the preferential size is less than 200 nm (Biswas *et al.,* 2014).Use of biological organisms such as microorganisms, plant extract or plant biomass could be an alternative to chemical and physical methods for the production of nanoparticles in an eco-friendly manner and extensive photocatalytic activity. Biosynthesis of nanoparticles is a sort of bottom-up approach, where the main reaction occurring is reduction (Prabu and Johnson, 2015).

Nanoparticle synthesis entails a combination of physical, biological and chemical approaches. Physical and chemical approaches are too expensive. Employing biological methods to synthesize nanoparticles would alleviate the requirement for rigorous processing conditions, as it enables synthesis to occur at physiological pH, temperature and pressure, while also being cost-effective. Biological approaches would aid in removing brutal processing conditions by allowing for the creation of nanoparticles at physiological pH, temperature, pressure and a small cost. Green techniques make the upscaling of nanoparticles production simple and cost-effective (Puri *et al.,* 2024).

The reliable, toxicity free and green chemistry process for the synthesis of nanomaterials are an important aspect of nanotechnology. There is a growing need to develop simple, low-cost, eco-friendly and size control approaches for the synthesis of metal nanoparticles. The secrets gleaned from nature have led to the development of biogenic approaches for the production of advanced nanomaterials, showing an alternative process for researchers. Biological resources are enormous, diversified and richest which could be employed for the synthesis of metal nanoparticles as reducing and stabilizing agents viz., bacteria, fungi, yeast and plant extracts leading to their own advantages over the conventional methods (Yallappa *et al.,* 2015). Nanomedicine has numerous new and exciting advancements that utilize nanoscale materials as diagnostic tools or medicinal drug delivery systems to specific targeted areas. The use of nanotechnology has proven to be advantageous in the diagnosis and treatment of diseases, regenerative medicine, gene therapy, oncology and targeted drug delivery such as therapy (Puri *et al.,* 2024).

Zinc oxide nanoparticles have been considered as potential candidates for targeted drug delivery due to their easy synthesis from low cost precursors, biocompatible nature and effective cellular internalization through endocytosis. Another unique advantage of zinc

oxide nanoparticles is their availability in different nanostructure forms, like nanosphere, nanosheet, nanorod, nanobelt and quantum dot. The various types of interactions involved in nanoparticle-mediated targeted drug delivery are ligand-receptor recognition, hydrophobic and coulombic interactions, etc. Besides, the various stimuli-responsiveness which guide the drug release from nanoparticles are temperature, pH, enzyme, light and biomolecules (glucose, GSH). So far, most of the researchers used several zinc oxide nanoparticle-based smart drug delivery systems for the targeted delivery of doxorubicin in cancer cells. After successful doxorubicin loading inside the channel-like pores of mesoporous silica nanoparticles, amine-functionalized zinc oxide quantum dots were employed to seal the pores via covalent amide bond formation with carboxylic acid groups anchored on the outer surface mesoporous silica nanoparticles. After internalization of the nanoassembles into HeLa cells, the zinc oxide quantum dot lids were dissolved rapidly in the weakly acidic intracellular compartment leading to the release of doxorubicin into the cytosol. Besides, the zinc oxide nanoparticles also imparted synergistic antitumor effect in HeLa cells due to its inherent anticancer activity (Singh *et al.*, 2020).

The toxicity of five kinds of nano-metal oxides on mouse brain neuroma cell line neuro 2a has also been studied and compared, suggesting that compared with nano-Al_2O_3, Nano-TiO_2, nano-Fe_3O_4 and nano-CrO_3 having similar particle sizes, nano-zinc oxide is the most toxic particle, which could cause mitochondrial function decrease, lactate dehydrogenase leakage increase and cell apoptosis. It has also been found that after exposing the nano-zinc oxide particles, the activity of mouse neural stem cells was decreased, DNA was damaged and cell apoptosis was induced. Recent research on nano-zinc oxide neurotoxicity has mainly focused on the toxic damage to hippocampus, cortex and the cognitive function. However, the response of striatum and dopaminergic neurons to nano-zinc oxide especially companion of *in vivo* or *in vitro* studies. Neurodegenerative diseases are often accompanied by damage to the main functional brain areas, such as hippocampus and striatum, which further damages the corresponding nerve function. Patients with neurodegenerative diseases are susceptible to environmental toxicants (Liu *et al.*, 2020).

2.4.1. Targeted drug delivery

An ideal nanoparticle drug delivery system (Figure. 6) should be able to reach, recognize, bind and deliver its load to specific pathologic tissues and minimize or avoid drug

induced damage to healthy tissues. Thus, coating specific targeting ligand(s) on the surface of nanoparticles is the most common strategy. These targeting ligands could be in the form of small molecules, peptides, antibodies, designed proteins and nucleic acid aptamers (short single-stranded oligonucleotides that are capable of binding various molecules with high affinity and specificity) (Rizvi and Saleh, 2018).

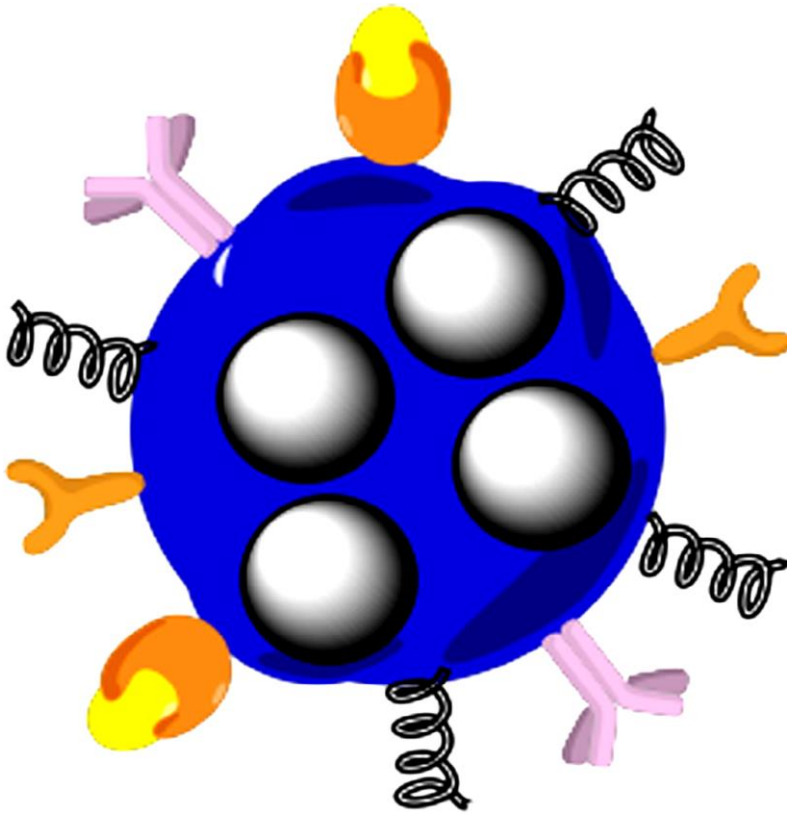

Figure 6. Representation of a smart multifunctional drug loaded nanoparticle, decorated with various moieties for targeting, imaging and stealth properties. **(Rizvi and Saleh, 2018)**

Recently, neuroprotective effects of resveratrol were evaluated by preparing solid lipid nanoparticles decorated with apolipoprotein E for low-density lipoproteins receptor recognition on the blood-brain barrier (Neves *et al.*, 2016). Zinc oxide is a potential photocatalyst instead of titanium dioxide due to its band gap energy and stability. By its wide band gap, zinc oxide can be applied in a broad range of applications, including self-cleaning, photocatalysis and environmental purification. In order to enhance the activity of zinc oxide in such application in photocatalysis, synthesis of zinc oxide to form nanoparticles is widely investigated (Fatimah *et al.*, 2016).

Zinc oxide nanoparticles, in particular, are environment friendly, offer easy fabrication and are non-toxic, biosafe and biocompatible making them ideal candidates for biological applications. Additionally, as per the United States, Food and Drug Administration, zinc oxide with other four zinc compounds have been listed as safe materials, less time consuming and large-scale synthesized. Various chemical methods have been proposed for the synthesis of zinc oxide nanoparticles, such as reaction of zinc with alcohol, vapor transport, hydrothermal synthesis, precipitation method and so on. However, these methods have various disadvantages due to the involvement of high temperature and pressure conditions and the use of toxic chemicals. Green synthesis approaches are gaining interest circumventing the high costs and usage of toxic chemicals and harsh conditions for reduction and stabilization (Jamdagni *et al.,* 2018).

The core advantage of targeted drug delivery arises from their easy administration to specified domains and their in vivo biocompatibility, sufficient protection of entrapped bioactive molecules, sustained cargo release, low cost and ease of scale-up procedures (Kulbacka *et al.,* 2016). An elegant nanomaterial capable of exhibiting high drug loading capacity, drug release, suitably collected for targeted delivery to malignant cells, zero premature release and obviously low toxicity of the native nanocarrier is highly desirable for therapeutic applications. Therefore, porous nanomaterials with higher surface area and porosity and lower density are privileged towards targeted drug delivery. Recently a plethora of porous zinc oxide-based nanostructures have come to the mainstream research of drug delivery systems. Zinc oxide is considered to be one of the five metal oxides that are generally recognized as safe and approved by the United States Food and Drug Administration. Therefore, zinc oxide based nanostructures are now being established in drug delivery systems overcoming the hurdles (Vimala *et al.,* 2014).

A high activity of this protein has been reported in many human diseases such as inflammatory processes, neurodegenerative disorders, viral infections and cardiovascular diseases (Perret *et al.,* 2013). In recent years, there has been increased interest in zinc oxide nanoparticles. This is mainly due to their smallest particle size, which enhances their chemical reactivity. Consequently, this has extended the wide application of zinc oxide nanoparticles in electronics, optics, biomedicine and agriculture (Yusof *et al.,* 2019).

2.4.2. Zinc oxide nanoparticles in neuroprotection

Zinc oxide nanostructures have a great advantage to apply to a catalytic reaction process due to their large surface area and high catalytic activity. Since zinc oxide shows different physical and chemical properties depending upon the morphology of nanostructures, not only various synthesis methods but also physical and chemical properties of synthesized zinc oxide are to be investigated in terms of its morphology. Many methods have been described in the literature for the production of zinc oxide nanostructures such as laser ablation, hydrothermal methods, electrochemical depositions, sol–gel method, chemical vapour deposition, thermal decomposition and combustion method. Recently, zinc oxide nanoparticles can be prepared by ultrasound, microwave-assisted combustion method, two step mechanochemical thermal synthesis, anodization, co-precipitation and electrophoretic deposition (Ding *et al.,* 2020).

A wide variety of natural agents, including extracts or monomers isolated from plants, animals, microorganisms and minerals, have been applied for prevention or treatment of neurological damages and diseases (Sharifi-Rad *et al.,* 2020). Due to their ability to protect neurons, some specific kinds of natural products have showed potentially optimum effects in spinal cord injury model to help the lesion recover and restore the internal homeostasis (Zhang *et al.,* 2016; Tao *et al.,* 2020). There in zinc oxide, as one of the most widely used metallic oxide materials, occurs naturally as the mineral zincite, has been well-studied in biomedicine, including drug or gene delivery, antimicrobial therapy and biosensors, due to its huge amount of storage and simple processing technology (Liu *et al.,* 2016; Wang *et al.,* 2017). Emerging of nanotechnology endows some positive therapeutic effects of zinc oxide in the form of nanoparticles, which have the capability to improve the reconstruction and repair of injured neural tissues (Pan *et al.,* 2020). For example, zinc oxide nanoparticles have been reported to show neurogenic and neuroprotective properties, thereby improving the synaptic connectivity/synaptic plasticity of cortical neurons and finally promoting neurogenesis in a rat model of cerebral ischemia (Barui *et al.,* 2020).

Zinc oxide nanoparticles have shown different features and applications from antibacterial and antifungal, towards gene therapy and neuroprotection (Bharathi and Bhuvaneshwari, 2019; Bharathi *et al.,* 2019). Recently, it has been revealed that zinc

nanoparticles could decrease the neural inflammation as well as affecting on apoptosis and enhancing the neural antioxidant responses. In addition, it can be able to increase the infiltrating the immune cell, which makes it a good candidate for neuroprotective effect against different range of neurotoxins. In addition, the use of some physical techniques such as high-gravity has been considered widely to increase the potential of fast electron transfer in synthesis methods as well as enhancing the effective collision between different particles (Rabiee *et al.*, 2020).

Figure 7 shows the effect of green synthesized zinc oxide nanoparticles using *Moriga olifera* leaf extract (MO-ZnONP) against acrylamide-induced neurobehavioral and neurotoxic impacts in rat (Dahran *et al.*, 2023).

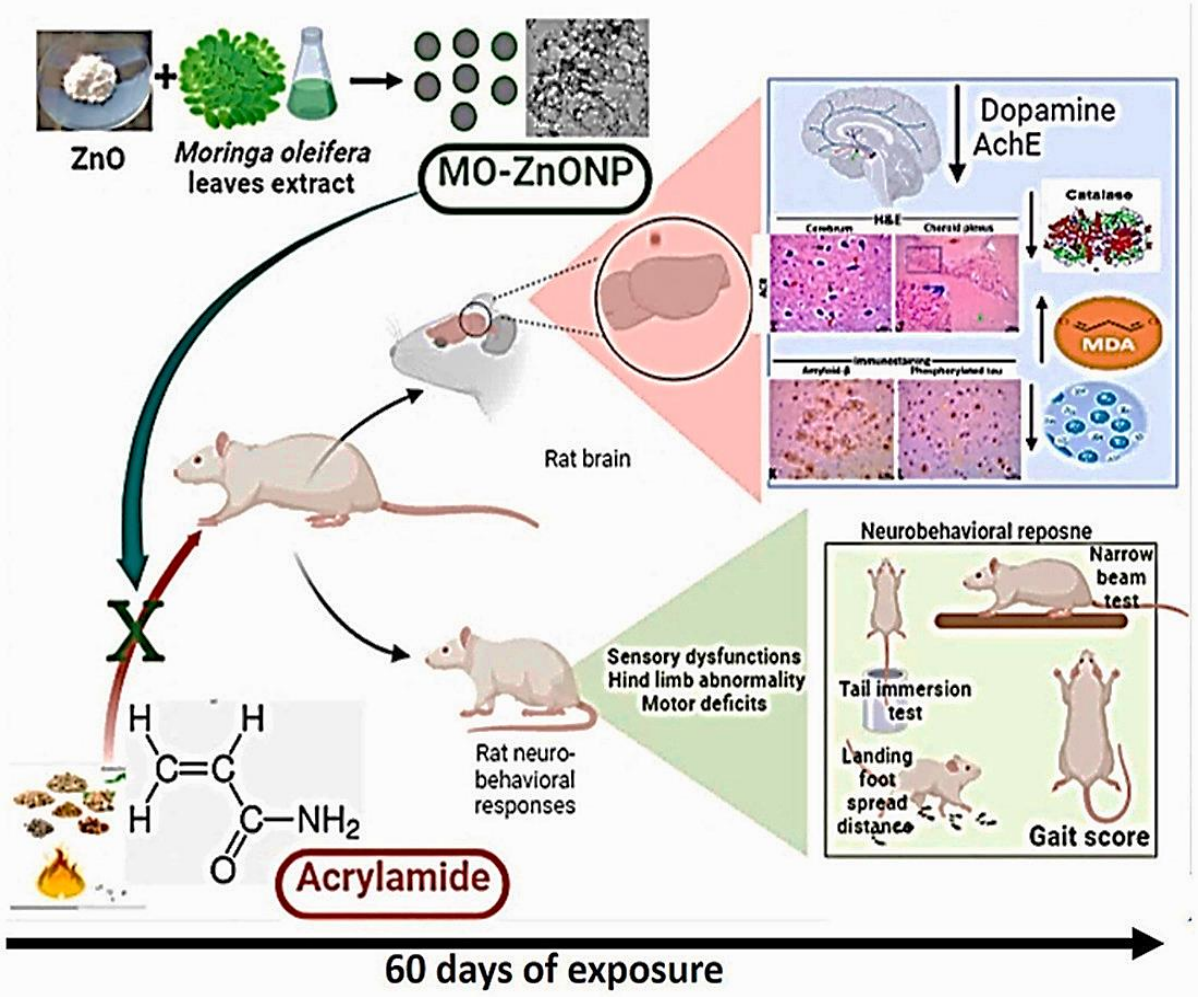

Figure 7. Mechanism of action of zinc oxide nanoparticles

(Dahran *et al.*, 2023)

The MO-ZnONP significantly reduced acrylamide-induced sensory dysfunctions, hind limb abnormality and motor deficits. Additionally, the acrylamide-induced increase in dopamine and AChE were significantly supressed by MO-ZnONP (Dahran *et al.,* 2023).

Tea polyphenol-zinc complex could change the distribution of electron cloud and had higher antioxidant activity and bioactivity than tea polyphenol alone since the aromatic hydroxyl group in tea polyphenol was activated after zinc and tea polyphenol were combined (Zhang *et al* 2015). No study had evaluated the encapsulation of CAT or CAT-zn complex using β-chitosoan nanoparticles. Chitosan nanoparticles have high encapsulation efficacy and can control the releasing rate of encapsulation bioactive compounds (Soltanzadeh *et al.,* 2021). The flavonoid catechin has been used to treat neurodegenerative diseases. So fabricated hybrid catechin-silica nanoparticle have confirmed their efficient protectant effect against neuronal cell loss (Sathishkumar *et al.,* 2018)

Several researchers have reported the application of synthesized zinc oxide nanoparticles from *Camellia sinensis* Rao *et al.,* (2021) demonstrated that the synthesis of zinc oxide nanoparticles from *Camellia sinensis* is effective against pathogenic bacteria and serves as a photocatalytic agent for the degradation of organic dyes. Senthilkumar and Sivakumar, (2014) described the fabrication of zinc oxide nanoparticles with antibacterial and antifungal activities using an aqueous extract of *Camellia sinensis* leaves. AI-ghamdi *et al.,* (2022) described the production of zinc oxide nanoparticles from *Camellia sinensis* leaf extract. The Green leaves obtained from Ooty, Nilgiris and the leaf extract are rich in antibacterial, antioxidant and anticancer characteristics. Kumar *et al.,* (2023) investigated the synthesis and characterization of zinc oxide nanoparticles derived from *Camellia sinensis*. Sattar *et al.,* (2023) earlier reported on the synthesis of zinc oxide nanoparticles utilizing *Camellia sinensis* aqueous extract from leaf powder, as well as the assessment of ofloxacin and ciprofloxacin estimations in commercial formulations, in Alarfaj *et al.,* (2023). Ajayan and Hebsur reported the production of zinc oxide nanoparticles from powdered *camellia sinensis* leaves in 2021. Irshad *et al.,* (2018) found that zinc oxide nanoparticles synthesized from powdered dry *Camellia sinensis* leaves are more efficient against bacteria. Al-Ogaidi (2017) investigated the production of zinc oxide nanoparticles from powdered dry *Camellia sinensis* leaves. Batool *et al.* (2021) reported on the production of zinc oxide nanoparticles from *Camellia sinensis* and the successful early removal of malachite green dye.

2.4.3. Catechin-coated zinc oxide nanoparticles

Zinc oxide is one of the very promising inorganic oxides that have recently attracted the attention of many scientists for the biosynthesis of nanoparticles due to its unique properties and multiple applications such as drug delivery, solar cells, photocatalytic degradation and personal care products like sunscreens and cosmetics. Zinc oxide nanoparticles have been synthesized from several plant extracts such as *Cassia auriculata* (mature tea tree), Prasad *et al.,* (2020) *Aloe vera* (Kattraazhai), Mahendiran *et al.,* (2017) *Cinnamomum verum (*Cinnamon), Ansari *et al., (*2020), *Bauhinia tomentosa* (Nilattiruvatti), Sharmila *et al.,* (2018) *Vitex trifolia* (Lemuning), Vitextrifolia Elumalai *et al.,* (2015) *Moringa oleifera (Murungai),* (Ngom *et al.,* 2021; Elumalai *et al.,* 2015) *Azadirachta indica* (Nimtree), (Singh and Kaushik, 2019; Haque *et al.,* 2020, Handago *et al.,* 2019), *Artocarpus gomezianus* (Mon-jack Paper mulberry) Suresh *et al.,* (2015) and *Olea europaea* (Common olive) and (Genaidy *et al.,* 2020; Abdelbay *et al.,* 2022) *Phyllanthus Niruri* (Stonebreaker), Jahangir *et al.,* (2020). Phenolic compounds exhibit higher antioxidant potential and antioxidants are very good reducers of metal ions, thus favouring the green synthesis of nanoparticles. Further, higher contents of proteins, lipids and amino acids help to stabilize the growth of nanoparticles and inhibit particle agglomeration (Senthilkumar and Sivakumar, 2014).

Many antioxidants exhibit inherent electro activity and therefore, the use of electrochemical methods could be a viable approach for evaluating the overall antioxidant activity of a matrix of nutraceuticals without the need for adding reactive species. Green tea is believed to be a healthy beverage due to a number of therapeutic benefits. Catechin, one of its constituents, is an important antioxidant and possesses free radical scavenging abilities Catechin has remarkable medicinal value due to the antibacterial, antitumor, anti-inflammatory and anti-diabetic properties. At the same time, this compound is recognized for its antioxidant properties, having the ability to neutralize free radicals and its an important antioxidant and possesses free radical scavenging abilities (Munteanu and Apetrei , 2022).

The minimal particle size and significant surface area give nanostructured materials exceptional properties and capabilities for their application in the food industry. Nanoencapsulation associated with natural compounds improve the efficiency of food processing and food safety (Taouzinet *et al.,* 2023). Nanoencapsulation offers a large number

of benefits and there is increased awareness now about the possible negative side effects, since some materials can be harmful to human health and in fact, some substances that are well known and previously used in diverse products present new potential risks due to fact that nanomaterials have novel properties and different molecular and atomic dimensions. The most appropriate nanoscale carrier materials for food applications are carbohydrates and protein or lipid-based alternatives (Pateiro *et al.,* 2021).

The absorption of catechins in the small intestines is relatively low and within 1 hour, around 80 % of epigallocatechingallate degrades in simulated intestinal fluid at pH 7.4 and 37°C. Therefore, protection of catechins from the external environment may improve their health-promoting effects (Kasote *et al.,* 2018). The components in green tea that are the most medically relevant are the polyphenols. The most pertinent polyphenols are the flavonoids and the most pertinent flavonoids are the catechins. The catechins comprise 80-90 % of the flavonoids and around 40 % of the water-soluble solids in green tea. Green tea contains more catechins than the other teas, mainly because of the way it is processed after harvesting. The amount of catechins in green tea can also be affected by factors like where the tea is grown, the growth conditions, time of harvest, how the leaves are processed and the brewing temperature and length of time of brewing. These factors lead to a huge variation in catechin content among the varieties and brands of green tea consumed (Reygaert, 2018).

Catechin, has many beneficial properties for human health such as anticancer, anti-obesity, antidiabetic, anticardiovascular, anti-infectious, hepatoprotective and neuroprotective effects (Isemura, 2019). Zinc oxide nanoparticles have a special place in almost every part of life such as delivery of controlled drug, electronic devices of sensors and photons, electrocatalytic water decomposition, nanocomposites for storage of energy and so on. Its potential applications are because of its electrical, optical, photochemical, catalytic properties and environmentally friendly nature of zinc oxide nanoparticles that have been widely investigated. Zinc oxide nanoparticles have a wide application ranges such as good antibacterial, photocatalytic, anti-oxidant and anti-cancer, biosensors, gas sensors, solar cells, ceramics, optical detectors, nanogenerators, active fillers for rubber, catalysts, plastics, UV absorbers, cosmetics, antiviral coatings, pigments of optical materials, optical and patriotic materials, optical probes, additives in high products of industrial and water and wastewater treatment (Shafiee *et al.,* 2021; Huang *et al.,* 2021).

2.5 *In silico* studies

2.5.1. Acetylcholinesterase

The hydrolysis of the neurotransmitter acetylcholine is involved as the principal mechanism of cholinergic signaling. The hydrolytic enzyme involved is acetylcholinesterase (AChE) which catalyzes acetylcholine (ACh) into choline and acetic acid. By inhibiting acetylcholinesterases, the amount of acetylcholine increases and possesses greater neuromuscular and other blocking effects. The basic mechanism behind the functioning of acetylcholinesterase inhibitors is to slower the hydrolysis rate of the acetylcholine. The effects produced are similar to the excessive stimulation of the cholinergic system. Anticholinesterase inhibitors have a wide range of utilization and a large diversity of drugs are involved. The newer acetylcholinesterase inhibitors have greater and potential effects than the classical ones. Because of the greater diversity for the anticholinesterase compounds, there has been complexity in understanding the various interactions as well as health consequences involved the effects of the acetylcholine inhibitors in various neurodegenerative diseases (Kaur *et al*., 2019).

There are two types of cholinesterase - acetylcholinesterase and butyrylcholinesterase. Acetylcholinesterase is found primarily in the blood and neural synapses. Acetylcholinesterase is predominantly observed in the neuronal synapses and blood, whereas, at the level of the human brain, butyrylcholinesterase is located close to glial cells and neurons or in tangles and neuritic plaques in Alzheimer's individuals (Stanciu *et al.,* 2019).

Acetylcholinesterase inhibitors in pre-clinical and clinical developments are shown in Table II.

Table II

Acetylcholinesterase inhibitors in preclinical and clinical development

S.No	Drug	Disposition
1.	Donepezil	Highly selective acetylcholinesterase inhibitor approved for mild, moderate and severe neurological disorders
2.	Rivastigmine	Have dual butyrylcholinesterase inhibitory and acetylcholinesterase inhibitory properties. Approved for mild-to-moderate neurological disorders. Patch formulation has reduced cholinergic-related side effects
3.	Galantamine	A lower potency acetylcholinesterase with allosteric nicotinic receptor modulation properties
4.	Tacrine	1. The beta-carboline derivatives (2A, 2B, 2C) and tacrine/ferulic acid hybrids (1A, 1B) were shown to have no efficacy *in vivo* and 1B actually worsened the impairment in an scopolamine-induced *in vivo* model. Clinical development has not been pursued further. 2. The tacrine-8-hdroxyquinoline hybrids showed potential *in vitro*, but the effects have yet to be shown *in vivo*

2.5.2. *In silico* Acetylcholinesterase constituents

According to data from the World Health Organization (WHO), 80 % of the world's population benefits from plants in the treatment of diseases. This rate can be said to be higher in developing countries. The use of plants for therapeutic purposes is more preferable since plant materials are more affordable and accessible than synthetic drugs in these countries such as United States and Europe. Many phytochemicals derived from the medicinal plants such as phenolics, phenolic acids, quinones, saponins, flavonoids, tannins, coumarins, terpenoids and alkaloids are the major classes of chemical constituents that possess many bioactive potential especially radical scavenging effect (Suntar, 2020; Hochma *et al.*, 2021). Innovative drug design from natural products is needed to combat global health challenges with the assistance of technological innovation. Most importantly is the need for new and innovative computational and analytical methods to identify chemical components of crude plant extracts in order to identify compounds causing the desired therapeutic effect and optimized extraction to exclude interfering components (Thomford *et al.*, 2018). It has also been noted that Linarin, a representative active ingredient of flavonoid glycoside in *Flos Chrysanthem Indici,* (wild chrysanthemum flower) has also been reported to have anti-

inflammation, AChE inhibitory and neuroprotection activities. The two inhibitors for Alzheimer's disease, galantamine and rivastigmine and the four compounds in the clinical trials, huperzine A, caffeine, nicotine and indomethacin, are in fact alkaloids (Innok *et al.,* 2021; Kumar *et al.,* 2023). The potential of plant derived alkaloids are have therapeutic effects against neurodegenerative diseases. Alkaloids include isoquinoline, indole, pyrroloindole, oxindole, piperidine, pyridine, aporphine, vinca, β-carboline, methylxanthene, lycopodium and erythrine by products (Hussain *et al.,* 2018). The majority of people merely use crude extracts to treat their health issues, never knowing the scientific background. Numerous investigations have extracted bioactive substances from natural sources. However, in-depth analyses is necessary to ascertain their molecular interactions.

Molecular docking is a powerful approach that may be used to explore protein-ligand interactions and predict the orientation of a ligand at a certain protein site. Molecular docking simulation can provide detailed structural information on the fluctuations and conformational changes of biological molecules Pinzi and Rastelli, 2019. While molecular docking simulation has been utilized to explore the structural characteristics of acetylcholinesterase-ligand binding, numerous recent researches have employed the molecular docking method to study the interactions and conformations of ligand against acetylcholinesterase target (ElKhatabi *et al.,* 2021; Makarian *et al.,* 2022; Terali, 2018). Here, an integrated strategy of structural modeling and experimental research to investigate the lead molecule from various natural sources for their medicinal potentials as therapeutic agents against neurological disorders.

Using molecular docking, fifty alkaloids and thirty-five flavonoid bioactive compounds that were isolated from various plant species were tested against acetylcholinesterase. Then, using molecular docking simulations, the dynamics information of the selected molecules were examined. In order to assess the compounds ability to inhibit acetylcholinesterase *in vitro* in comparison to crude extract, it was necessary to isolate the compound of interest that had been chosen based on docking results and references from its natural source. In-depth knowledge of the ligand-protein interaction and its inhibitory impact against acetylcholinesterase may prove beneficial for the development of new drugs for the treatment of neurological disorders (Somani *et al.,* 2015).

2.5.3. ADMET properties

A good drug candidate can be consumed in the desired time as well as distributed through the entire body for its efficient action and metabolism. Another factor is toxicity which is often very vital and dominates the Absorption, Distribution, Metabolism, Excretion behaviour of the drugs. *In silico*, ADMET and drug-likeness prediction help in the discovery of new targets and compounds with anticipated biological activities (Srivastava *et al.*, 2022).

2.5.3.1. ADMET properties of commercial drugs

Galantamine is an allosteric modulator of nicotinic acetylcholine receptors, enhancing their expression and activity in central cholinergic neurotransmission in addition to its inhibitory action on AChE. Abnormalities in the septo-hippocampal cholinergic pathway that are frequently observed in neurological disease patients are partially restored by this effect (Haake *et al.,* 2020). Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are slowly reversible dual inhibitors that are inhibited by **rivastigmine** (Blanco-Silvente *et al.,* 2017). The latter's involvement in subcortical regions associated with executive and focused activities suggests that dual inhibition may have advantages. On the other hand, donepezil is metabolized by CYP-450 and is selective for solely AChE inhibition. However, when rivastigmine is taken orally, compared to other cholinesterase inhibitors, it has been linked to a higher incidence of side effects and a worse outcome for all-cause discontinuation (Campbell *et al.,* 2017). **Tacrine** was the first medicine released in the United States market for the treatment of neurological disease, inhibiting both the acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes. This prevents acetylcholine from being metabolized, which increases its availability for binding to muscarinic receptors. Given that the study indicates that as neurological disease develops, AChE levels decrease while BChE levels either grow or stay stable, dual cholinesterase inhibitors may be more successful in treating the condition (Nordberg *et al.,* 2013). Neurotoxicity associated with neurodegenerative diseases can be treated by moderated **memantine** as a commercial compound (Shafiei-Irannejad *et al.,* 2021 and Kuns *et al.,* 2018). Pharmacological effects of memantine have been revealed and applied for clinical applications, such as neuroprotection in ischemic brain injury after cardiac arrest, treatment of vascular dementia, treatment of ischemic stroke, treatment of Parkinson's disease, dementia and alleviation of acute lung injury.

Donepezil increases the quantity of acetylcholine in the synaptic cleft by functioning as a highly selective, centrally acting, reversible inhibitor of acetylcholinesterase (Szeto and Lewis, 2016). This method of action is based on the cholinergic theory of neurological disease such as Alzheimer's disease, which states that elevating acetylcholine concentrations enhances neuronal function (Sharma *et al.,* 2019). Blanco-Silvente *et al.,* (2017) did a meta-analysis and showed that donepezil was more effective than galantamine or rivastigmine in treating global neurological disease symptomatology. Primary reported side effects when administered donepezil are indicative of its mode of action, which involves an increase in cholinergic activity.

2.5.3.2. ADMET properties of natural compounds

Cyanidin-3- glycoside also known as kuromanin, is the most notorious and investigated among cyanidin-glycosides. It was entirely different from the other flavonoids according to the initial report and the fact that Cyanidin-3- glycoside mostly resides in free form would undoubtedly enhance its antioxidant activity (Rupasinghe *et al.,* 2018). **Catechin** contains two benzene rings (A ring and B ring) alongwith dihydropyran (C ring) onto which the hydroxyl group is attached to carbon 3. The presence of two chiral centre molecules at carbon 2 and 3 is accountable for the generation of diastereoisomers. Two isomers with trans configuration are termed as catechin; while the other two with cis configuration are known as epicatechin. These isomers can be distinguished by chiral chromatography (Rinaldo *et al.,* 2010). The antioxidant mechanism of catechins played a significant role in mediating nephroprotective effect (Sardana *et al.,* 2015). **Kaempferol** is a natural flavonol-type flavonoid which has neuroprotective effects in Parkinson's disease is the second most common disorder of the central nervous system (Reeve *et al.,* 2014). Its pathological features are the loss of dopaminergic neurons and the formation of cytoplasmic inclusions in the substantia nigra. When Parkinson's disease is present, ~80 % of striatal dopamine is lost and damage to the terminal region may pre-empt the loss of cell bodies in substantia nigra (Tian *et al.,* 2012). **Genistein** inhibited lipid peroxidation thiobarbituric acid reactive substance method induced by hydroxyl radicals in 90.5% in the used C6 rat glioma cell line. Several studies using polymeric hemodialysis membranes such as polysulfone, polyethersulfone and polyvinylpyrrolidone modified with genistein show that various forms of encapsulation of hydrophobic isoflavone can be employed to treat a variety of disorders. Modified forms of genistein demonstrated higher antioxidant properties when compared to

mangiferin (Chang *et al.,* 2014). The main beneficial effects of (−)-**epicatechin** is via its ability to directly or indirectly scavenge reactive oxygen species by chemically reacting with reactive oxygen species or by modulating pathways that regulate reactive oxygen species scavenging compounds and enzymes, identified hydroxyl groups as the crucial structural feature of flavonoids responsible for reactive oxygen species scavenging (Shay *et al.,* 2015). The bioavailability of **quercetin-3-glucoside** is similar to that of quercetin-49-glucoside. Quercetin-3-glucoside itself also occurs commonly in foods such as tea, tomatoes and apples (D'Andrea, 2015). **Epicatechin gallate** is a main effective catechin widely existing in natural plants and food, with well-known health benefits. The neuroprotective effects of Epicatechin gallate -Exo were evaluated on Rot-induced SHSY5Y cells (neuronal cell line) and compared with free Epicatechingallate (Luo *et al.,* 2021). **Rutin** (3, 3',4',5,7-pentahydroxyflavone-3-rhamnoglucoside) is a flavonol, abundantly found in plants, such as passion flower, buckwheat, tea and apple. Chemically it is a glycoside comprising of flavonolicaglycone quercetin along with disaccharide rutinose. It has demonstrated a number of pharmacological activities, including antioxidant, cytoprotective, vasoprotective, anticarcinogenic, neuroprotective and cardioprotective activities (Javed *et al.,* 2012; Nassiri-Asl *et al*., 2010). **Hesperetin**, a flavanone class of citrus flavonoid is a derivative of hesperidin found in citrus fruits such as oranges, grapes and lemons. It has been extensively reported that hesperetin exerts neuroprotective effects in experimental models of neurodegenerative diseases. Hesperetin, an important bioactive compound in Chinese traditional medicine, has antioxidant and anticarcinogenic properties (Ikram *et al.,* 2019). Currently, it has been indicated that hesperetin confers marked antioxidant, anti-inflammatory and neuroprotective effects in different models of neurodegeneration (Muhammad *et al.,* 2019). Among the different types of phenolic compounds, **apigenin** is one of the most renowned, with countless nutritional and organoleptic characteristics. Nonetheless and more interestingly, it can also contribute with its beneficial health properties, which could lead to a possible inclusion in nutraceutical formulations (Hostetler *et al.,* 2017; Grumezescu *et al.,* 2018). Due to apigenin's variety of pharmacological activities and importance for human health, a deepen knowledge of its mechanism of action would be of utmost importance for possible nutraceutical applications. **Zinc** essentiality was established in 1869 for plants, in 1934 for experimental animals and in 1961 for humans. Anemia, hypogonadism and dwarfism was reported in a 21- year- old Iranian farmer in 1961 who was subsisting on a diet of unrefined

flat bread, potatoes and milk (Ross *et al.,* 2020). Although, zinc- dependent biochemical mechanisms in physiologic functions have received extensive study, clear relationships have not been fully established. Zinc is ubiquitous within cells in contrast to iron, which is contained in defined cellular components and has defined physiological roles. In a model of aluminum chloride-induced excitotoxicity which results in cell death and concomitant hyperactivation of microglial cells, the neuroprotective effects of **hesperidin** against hippocampus excitotoxicity were evaluated (Jovanova-Nesic *et al.,* 2012). The cytoprotective effects of hesperidin are largely dependent on its antioxidant and anti-inflammatory activities (Justin Thenmozhi *et al.,* 2018), which indicates that cognitive impairments are ameliorated by hesperidin treatment. Hesperidin treatment is also associated with decreased levels of nitrate/nitrite and increased levels of brain-derived neurotrophic factor in the mouse hippocampus (Donato *et al.,* 2014) as well as the inhibition of glutamate release following kainicacid-induced excitotoxicity in the rat hippocampus (Chang *et al.,* 2015). **Kaempferol-3-O-rutinoside** is an effective component that is present in some medicinal plants, such as *Carthamus tinctorius*. It is a flavonoid glycoside and takes the form of a yellow amorphous powder, Kaempferol-3-O-rutinoside has been found to be effective in the prevention and treatment of ischemic brain damage. It is also found to be effective for the treatment of neuronal inflammation by inhibiting the activation of transcription factor nuclear factor, a signal transducer and activation of transcription 3 (Yu *et al.,* 2013). Kaempferol-3-O-rutinoside can significantly reduce the neurotoxicity of beta-amyloid-induced nerve cells (Mira *et al.,* 2015). Multi-infarct dementia model rats, Kaempferol-3-O-rutinoside can improve rat memory function and reduce damage due to oxidative stress on the nervous system. **Quercetin** is found to be active free radicals. Cigarette tar is a source of free radicals which has been found to damage erythrocyte membranes. It was also found that quercetin and its conjugate metabolites could protect erythrocytes from the membranous damage that is caused by smoking. Neurodegenerative diseases as well as neuronal injury associated with stroke are associated with neuroinflammatory processes in the central nervous system. There has been intense interest recently in the potential of flavonoids to modulate neuronal function and prevent against age- related neurodegeneration, it also has been the effective in preventing neurons from neurotoxins (Choi *et al.,* 2012). The consumption of flavonoid rich food limits neurodegeneration.

Theaflavins comprise of a large group of polyphenols abundantly present in black and oolong teas. Theaflavins are the biflavonoid class consisting of a benzotropolone skeleton and accounting for about 2 % of dried tea leaves. Theaflavins are the main oxidation products of catechins and aggregates during the fermentation steps (Fatima *et al.*, 2013). Theaflavins are formed by the oxidation of selected catechins (epicatechin and epigallocatechin-3-gallate) in presence of polyphenol oxidase and peroxidase enzymes. During fermentation, the catechins get converted to Theaflavins primarily the theaflavin, theaflavin-3-gallate, theaflavin-3′-gallate, theaflavin-3,3′-digallate. **Gallic acid**, a type of phenolic acid, has been associated with a wide range of neurological ailments. It is a secondary metabolite found in many different parts of the upper plant kingdom in free-state or ester. It is a colorless to slightly yellow crystalline substance and is used in the food and medicine industries (Fernandes *et al.,* 2016). Coffee is one of the major sources of antioxidants in people's daily diet, which possesses chlorogenic, ferulic, caffeic and n-coumaric acids. In roasted coffee, melanoidins (brown pigments) are synthesized these are strong antioxidants, in some research revealed that **caffeine** and trigonelline are considered to be antioxidants also phenylalanines which are formed during the roasting process show high antioxidant activity (Yashin *et al.*, 2013). **Kaempferol** is one of the flavonoids commonly found in some vegetables, fruits and traditional medicine. In nature, almost all dietary flavonoids exist in their glycosidic forms (Xiao *et al.,* 2014). Kaempferol usually bonds with glucose, rhamnose, galactose and rutinose to exist in its glycoside form (Calderon-Montano *et al.*, 2011). The antioxidant and hepatoprotective effects of kaempferol 3-O-b-D- (2,6- di-O-a-L-rhamnopyranosyl) galactopyronoside isolated from unripe soybean leaves has been reported (Zang *et al.*, 2017).

With this background information, the experimental design for the study was formulated as given in the following chapter.