"TARGETTING BRAIN DERIVED NEUROTROPHIC FACTOR IN ALZHEIMER'S DISEASE USING FLAVONOIDS OF *Acorus calamus.*"

Dissertation submitted to **MAHATMA GANDHI UNIVERSITY** *in partial fulfillment for the award of the degree of*
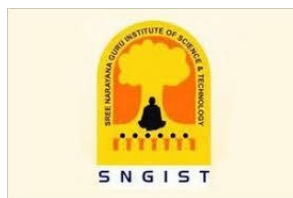
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IN

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SUBMITTED BY

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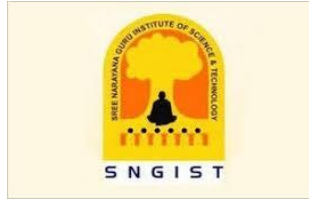*(Affiliated to Mahatma Gandhi University)*

NORTH PARAVUR

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Under the guidance of

Mr. Dr. Rajesh Ramachandran

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BONAFIDE CERTIFICATE

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This is to certify that the Project work entitled **"TARGETTING BRAIN DERIVED NEUROTROPHIC FACTOR IN ALZHEIMER'S DISEASE USING FLAVONOIDS OF** *Acorus calamus."* is a bonafide record of original work carried out by **Ms. ANJANA UNNIKRISHNAN (180011009167)**, IV semester, SNGIST Arts and Science College, N. Paravur to the Mahatma Gandhi University in partial fulfillment of requirements for award of the Degree of **Master of Science in Biotechnology** is an authentic record of research work carried out by her during the period of study.

Internal Guide   **Head of the Department**

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Place:

Date:

DECLARATION

I ANJANA UNNIKRISHNAN (Reg no: 180011009167), hereby declare that the dissertation work entitled **"TARGETTING BRAIN DERIVED NEUROTROPHIC FACTOR IN ALZHEIMER'S DISEASE USING FLAVONOIDS OF *Acorus calamus."*** Submitted to Mahatma Gandhi University, Kottayam, in the partial fulfillment of the requirement for the award of degree of Master of Science in Biotechnology, SNGIST College N. Paravur is a record of original research work done by me during the period from March to May 2020 under the supervision and guidance of **Mr. Dr. Rajesh Ramachandran**, Director of Biogenix Research Centre, Trivandrum. The thesis has not formed on the basis of award of my degree, diploma, fellowship or similar to any candidate of my university.

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ABSTRACT

The brain derived neurotrophic factor (BDNF) belongs to the family of neurotrophins that have a crucial role in survival and differentiation of neuronal populations during development. In the adult brain, BDNF also maintains high expression levels and regulates both excitatory and inhibitory synaptic transmission and activity-dependent plasticity. Previous findings has shown that gradual dysregulation of neurotrophic factors like neurotrophic growth factor (NGF) and brain derived neurotrophic factor (BDNF) have been reported during Alzheimer's disease (AD) development thus intensifying further research in targeting these factors as disease modifying therapies against AD. In this aspect the present study aims to validate the effects of flavonoids from *Acorus calamus* on experimental neurodegeneration induced by b amyloid on IMR 32 human neuroblastoma cells.

Keywords: Brain derived neurotrophic factor, Alzheimer's disease, Acorus calamus, Flavonoids, neurodegeneration, β amyloid, IMR 32 human blastoma cells.

INTRODUCTION

INTRODUCTION

Neurodegenerative disorder represents major threat to human health and is an age-dependent disorder that has been increasingly widespread (Aaron et al.). They are characterized by the accelerating loss of vulnerable group of neurons. It is an incurable and enfeebles condition leading to progressive degeneration or death of neurons. Neurodegenerative diseases are classifies on the basis of its clinical features, anatomical distribution or molecular abnormality. Common neurodegenerative disorders include; amyloidosis, tauopathies, α- synucleinopathies, and TDP-43 proteinopathies. It is typically defined by specific protein accumulation and anatomic vulnerability, neurodegenerative disorder deals with many basic phenomena associated with neuronal disability and death such as oxidative stress, programmed cell death and neuroinflammation (Gibb and Lees 1988).

Furthermore, it is important to note that the protein accumulation that cause neurodegenerative disorder can be present before the onset of clinical feature and more than one neurodegenerative disease may occur in an individual (Uchikado et al. 2006). Also genetic mutation can cause the disorder (Ghasemi et al.2016). Cross- sectional evaluations of large number of human brain from patients have suggested that many neurodegenerative disorders have a stereotypic progression that can be defined by stages. Staging scheme has been invent for Alzheimer's disease (AD) (Braak and Braak 1991; Thal et al. 2002), Parkinson's disease (PD) (Braak et al.2003), dementia with Lewy bodies (DLB) (Kosaka et al.1984) and chronic traumatic encephalopathy (CTE) (McKee et al.2013).

SOCIO ECONOMIC BURDEN

Alzheimer's disease (AD) and related dementias (ADRD) is one of an expensive disease to society. It is a great concern of medical or social medical care cost for patients related to their illness. The medical cost care includes medical practitioner/health professional visits, hospital care, medical treatments or medications and specialized aids and equipment. Non-medical costs include social services (such as transportation, peer support), nursing homes, patient income, welfare support and household expenses. It is also realize that caregivers who look-after the patients develop certain health issues (e.g., depression, low immunity, and cardiovascular problems).

AMYLOIDOSES

They are insoluble fibrous proteins having specific structural characteristics. The protein characteristics of almost all neurodegenerative disease have few features of amyloid. A defining feature of non-neuronal amyloidosis is having abundant extracellular protein aggregates (Westermark et al. 2005). In certain type of disorder, amyloid-like filamentous aggregates are mostly found within cytoplasm of neuron and glia. Amyloid plaques have a wide range of morphologies and have been further subcategorized into; diffuse plaques,

dense-cored plaques, 'classical' plaques, and cotton wool plaques (Dickson 1997). The most common type of amyloid is the proteolytic product of amyloid precursor protein (Kang et al. 1987) which is referred as β-amyloid or Aβ.

TAUOPATHIES

Disorder associated with pathological accumulation of tau proteins in neurons and glia (Lee et al. 2001). Tau is a microtubule associated phosphoprotein excessively in axon contributes to the development of polymerization and stabilization of microtubules (Binder et al. 1985).

ALZHEIMER'S DISEASE

Alzheimer's disease is a critical public health condition affecting elderly population. It was defined by Alois Alzheimer in 1906 using the criteria of progressive memory loss, disorientation, and pathological markers (senile plaques and neurofibrillary tangles).

AD attacks nerves and brain cells as well as neurotransmitters. The destruction of these parts leads to the formation of protein clumps around the brain's cell, these clumps are known as 'plaques' and 'bundles'. Presence of these 'plaques' and 'bundles' starts to destroy other connections between the brain's cells, which make the condition worse.

ACORUS CALAMUS

Acorus calamus (sweet flag) is a tall perennial wetland monocot plant from *Acoraceae* family. Its leaves and rhizomes have been traditionally used as a medicine and dried and powdered rhizome has a spicy flavor and is used as substituent of certain food items. In Ayurvedic medicine *Calamus* is an important herb and is valued as a 'rejuvenator' for the brain and nervous system. Active principle involved in Acorus are; alpha- asarone (volatile oil) and beta- asarone (potential candidate for development as a therapeutic agent to manage cognitive impairment associated with conditions such as AD) and Eugenol (Asmita et al.).

Major Phytochemicals of Acorus involves flavonoids, phenols, alkaloids, terpenes, tannins and saponins.

Flavonoids are an important class of natural products; have excellent role in biochemical and antioxidant effects associated with various diseases such as cancer, Alzheimer's disease (AD), Atherosclerosis, etc. (Burak & Lee Y et al. 2009). They are extracted from plants and they are used by plants for their growth and defence against plaques (Havsteen B. 2002). Flavones are one of important subgroups of flavonoids.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Alzheimer's disease (AD), a chronic neurodegenerative disorder associated with intensifying dementia and mild cognitive disability (R Venkatesan et al., 2015) which has been reported to be an serious sociomedical problem. Attenuation of neurogenesis in the brain is one of the major reasons for dementia in AD. (S Gopalkrishnashetty et al., 2017) while modulating hippocampal neurogenesis led to the formation of new neurons have an improvement in AD patients.

Neurotrophic factors or neurotrophins such as Brain derived neurotrophic factor (BDNF) plays an important role in AD and their consumption accelerates the progression of the disease. Neurotrophic factors are small group of dimeric proteins (G R Lewin, Y A Barde. 1996.) or soluble proteins specific to cell surface receptors. There are four mammalian neurotrophic factors, i.e. Nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), neurotrophins-3 (NT-3), neurotrophins-4/5 (NT-4/5) they bind and activate Trk family receptor tyrosine kinase and p75 receptors. Among these NGF and brain derived neurotrophic factors (BDNF) have a role in synaptic transmission and neuronal development (Huang E.; 2001). Messenger RNA transcript level of BDNF had a strong influence on neurodegenerative disease (Heidi, P. 1991) and also BDNF have importance in intracellular signaling pathway by regulating neurogenesis in parietal cortex, temporal cortex, hippocampus, frontal lobe etc (Narisawa-saito, M.; 1996). Neutrophins activate signaling pathways, mediated by ras and members of cdc-42/ras/rho G proteins and MAP kinase. (Louis.F.R. 2001).

Weinstein et al. found that greater peripheral BDNF levels secure older adults against AD. By having BDNF levels increasing by one standard deviation, the complication for AD or dementia was decreased by 33%. Naegelin et al. have proposed that BDNF can in fact reliably measure in human serum (Naegelin et al. 2018). The study by Leyhe et al. revealed that by treating patients with AD with donepezil for 15 months, serum BDNF level can put back to the levels similar to those of healthy controls (Shimizu et al. 2003). Studies put forward by Connor et al. proposed that the administration of corticosterone decreased BDNF mRNA by as much as 70% (Connor B., et al).

Brain-derived neurotrophic factor (BDNF) is a critical regulator of neural development (Lewin & Barde, 1996) and have a widespread distribution in the central nervous system. However, some studies showed endogenous BDNF is essential for peripheral nerve degeneration and remyelination (a natural repair mechanism of demyelination) after nerve injury. Evidence that BDNF upregulation is associated with seizure activity (sudden, uncontrolled electrical disturbance in brain) proposed it play a vital role in epileptogenesis.

Acorus calamus (a sweet flag) also known as Vacha is an herb that is used extensively in Indian system of medicine belongs to Araceae family (Patekar et al). It is a traditional medicinal plant that can be used to treat many neuronal and non-neuronal based diseases (V& R Sharma and D Singh). Phytochemicals such as fatty acids, phenols, alkaloids, flavonoids etc. from medicinal herbs play a major role in stabilizing brain's chemical balance by disturbing the activity of receptors for the essential inhibitory neurotransmitters. They are also used to cure learning disability as herbal medicines contain phytochemical having neuroprotective effect on neuropsychiatric & neurodegenerative diseases (G Phani Kumar & F Khanum. 2012).

The ethanolic extracts of A. *calamus* were studied for learning and memory-enhancing activity. The subjects used consist of male rats, through Y maze and shuttle box tests models. The results showed an increase an acquisition-recalling and spatial recognition data. The ethanolic extracts potentiated pentobarbitone-created sleep periods, which caused excessive inhibition of conditioned avoidance response in rats and marked protection against PTZ-induced convulsions, although it doesn't show any motor activity and impact the fighting behavior response in male rat pairs (Vineeth Sharma et al. 2020).

The annual incidence of AD increases rapidly with age, from 53 new cases per 1000 people aged 65-74, to 170 new cases per 1000 people aged 75-84, and 230 new cases per 1000 people aged over 85 (Alzheimer's, 2011). Additionally, its commonness increases exponentially with age, in the U S. Roughly 5% of cases occur as an inheritable form passed on in a Mendelian manner whereas the onset of symptoms occur early in life, termed as early onset AD (EOAD) (Van Gool & Eikelenboom et al., 2000). In contrast, majority cases develop sporadically as late onset AD (LOAD).

Over 80% of cases of EOAD are accounted for by mutations in either the gene for amyloid precursor protein (APP) or one of two other proteins presenilin-1 (PS1) and presenilin-2 (PS2). These mutation lead to the continuous Aβ secretion and finally results in the accumulation in brain. Perhaps, LOAD occurs by impaired clearance of Aβ as a result of several factors (Zetzsche et al., 2010).

Old age is a prominent risk factor for AD (Swerdlow et al.). Pathophysiological changes occur in the aging brain and these changes may vary among individuals. Pathophysiological alterations include higher oxidative stress, inflammation, vascular impairment, gliosis, Aβ accumulation and tau hyperphosphorylation. Targeting a single change in the brain causes nerve cell damage and even death. Mainly, these changes interact with lifestyle, environmental, and genetic risk factors with varying degrees. One mouse model of aging that exhibit the features of AD is the senescence-accelerated prone 8 (SAMP8) mouse and it was developed in Japan. These mice shows ongoing, age- associated decline in brain activities similar to human AD patients (Pallas et al.).

Key neuropathological characteristics on which the conclusive diagnosis of AD relies, viewed at post-mortem in the AD patients. They are atrophy of the cortex, neuron and synaptic loss, extracellular plaques composed of insoluble β-amyloid (Aβ), and intraneural neurofibrillary tangles (NFTs) containing hyperphosphorylated tau (Perl, 2010). Aβ , presently the most widely studied neuroimaging biomarker for the diagnosis and predicting the disease.

AD, a diverse and complex disorder characterized by the presence of amyloid β (Aβ) plaques and neurofibrillary tangles (NFT) (Syed Faraz Kazein.2016). Common pathology of neurodegeneration is deposition of proteins with altered physiochemical properties in the human being. These pathological conforms are called misfolded proteins such as accumulation and aggregation of amyloid β in AD. Mechanism of action of herbal drug is not yet determined but some of them have the ability to prevent the formation of amyloid plaques, promote nerve growth and some inhibit acetylcholine esterase (AchE) (Supriya.R & Himani.A., 2017).

Flavonoids are phytochemicals with wide range of potential therapeutic activities including AD. Naturally occurring flavonoids scaffolds have shown to be beneficial influence in experimenting models of AD through one or more mechanisms (P Anand & B Singh., 2013). Interaction of flavonoids in these mechanisms can reduce the progression of the disorder and improve cognitive performance. Some mechanisms involve the interaction with signaling pathways in brain such as phosphatidylinositol 3-kinase/ Akt and mitogen-activated protein kinase pathway, Other process include damaging amyloid β aggregation alteration in amyloid precursor (Filipa et al., 2014).

Some epidemiologic studies indicates cardioprotective role of flavonoids against coronary heart disease & reduce mortality from the disease (Hertog et al.1993). The study of flavonoids is complicate because of heterogeneity molecular structures and shortage of data on bioavailability. Data on long- term impact of flavonoid ingestion are chiefly short. Currently, the consumption of fruits, vegetables and beverages containing flavonoids is supported (Formica et al.1995).

Saponins are either triterpenoid or steroidal glycosides that proved to be a major phytoconstituents with various pharmacological processes such as antiallergic, cytotoxic antitumor, antiviral and antifungal. Recent study showed that three diosgenyl saponins were isolated from *Paris polyphylla* reported to have immunostimulant properties (Z. Xiu-feng. 2007). Immunostimulatory activities of terpenoid compounds such as glycyrrhizinic acid, ursolic acid, oleanolic acid and nomilin have been reported (T. Raphael et al. 2003). The preparations of Indian traditional medicine may accelerate immunomodulation due to their content of herbs with immunomodulatory activities that possibly act synergistically. Certain reviews predict that there are many medicinal herbs which exert this activity on experimental models at a significant dose.

Xiong et al. describes that by lowering cortisol levels, the level of BDNF can be improved (Xiong G. L., 2009).

The crucial oil of Indian *A. calamus* (Vacha) exhibit potential for stored-product pest control. It has an active element, β-asarone, has harmful and sterilizing effects. Now, the beetles are collectively span in Africa were harvested maize is damaged. For this reason the A. calamus oil and β-asarone was checked as control agents. The corn grains were treated with acetone solutions of oil and after evaporation of the liquid the grains is widespread and invade with beetles. Besides, the grains also treated with rhizome powder of A. calamus. The destruction of grains was calculated on weight of corn dust collected from infested grains. Within 21 days, treatment with 0.01% oil only can reduce grain loss significantly. The temperature was very critical and was observed at 30°C (Sudarshan Reddy et al. 2015).

In the adult brain BDNF also maintains high expression levels and regulates both excitatory and inhibitory synaptic transmission and activity-depended plasticity (Tyler et al., 2002). The BDNF expression is balanced during transcription, translation and also by post-translational modifications. There are at least four BDNF promoters in rats were identified (Timmusk et al., 1993). Each one managing transcription of mRNAs having one of the 8 non-coding exons spliced to the common 30 coding exons, synthesizing heterogeneous population of BDNF transcripts.

There is a growing body of evidence to describe that flavonoids and other polyphenols able to frustrate the neuronal injury, thereby slow down the progression of these brain pathology (Mandel & Youdim et al., 2004). For example, a Ginkgo biloba extract reveal to protect hippocampal neurons against nitric oxide- and beta-amyloid- induced neurotoxicity (Luo & Smith et al., 2002.). Flavanones, such as hesperetin and its metabolite, 5- nitro- hesperetin, that have been showed to inhibit oxidant- induced neuronal apoptosis via a mechanism involving the activation/phosphorylation of signaling proteins essential for pro-survival pathways (Vauzour et al., 2007).

The most common phenolic group compounds contained in human diet is flavonoids. It distributes a common feature consisting of two aromatic carbon rings, benzopyran (A and C rings) and a benzene ring (B ring) and may be divided in various subgroups based on the degree of the oxidation of the C ring, the hydroxylation pattern of the ring structure and the substitution of the 3- position. Bioavailability and biological effects of flavonoids vastly depend on their biotransformation by the liver and by gut microbiota (Rodriguez-Mateos et al., 2014).

Other flavonoids such as resveratrol, fisetin and hyperin which is also regarded as potential drugs with neuroprotective effects (Bhullar & Rupasinghe et al., 2013). Recent studies verified different flavonoids and studies between different flavonoids are rare. An example Resveratrol, exhibit neurological and cognitive enhancement features in a clinical

trial (Witte A.V & Kerti L., 2014). In AD flavonoids represent an interesting phytochemical class which has been widely used as phytotherapy medicines, like Ginkgo biloba, which includes quercetin and kaempferol (Chan P. C & Xia Q et al., 2007).

Another hallmark characteristic of AD is associated with lowered cholinergic neurotransmission which has an impact on cognitive decline, leading to dementia and behavioral problems (Parsons C. G, & Danysz W et al., 2013). A rise in cholinergic neurotransmission is a vital focus on drug discovery and inhibit acetylcholine's degrading enzyme, acetyl cholinesterase (AchE). Besides, both quercetin and rutin are the targets of present studies on acetylcholinesterase inhibition, which is an important target on AD drug therapy where quercetin is a strong AchE inhibitor (Abdalla F. H & Baldissarelli et al., 2014). Liu et al., 2013 investigated in vivo the protective effects of quercetin against Abeta-induced toxicity, on both endothelial cells and neurons.

The neuropathological studies of AD & dementia were largely limited to presenile dementia that develops before the age of 65. The studies of Edgar Miller shows that the common behavioral symptom of presenile dementia/AD is memory disorder, a condition in which recently gained information fails to reach long-term memory storage. He also suggested the poor retrieval of information from long-term memory storage (Miller et al., 1971).

IMPACT ON SOCIETY

Dementia is emerging as a major public health concern. Because dementia is largely a disease of older age (above 70), the demographic changes in the United States have resulted in increasing numbers of people at risk of developing the disease. The prevalence of dementia in the age group over 85years may be as high as 20 per cent. It is currently found out that most common cause of severe intellectual impairment in the elderly is Alzheimer's disease (AD). AD may lead to death in 5 to 10 years, decreasing life expectancies of one-half to one-third of healthy persons of the same age.

Families caring for a person with AD soon discover that it is unlike any other illness. It is more disruptive to, and has greater impact on, family than other chronic disease (Lisa. P. 1998).

These pathologies have an economic impact in society, too. Economic burden is particularly evident in more advanced pathologies with more severe symptoms, where poor quality of life, reduced productivity, drug therapy increase and even greater need of healthcare services increase direct and indirect costs. But, it is very difficult to make confident projections of future economic costs.

The studies examined both positive and negative impact of caregiving in a sample of 110 caregivers to an aging family member suffering from AD (Shannon et al. 1994).

Impact on health-related quality of life (HRQL) and perceived burden of informal caregivers of individual with AD in Canary islands shows that HRQL was higher for more educated caregivers (PG Serrano et al, 2006).

DISCUSSION

DISCUSSION

Since Acorus calamus is a traditional medicinal herb and numerous phytochemicals like alkaloids, flavonoids, phenols etc are available in different parts of the plant. We review the antioxidant and anti-inflammatory effects of these phytochemicals. Therefore, many phytochemicals have multiple potential neuroprotective approaches which contribute to therapeutic benefit for pathogenesis of neurodegenerative diseases. One of the chief benefits is that they have low toxicity compared to pharmaceutical agents. Sooner the treatment is started, better will be the outcome.

This plant species were easily available in many region of the world and the traditional use of Acorus calamus in Indian Ayurvedic system is strongly accepted. Not only in neurodegenerative diseases but also in western herbal medicine, the plant is mainly employed for gastrointestinal related problems like gas, bloating and poor digestive function. The Acorus calamus extracts used as a Chinese medical prescription, beneficial for memory loss, on learning performance, lipid peroxide content and anti-aging effects in senescence.

Flavonoids comprise most common group of polyphenolic compound present in our diet, present in Acorus calamus. Studies indicate that after ingestion of flavonoids they metabolized in gastrointestinal tract and liver, are absorbed in blood stream and can reach the CNS by crossing blood-brain barrier (BBB).

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