1-1-2016

Histological Evidence Of The Effect Of Oil Palm Phenolics In Atherogenic Diet Induced Rat Model Of Alzheimer's Disease

Kenechukwu Monplaisir Monplaisir
Wayne State University,

EFFECT OF OIL PALM PHENOLICS ON BETA AMYLOID DEPOSITION IN CHOLESTEROL INDUCED RAT MODEL OF ALZHEIMER'S DISEASE: HISTOLOGICAL EVIDENCE

by

KENECHUKWU UCHENNA MONPLAISIR

THESIS

Submitted to the Graduate School

of Wayne State University,

Detroit, Michigan

in partial fulfillment of the requirements

for the degree of

MASTER OF SCIENCE

2016

MAJOR: NUTRITION AND FOOD SCIENCE

Approved By:

_____

Advisor Date

DEDICATION

I dedicate this thesis to my family who have been highly supportive of my journey through graduate school thus far and to the memory of my baby, Udo who passed this year , I love him deeply and appreciate all the life lessons learnt from his short stay with us.

ACKNOWLEDGEMENTS

I would humbly like to acknowledge my advisor Dr. Smiti Gupta for her support, understanding and patience with me through the process of research and thesis writing. I would like to express appreciation to my advisory committee: Dr. Pramod Khosla and Dr. Ahmad Heydari. I sincerely thank the doctoral student that I worked with , Yan Wu, an exceptionally hardworking and brilliant individual who was diligently willing to teach and guide me through the research process. My gratitude also goes to all my fellow labmates Dr. Nadia Saadat, Dr. Arvind Goja, Lichavi Dhananjaya Rajasinghe, Rohini Sri Harshini Pindiprolu, Vindhyaja Srirajavatsavai, Nurul Razalli, Dr. Andreea Geamanu for their insight and productive contribution to the progress of my research project.

I wish to express my sincere thanks to the faculty members such as the former chair of the biomedical engineering department, Dr. Juri Gelovani and other departments of Wayne State University for their help, support, laboratory advice and assistance during my research. I ultimately thank God for seeing me through the process and my family for their unconditional love and support.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

DEMENTIA

Dementia is not a disease in itself but is a general term used to describe a variety of diseases and conditions that emerge when nerve cells in the brain die or no longer function normally. This thereby causes death or malfunction of these nerve cells (neurons), leading to changes in one's memory, behavior and cognitive abilities [1]. According to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) [2], a patient has dementia when they present a decline in memory and at least one of the following symptoms:

1) Ability to generate coherent speech or understand spoken or written language.

2) Ability to recognize or identify objects, assuming intact sensory function.

3) Ability to execute motor activities, assuming intact motor abilities and sensory function and comprehension of the required task.

4) Ability to think abstractly, make sound judgments and plan and carry out complex tasks.

1.1.1 TYPES OF DEMENTIA

There are different types of dementia, categorized by their symptoms and brain abnormalities [1][2][3].

a. Alzheimer's disease: This is the most common form of dementia.

   Early symptoms: Difficulty remembering names and current events; apathy and depression

   Late symptoms: Impaired judgment, disorientation, confusion, behavior changes and difficulty speaking, swallowing and walking.

Brain abnormalities: Deposition of beta-amyloid (plaques) and twisted strands of the protein tau (tangles) along with proof of nerve cell damage and death in the brain.

b. Vascular dementia: This form of dementia was formerly known as multi-infarct or post-stroke dementia (dementia secondary to stroke).

   Symptoms: Impaired judgment or ability to make plans

   Brain abnormality: Localized brain injury leading to microscopic bleeding and blockage of blood vessels.

c. Dementia with Lewy bodies(DLB): Lewy bodies are abnormal clumps of the protein called alpha-synuclein.

   Symptoms: Vivid hallucinations, sleep disturbances in additions to the symptoms seen in Alzheimer's disease.

   Brain abnormality: Deposition of alpha-synuclein in the cerebal cortex of the brain.

d. Mixed dementia: As its name goes, it's symptoms include a combination those seen in Alzhiemer's disease, vascular dementia and DLB.

   Brain abnormality : There's more of beta amyloid deposition and injury to the brain seen.

e. Parkinson's disease: Presentation is similar to that of DLB or Alzheimer's disease.

   Symptoms: Difficulty moving around.

   Brain abnormality: Alpha-synuclein is deposited in a deep area of the brain called the substantia nigra. This deposition is believed to destroy nerve cells that produce dopamine thereby resulting in Parkinson's disease.

f. Frontotemporal lobar degeneration (FTLD): There are subtypes of FTLD such as as behavioral variant FTLD, primary progressive aphasia, Pick's disease and progressive supranuclear palsy.

Symptoms: Difficulty with languages, altered behavior and personality

Brain abnormality: There is no distinct abnormality seen, however the neurons on the front and side regions of the brain seem to be affected.

g. Creutzfeldt-Jakob disease: There is a variant form of this disease which results from the feeding on products from cattle infected with the mad cow disease. This disease is rapidly fatal.

Symptoms: Memory and coordination impairment, altered behavior.

Brain abnormality: Malfunction of protein of the brain. This is as a result of prions (misfolded proteins) causes other proteins of the brain to misfold.

f. Normal pressure hydrocephalus: This type of dementia can be corrected by the insertion of a shunt in the brain to drain fluids.

Symptoms: Urinary incontinence, loss of memory and impaired coordination

Brain abnormality: Accumulation of fluid in the brain.

1.1 ALZHIEMER'S DISEASE

Alzheimer's disease (AD) accounts for 50 to 70 % of dementia presentations. It was initially identified and brought to light in 1906 by Aloysius 'Alois' Alzheimer, a German psychiatrist and neuropathologist as amyloid plaques (Aβ) and neurofibrillary tangles (NFTs) from a 51-year old patient named Auguste Deter. This patient presented impaired mental function with significant cognitive decline [4]. AD is the sixth leading cause of death in the United States and the fifth leading cause of death in people over the age of 65, as determined by the Centers for Disease Control and Prevention (CDC) [5]. The risk of developing AD increases exponentially with age. This disease has since been diagnosed in more than 5.1 million people in the United States alone. In 2015, the direct

costs to the United States for caring Alzheimer's patients estimated at total of $226 billion, with half of the costs borne by Medicare. People 65 years of age and older with Alzheimer's and other dementia spend three times higher on average per-person Medicare expenses than for seniors without dementia. The Medicaid payments are 19 times higher. Approximately one in every five Medicare dollars is spent on people with Alzheimer's and other dementias. In 2050, it will be one in every three dollars [6]. There are two forms of AD; early onset (EOAD) and the late onset also known as sporadic AD (LOAD/SAD). The late onset AD accounts for about 87% of the cases and early onset accounts for about 13% of cases. It has stimulated several hypothesis as its possible cause (although actual process of development is still yet to be understood). It is characterized by a significant deficit of hippocampal neurons which leads to decrease in cognition, memory loss ultimately, dementia. There is the cholinergic, tau and amyloid hypothesis, in our study we explored the amyloid hypothesis [7].

The Cholinergic hypothesis:

It has been discovered that AD patients have low concentrations of acetylcholine. Acetylcholine is a neurotransmitter that is metabolized by an enzyme (a serine protease) called acetylcholine esterase in the central nervous system (CNS) into acetate and choline as seen in figure 1 below[8]. This chemical serves as a neuro-modulator in the CNS and is located in the pre-ganglionic and parasympathetic neurons. It is also the neurotransmitter in the sweat glands and piloerector muscles of the sympathetic autonomic nervous system.

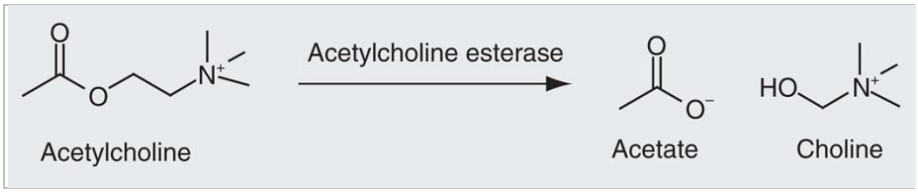

Figure 1: Breaking down of acetylcholine by acetylcholine esterase.

The brain of mammals contains two major forms of cholinesterases: acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). The two forms differ genetically, structurally, and for their kinetics. Butyrylcholine is not a physiological substrate in mammalian brain, which makes the function of of difficult interpretation. In human brain, BuChE is found in neurons and glial cells, as well as in neuritic plaques and tangles in AD patients. Whereas, AChE activity decreases progressively in the brain of AD patients, BuChE activity shows some increase [9].

The Tau Hypothes

Tau is a microtubule-associated protein (MAP) required for stabilizing microtubules and neurite outgrowth [10]. Normal Tau interacts with tubulin, facilitates its assembly into microtubules and stabilizes their structure [11]. Tau-based neurofibrillary pathology is found in more than 20 neurodegenerative diseases. Phosphorylation of Tau within the microtubule binding repeats (R) is necessary for appropriate neurite outgrowth. The ratio of 3R and 4R Tau isoforms is generally 1:1 in the adult brain, but deviations from this ratio may cause Tauopathies (Tau pathologies) [12]. Hyperphosphorylated Tau spontaneously aggregates into paired helical filaments (PHF), which can subsequently form NFTs. In AD, hyperphosphorylated Tau accumulates, prompting its dissociation from microtubules, thus leading to their destabilization and the disruption of neuronal transport.

The Amyloid Hypothesis:

One of the hallmarks of AD is the accumulation of amyloid plaques between nerve cells (neurons) in the brain. The amyloid hypothesis indicates that is accumulation leads to a chain of events that culminate the AD pathology. These events damage synapses and neurons thereby producing neuroinflammation, neurodegeneration, loss of synapses, formation of neurofibrillary tangles and aggregation of beta amyloid (Figure 1). Amyloid are protein fragments that the body produces normally. Beta amyloid is a protein fragment cleaved from an amyloid precursor protein (APP), which are involved in neuro-genesis and neural development. In a healthy brain, these protein fragments are broken down and eliminated [13]. In AD, the fragments accumulate to form hard, insoluble plaques.

The cleavage of APP takes place at the N-terminal of a beta amyloid sequence, this gives rise to a C -terminal APP fragment, C99 and a large secreted N-terminal product called sAPP-β. The sAPP-β is a soluble fragment. C99 is further cleaved by gamma secretase (containing catalytic subunits; presenilin, Aph-1, Nicastrin, and Pen-2 protein) forming a beta amyloid fragment and an APP intracellular domain (AICD) [14]. The end result is beta amyloid (Aβ), a peptide of amino acids ranging from 36 to 43, the main component of amyloid plaques. Aβ40 is soluble and turns out to be the most abundant species accounting for 90% of the total Aβ peptide in normal brains and AD brains. Aβ42 is the primarily abundant in patients presenting with AD. Aβ42 possess 2 hydrophobic ends therefore this species has a very high propensity for clustering or aggregating. It forms toxic oligomers which facilitate the formation of plaques [15]. The accumulation of Aβ42 is highly due to mutations in presenilin genes present in vertebrates (which encode for PS-1 and PS-2) which are key catalytic subunits of gamma secretase intramembrane protease complex (Figure 3). The

mutations in presenilin genes and APP play a major role in the accumulation of Aβ42 which subsequently leads to the formation of fibrils and eventually neurotoxity or cerebral beta amyloidosis [16]. These mutations predominantly accounts for the presentation of early onset AD which is referred to as familial AD. In AD patients the amyloid accumulation drives the deposition of the plaques because it happens to be the main component protein of the plaque. This subsequently causes formation of neurofibrillary tangles, neuronal loss, vascular damage and dementia. The severity of AD is directly proportional to the amount of amyloid plaque accumulated [17]. The aforementioned process describes the amyloidogenic processing of APP. It should be noted that physiologically beta amyloid peptides are generated at low concentrations and that a significant amount of APP is processed via the non-amyloidogenic pathway that involves cleavage of APP by alpha secretase.

Now the alpha site of APP is cleaved by alpha secretase (comprising of members of the disintegrin and metalloproteinase; ADAM family) producing C83, an α-secretase-generated C-terminal APP fragment (α-CTF) and N-terminal portion of APP (soluble APP-α; sAPP-α) [18]. The fragment, α-CTF undergoes further processing by the γ-secretase complex to generate AICD and p3 peptides. sAPP-α is found to be neurotrophic and neuroprotective properties [19]. Studies have revealed that the suppression of sAPP-α by conditions such as oxidative stress, abnormal lipid metabolism, abnormal glucose metabolism, physical inactivity, and cerebral hypoperfusion can lead to sporadic AD (SAD). sAPP-α is highly being considered for potential therapeutic properties [20]. It is unquestionable that the AD pathology is mostly due to the accumulation of Aβ and inhibition or suppression of sAPP-α .rather than altered expression of APP or its products, causes AD pathology.

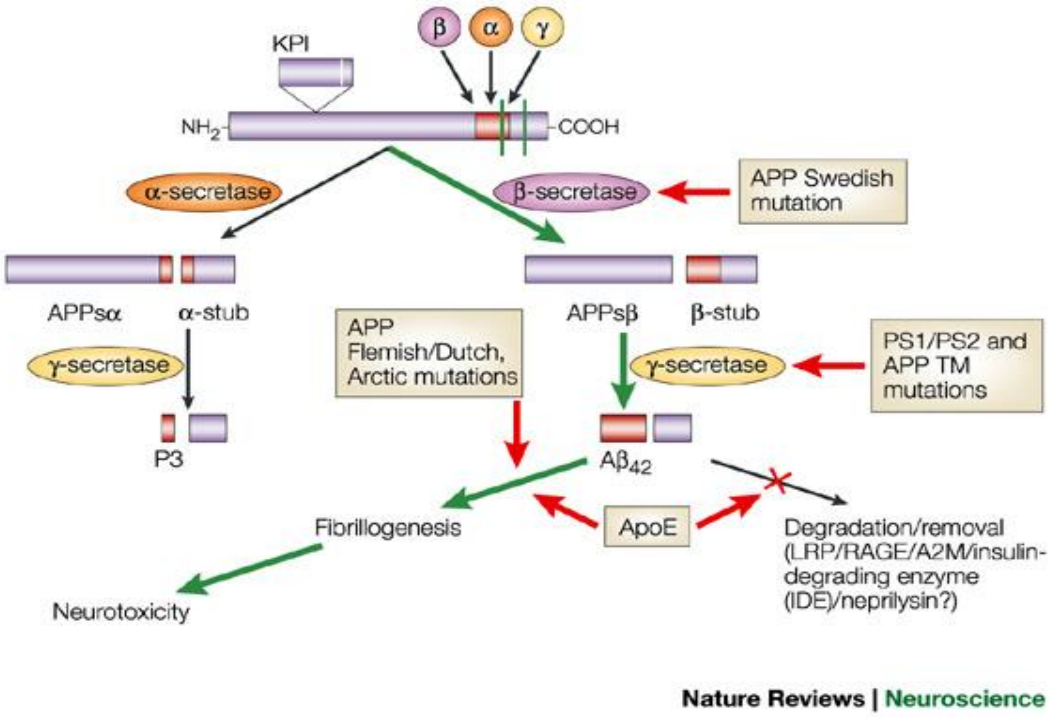

Figure 2: Generation of Aβ42

Epsilon 4 allele of the apolipoprotein E gene (APOE ε4):

Apolipoprotein E is a class of apolipoprotein that primarily break down and transport triglycerides, cholesterol and beta amyloid. It is a polymorphic gene with three alleles; ε2, ε3 and ε4. The allele; ε2 is protective against AD but may increase or decrease risk of an atherosclerosis diagnosis but it facilitates the metabolism of beta amyloid. The allele, ε3 is considered the neutral allele of ApoE while the ε4 form is linked with a high risk of getting AD [21]. ApoE-ε4 has been linked to late onset AD [17]. The allele, ε4 significantly increases the chances of sporadic AD by 3 fold in heterozygotes and by 15 fold in homozygotes [22]. In the presence of ApoE-ε4, there is a gain in toxic function due to inefficient metabolism of beta amyloid. Therefore, an accumulation of beta amyloid which leads to a cascade of damaging

processes which include decreased lipid metabolism, decrease in synaptic function , neuronal toxicity, atrophy of the brain and increased aggregation of beta amyloid peptides [23].

1.2.1 SYMPTOMS OF ALZHEIMER'S DISEASE

Clinical experts have categorized the symptoms of AD to fall under mild cognitive impairments. This includes significant attention deficits, decline in learning and memory, decreased executive function, declined speed in processing information, and semantic language. [24].

1.2.2 DIAGNOSIS OF ALZHEIMER'S DISEASE

Innovative early diagnostic tests have been developed to detect AD before symptoms begin to appear. One such test involves the analysis of cerebrospinal fluid for beta-amyloid or tau proteins, [25] both total tau protein and phosphorylated tau_{181P} protein concentrations. Searching for these proteins using a spinal tap can predict the onset of Alzheimer's with a sensitivity of between 94% and 100% [26][27]. When used in conjunction with existing neuroimaging techniques, doctors can identify people with significant memory loss who are already developing the disease [28]. Alzheimer's disease is usually diagnosed based on the person's medical history, history from relatives, and behavioural observations. The presence of characteristic neurological and neuropsychological features and the absence of alternative conditions is supportive [29][30]. Advanced medical imaging with computed tomography (CT) or magnetic resonance imaging (MRI), and with single-photon emission computed tomography (SPECT) or positron emission tomography (PET) can be used to help exclude other

cerebral pathology or subtypes of dementia [31]. Moreover, it may predict conversion from prodromal stages (mild cognitive impairment) to Alzheimer's disease [32].

1.2.3 TREATMENT OF ALZHEIMER'S DISEASE

There is currently no cure for AD. The interventions are palliative in nature and cover three categories of management of AD; psychosocial, pharmaceutical and caregiving.

Psychosocial: This intervention has insufficient data to proof its efficaciousness. It targets more dementia than AD and it is administered as an adjunct to pharmaceutical intervention [33]. Psychosocial options include emotion-oriented interventions, behavioral interventions and cognitive-oriented treatments [34].

Pharmaceutical: This method of managing AD borders on two approaches; inhibiting acetyl-cholinerase and N-Methyl-D-aspartate (NMDA). Four medications have been developed that inhibit acetyl-cholinerase, they include tacrine, rivastigmine,galantamine and donepezil[35]. Unfortunately, none of the medical treatments listed can cure AD. In fact, the improvement due to medical intervention are mild to moderate and last for 6 months to 1 year.

Nutritional: There has been several studies conducted showing the effect of bioactive components of food on AD; omega-3 fatty acids, antioxidants or B vitamins, and dietary patterns (Mediterranean diet) have demonstrated protective effects on age related chronic diseases [36]. Cassia Obtusifolia, a botanical that has been traditional used to treat inflammatory conditions and diseases of the liver and eye is currently being explored for neuro-protection in AD [37]. It's proposed mechanism of action in treating or preventing AD is through the inhibition of acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and β-site amyloid precursor protein (APP) cleaving enzyme 1 (BACE1) enzyme activity. There is also the possibility of treatment of AD with antioxidants due to the damaging effects of oxidative stress

on nerve cells and synapses. The question of whether or not antioxidants play a role in treating AD due to the effect of reactive oxygen species (ROS) which can subsequently lead to oxidative stress is still being explored. The presence of oxidative stress can cause high concentrations of protein oxidation, protein nitration, glycoloxidation and lipid peroxidation and the hallmark of AD; accumulation of Aβ [38]. There is justification in experimenting on the effect of nutrition high in antioxidants on AD.

1.3 NUTRITION AND ALZHEIMER'S DISEASE

Nutrition plays a role in the presentation of AD. Extensive studies have shown that supplementation of the diet with antioxidant, B vitamins, polyphenols and polyunsaturated fatty acids and increase in the ingestion of fish, fruits, vegetables, coffee and light to moderate alcohol not only help treat AD but also reduce the risk of getting this disease, figure 3 [39].

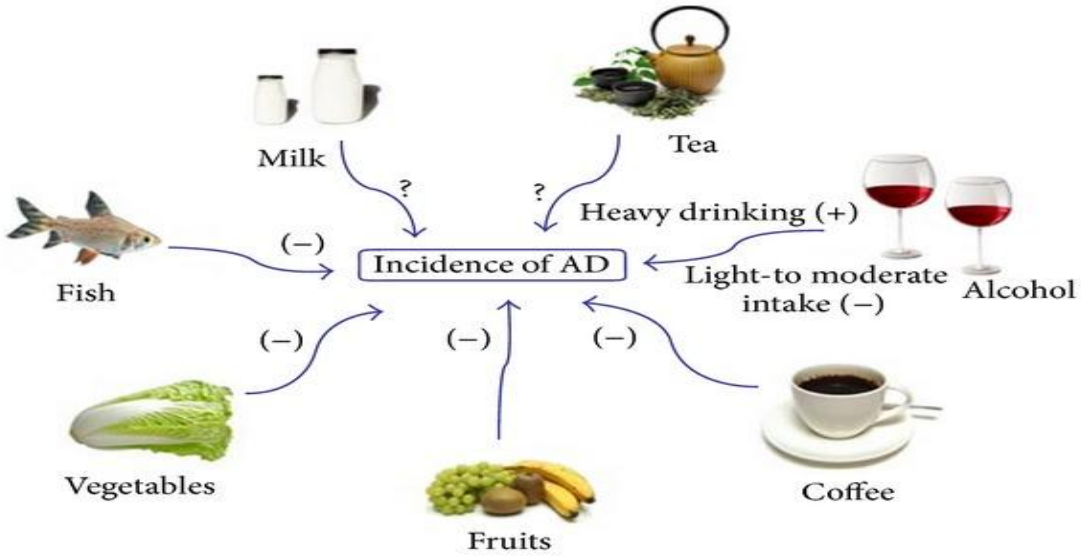

Figure 3: Nutrition

Foods and beverages that influence the incidence of AD. Fish, vegetables, fruits, coffee, and light-to-moderate alcohol intake are reported to reduce AD incidence. Milk and tea are reported to influencecognition, but their influence on AD is not clear.

It is postulated that there is a correlation between the development of AD and oxidative stress. The generation of Aβ could actually be protective in hiding sources of free radicals. The accumulation of the Aβ as indicated earlier will induce AD as a result of insufficient or inefficient clearing. Aβ accumulation can interfere with the normal electron flow through the respiratory chain by binding to mitochondrial membranes and interacting with heme. This subsequently leads to the production of reactive oxygen species (ROS) and diminished mitochondrial energy metabolism. This could justify the hypometabolism observed in patients with AD [40]. Data collected from studies conducted with flavonoid and non-flavonoid polyphenols have show promise in neutralizing excess ROS thereby suppressing their damaging effects.

Although there is limited data on the mechanism of action of antioxidants in AD, neuronal protection and potent scavenging of singlet oxygen, superoxide anions, hydroxyl radical and peroxyl radicals have been shown with the ingestion of epigallolcatechin (EGCG), resesveratrol and curcumin. The scientist, Schroeder demonstrated the effect of EGCG on the integrity of neuronal function on rat models and discovered selective protection of the rats cultured cerebullar granule neurons from oxidative stress [41]. EGCG has also shown protection from toxicity resulting from beta amyloid precursor protein (APP), 3-hydroxykynurenine, or 6-hydroxydopamine (6-OHDA) in SH-SY5Y human neuroblastoma cells [42]. Resveratrol (trans-3,4′,5-trihydroxystilbene) is the key non-flavonoid polyphenol that can be extracted from grapes and found in red wine. It has anti-inflammatory, anti-carcinogenic and antioxidant activities [43]. Beta amyloid induced intracellular accumulation of reactive oxygen species was

attenuated by resveratrol [44]. In vivo studies have also reported attenuation of the degeneration of neurons in the hippocampus of the inducible p25 transgenic mouse model of AD and tauopathy by reseveratrol [45].

1.3.1 CURCUMIN

Curcumin (diferuloylmethane) is a popular Asian spice is derived from *Curcuma longa*, turmeric. Over many years in has been utilized medicinally as a remedy for wound healing, inflammation and even as an anticarcinogen [46]. Epidemiological studies have revealed correlation between cognitive function and the intake of curcumin. One of these studies pulled a total of 1010 Asians between 60 and 93 years of age and subjected them to a standard test, MMSE that measures cognition. The subjects were placed into groups that consumed curry less frequently (less than once a month), rarely consumed curry and the consumed curry more often (more than once a month). The result of the study showed higher MMSE score for the group that consumed curry more often compared to those you either eat less or never eat curry [47]. Extensive research have shown that curcumin acts as an antioxidant, playing a major role in combating oxidative stress and health conditions related to oxidative stress like diabetes, obesity, cardiovascular disease and AD. It has shown to be a potent scavenger of different reactive oxygen species; like superoxide anion radicals, hydroxyl radicals [48], and nitrogen dioxide radicals [49][50]. It has been theorized that curcumin stimulates the activation of stress sensitive kinases in fructose fed rats by inhibiting phosphorylation of the kinases, thereby preventing cell damage [51]. A summary of the various revealed mechanisms of action of curcumin is displayed in figure 4. Studies conducted on cultured neuroblastoma cells focused on the effect of curcumin on the in vitro expression of PS1 and the enzyme glycogen synthase kinase-3, GSK-3β. GSK-3β presentation has been linked to the development of AD and

presenilin-1 has been identified as its substrate. Curcumin was reported to be successful in blocking the stimulation of presenilin-1 activity by GSK-3β, thereby reducing the production of beta amyloid peptides [52]. In addition, studies conducted at UCLA revealed that curcumin facilitates the metabolism or clearance of beta amyloid plaques in AD by macrophages [53]. This justified our use of curcumin as a positive control in our research.

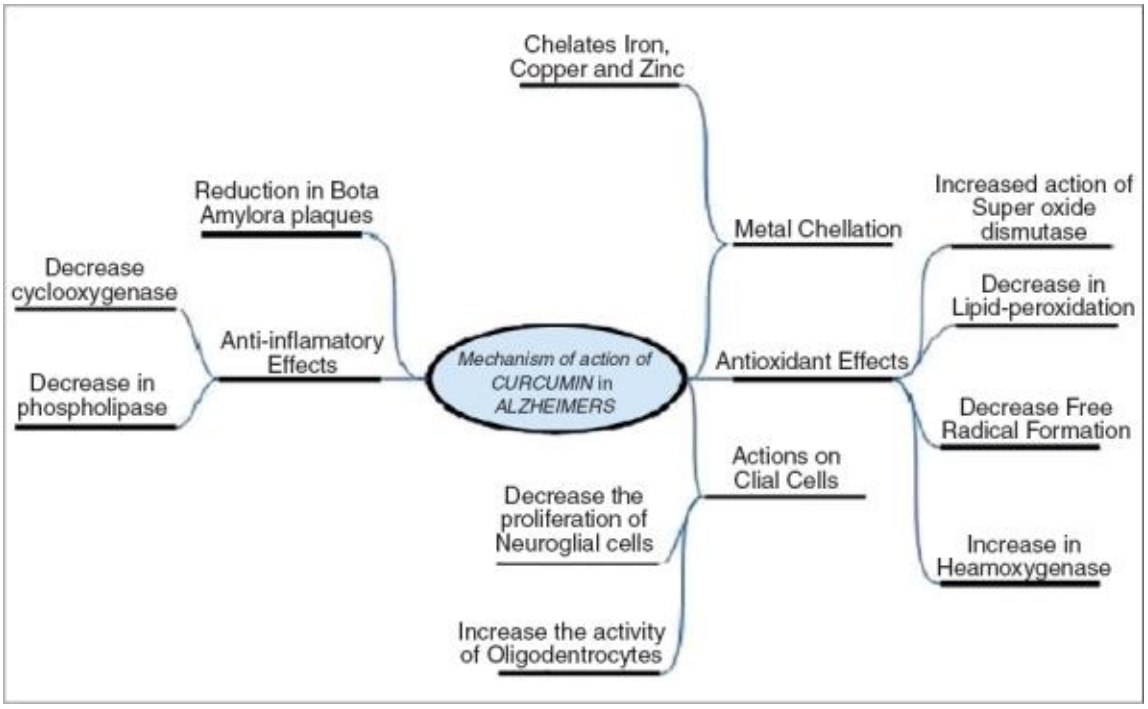

Figure 4: Various mechanisms of action of curcumin

1.4.1 OIL PALM PHENOLICS

Oil palm (Elaeis guineensis) is predominantly found in the tropics and contains a high concentration of water soluble phenolics. It is a high oil-producing tropical plant that appears to

have an effective anti-oxidative component to counter the oxidative stress exerted by high temperature and intense sunlight [54].

An estimate of 85% of the world's palm oil consumed as food. This has lead to further research on the nutritional contents and benefits of this food product. Oil Palm contains almost 50% saturated fatty acids (SFA). This percentage has presented in key area of research in determining its efficacy in relation to heart related diseases like coronary heart disease (CHD) risk. The constituents of oil palm are 44% Palmitic acid (44%), the key SFA in palm and monounsaturated oleic acid (39%) and polyunsaturated linoleic acid (11%).

In addition to the fatty acid component of oil palm, there is also a phytochemical component. Oil palm contains carotenoids, tocopherols, tocotrienols, sterols, squalene, coenzyme Q10, phospholipids, and polyphenols. The presence of these phytochemicals ensures the quality and stability of the oil [55]. Apparently, all the phytochemical present in palm oil possess antioxidant properties. Oil Palm Phenolic (OPP) or the palm juice is the aqueous stream extracted from oil palm that is rich in phenolic antioxidants. The therapeutic effects of OPP on cardiovascular diseases, diabetes and cancers have been reported by Sambanthamurthi et al [56]. They concluded that OPP could be used as a dietary agent for prevention of many oxidative stress related chronic diseases, including neurodegenerative ailments. Plant phenolics are major antioxidants due to their high redox potential which causing them to be effective as reducing agents, hydrogen donors, singlet oxygen quenchers and chelators for metals. Antioxidants that accumulate in neuronal tissues are potential candidates for the prevention and treatment of neuronal disorders involving oxidative stress [57]. As a result of extensive research that has revealed the neuroprotective potential of OPP and the finding that the hallmark of

Alzheimer disease is neuroinflammation and oxidative stress impacting neurotoxicity, we test the impact of OPP on rats fed a high fat diet using curcumin as the positive control.

CHAPTER 2

MATERIALS AND METHODS

2.1 Animals and diets

32 in-bred Brown Norway (BN) rats were obtained from the aged rodent colonies of the National Institute of Aging (Bethesda, MD). Since effects of estrogen on cholesterol metabolism might alter Aβ, we used male rats for this study. Upon arrival, 12 rats were 22 weeks old and 20 rats are 24 weeks old. They were assigned to 4 groups on a pseudo-random basis with the constraint that all diet groups had the same mean body weight. Four groups were named by their diets: control diet (n = 8), High fat diet (n=8), High fat with OPP (n=8) and High fat with Curcumin (n=8). The diet compositions are summarized in Table 1. Diets for four groups contained similar level of calorie. The purified diets were obtained in pelleted form from Dyets Inc. (Bethlehem, PA), and sufficient diet was obtained for the entire duration of the study. The diets were kept at -20°C, and sufficient diet was removed weekly as needed and kept refrigerated at 4°C. Animals were fed ad libitum and had free access to tap water.

Briefly, twice weekly food intake and weekly body weight were recorded to monitor health status (Table 2). Urine samples of each rat were collected once a month for urinary metabolomic profiling. Once a month, all rats were subjected to a five-day test of spatial learning/memory using Morris's water maze. Monthly urine collections were carried out for a urinary metabolomic study. All procedures and protocols were in accordance with and ratified by the Animal Investigation Committee of Wayne State University.

Table 1: Composition of purified diets

Ingredient	isocaloric Control	High Fat (2% Cholesterol)	High Fat+5% OPP	High Fat+ 2%Curcumin
		g/kg		
Casein	140	140	140	140
L-Cystine	1.8	1.8	1.8	1.8
Sucrose	100	77.5	77.5	77.5
Cornstarch	465.692	465.692	415.692	445.692
Dyetrose	155	155	155	155
Soyabean oil	40	40	40	40
t-butylhydroquinone	0.008	0.008	0.008	0.008
Cellulose	50	50	50	50
Mineral Mix#210050	35	35	35	35
Vitamin Mix#310025	10	10	10	10
Choline Bitartrate	2.5	2.5	2.5	2.5
Cholesterol	_	20	20	20
Cholic Acid	_	2.5	2.5	2.5
OPP	_	_	50	_
Curcumin	_	_	_	20
Total(g)	1000	1000	1000	1000

Diets were prepared and pelleted by Dyets Inc. (Bethlehem, PA).

Table 2: Study timeline based on the overall study

Procedures	Frequency of Measurement
Body weight, diet intake, water intake	Twice weekly (Week 1-6)
Urine collection	Once weekly (Week 2,4,6)
Blood and tissue collection	End of study (Week 6)

2.2 Spatial Learning Test

A Morris Water Maze test (MWM) was conducted to examine spatial learning and memory. Rats were tested in a circular pool, 60 inches in diameter and 30 inches deep, filled with 10 inches of water made opaque by the addition of a non-toxic dye and maintained at 24-25o C. The swimming pool was placed in a room surrounded by fixed spatial cues such as posters, floor lamp and desk. The rats were trained to locate the hidden escape platform submerged in the water. Latencies for rats to find the platform (sec) were analyzed as a measure of spatial learning. The Rats were given 3 trials/day for 5 consecutive days. Rats were allowed 90 s to swim around the pool and find the hidden platform. If the rat did not find the platform within this time, it was gently rescued from the water and placed on the platform. Each trial was separated by 45 min. The platform was always located in the same fixed spot during training and the starting point also remained the same among the trials for each rat. All trials were recorded by a video camera mounted above the pool and the behavioral measures were acquired by a computerized video-tracking system (EthoVision 2.0, Noldus Information Technology, Leesburg, VA).

2.3 Tissue collection

All rats were sacrificed by decapitation. Trunk blood was collected into K2EDTA coated tubes and kept on ice. Organs were excised and wet weight was recorded prior to flash freezing in liquid nitrogen. Brain was extracted immediately (generally within 3-5 minutes) and the left hemisphere was flash frozen in liquid nitrogen. Right hemispheres of all rats were immersion-fixed in 10% neutral buffered formalin (Fisher Scientific International) for 48 hours and then were transfer to 70% Ethanol before staining. Hippocampus and cerebral cortex were extracted from the rest of the right hemispheres and fixed in RNAlater (Ambion, inc. Austin, TX) for RNA extraction.

2.4 Histology

All the right hemisphere brain tissues preserved in 10% neutral buffered formalin were sent to Histology lab, Division of Human Pathology, Michigan State University for Hematoxylin and eosin stain (H&E) staining and Congo red staining. Nuclei were counter stained by H&E for visualizing the morphological changes of the neurons. Congo red staining was performed to visualize extracellular amyloid deposition in the hippocampus on the brown Norway rats. All the slides were observed under the microscope (Nikon Eclipse 80i).

2.5 Enzyme-linked Immunosorbent Assay

This assay involves a two part process; firstly the whole tissue extraction and running the assay. The Colorimetric BetaMark x-42 ELISA kit was purchased from Covance (Princeton, New Jersey, USA). All the reagents, standard dilutions, control and samples were reconstituted according Covance protocol. The whole brain tissues of all the rats in the four groups; control (C), high cholesterol (H), high cholesterol and oil palm phenolics (HP) and high cholesterol and

curcurmin (HC) were homogenized individually in TBS with protease inhibitors and EDTA. 70% formic acid was added to the homogenate, to mix we agitated the tissue by pipetting up and down. We spun the mixture of each group for 20 minutes at 350,000 g. The supernatant which represents the whole brain extract was retrieved for the assay. The supernatant was stored in -80° C. To start running the assay the sample were removed from -80° C and thawed on ice. 300 µL of 1X wash buffer were added to each well, then dumped out after which the plate is pat dried on clean paper towel. On day 1, 50µL of each standard was added to the plate in triplicate. 50µL of each sample was added in triplicate as well as 50 µL to each well of diluted HRP detection antibody. The plate was then covered with a plate sealer. The plate was mixed on a plate shaker for one minute and then incubated overnight at 8°C. On day 2, the plate was removed from the refridgerator and the contents are dumped out. The plate was then washed with 300µL of 1X wash buffer per well. The buffer was then dumped and the plate pat dried with clean paper towel. The plate was washed repeatedly with the 1X wash buffer 4 more times for a total of 5 washes. 200µL of TMB substrate was added to each well. The plate was then incubated for 50 minutes at room temperature in the dark. The optical density of each plate was determined using a Bio-tek Elx800 micro-plate reader set at a wavelength of 620nm. The concentration of the unknown (β42) was calculated using the standard curve.

2.6 Statistics

All statistical analyses were performed using SPSS 15.0 for Windows® and SmartWiewerTM, (SPSS Inc. Chicago, IL). Data were analyzed using one- or two-way ANOVA with the age and diet as between-subject factors followed by Tukey post-hoc tests. Comparisons between the diets in the same age group were analyzed using student t-test. For MWM test, the

trial day was the repeated within-subject factor and diet was the between-subject factors. Results are presented as the means ± SEM.

CHAPTER 3

RESULTS

3.1 DIET AND WATER MAZE

We were successful in feeding the rat models isocaloric diets across the control, high cholesterol, high cholesterol and OPP, high cholesterol and curcumin groups. There was no significant gain or loss of weight during the 23 week period of feeding and weighing of the animal models (figure 5 and 6). The Morris water maze experiment revealed longer escape latency time initially for the high cholesterol group but this improved from day 3 (figure 7). Subsequently, we observed significant improvement in escape latency time when month 1 was compared to month 5 (figure 8).

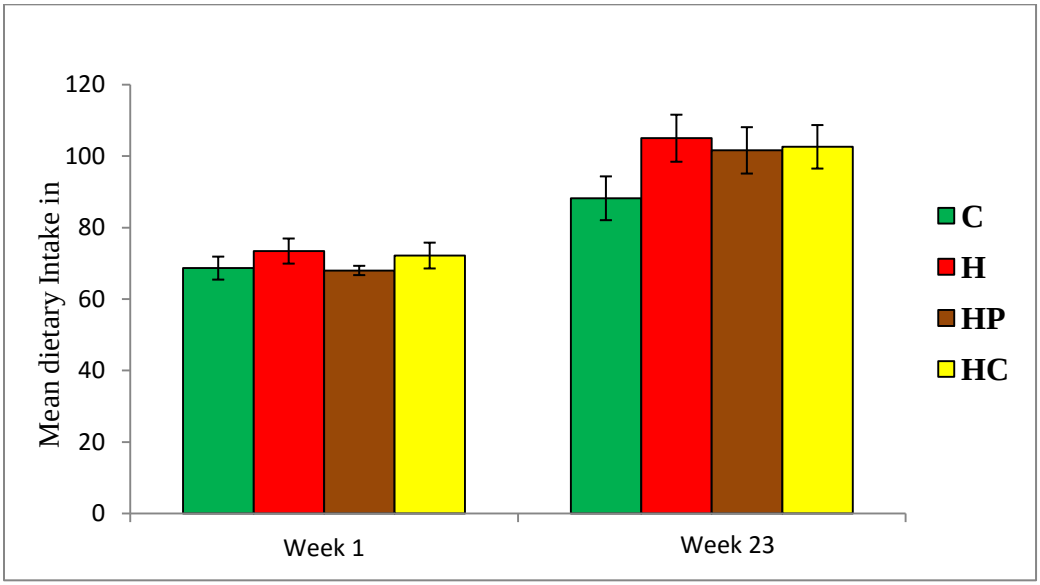

Figure 5: Mean dietary Intake per group consumed at week 1 and week 23.

Results represent mean + SE.

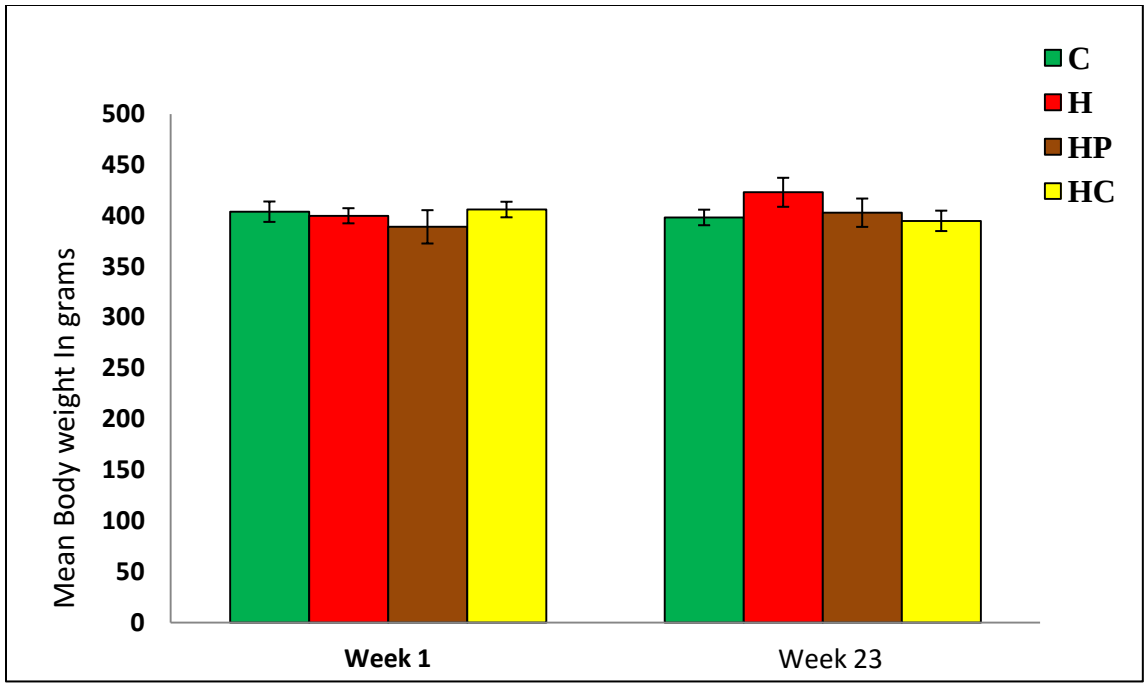

Figure 6: Mean body weight of rats per group at week 1 and Week 23.

Results represent mean + SE.

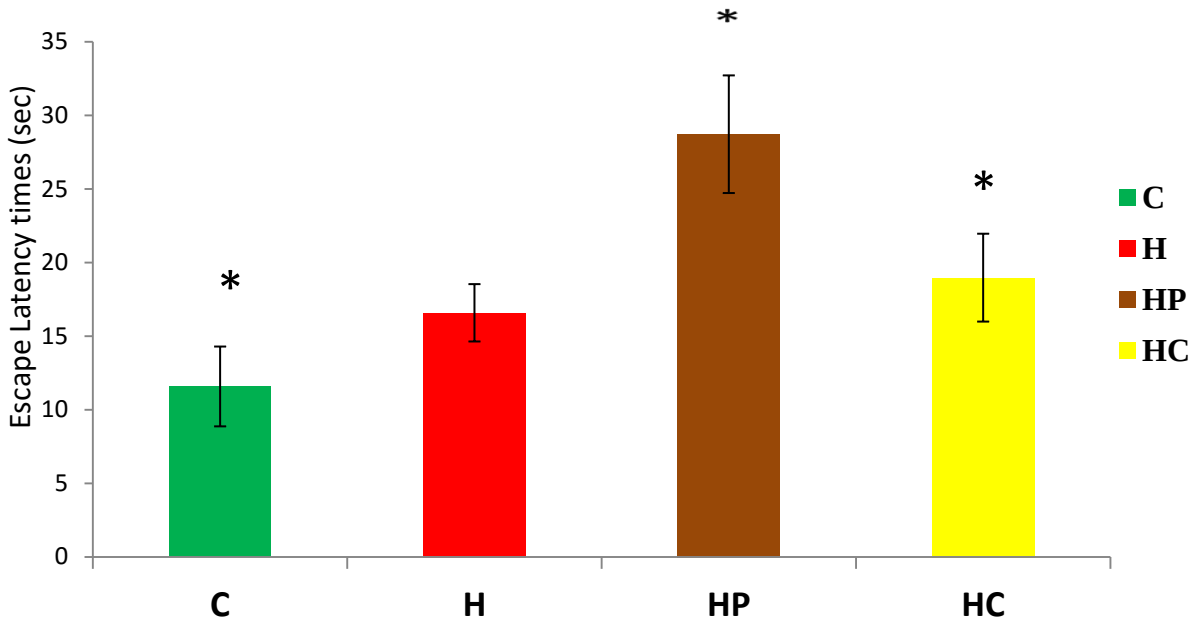

Figure 7: Escape Latency times at month 1 of Morris water maze.

* P < 0.05 when compared with control group. Statistical analysis ANOVA with Tukey's procedure was used SPSS software

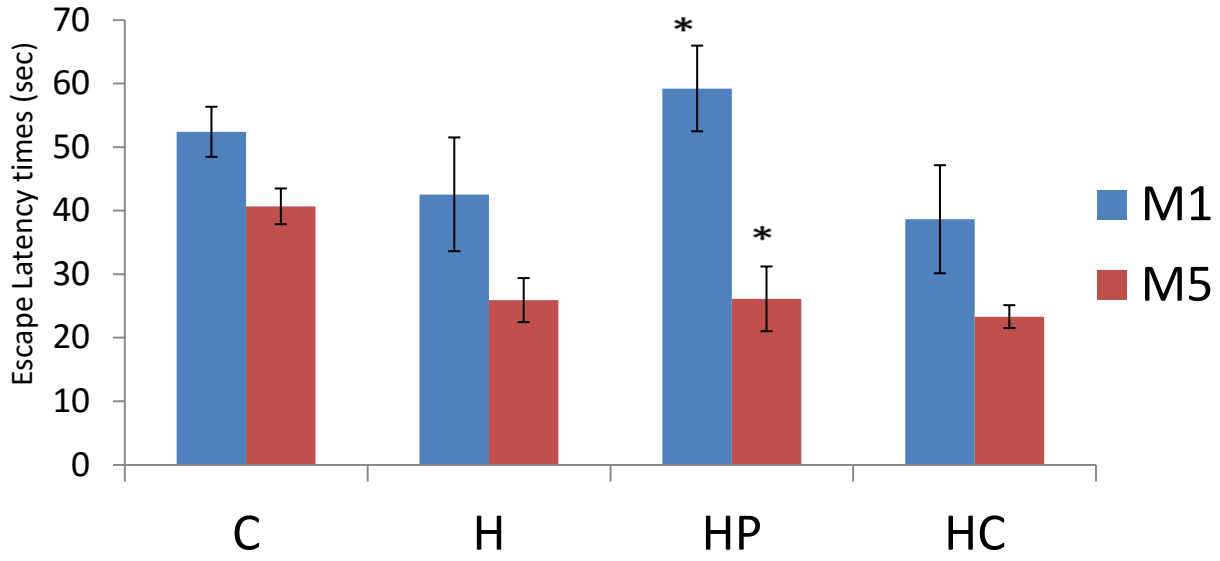

Figure 8: Morris Water Maze.

Improvement in Escape Latency (EL) using Morris water maze test in the OPP fed group (HP) from month 1 (M1) to month 5 (M5) of the study.

3.2 HISTOLOGY AND ELISA

When viewed under the Nikon microscope the H & E stained slides reveal more dead neurons and neuronal in the control and high cholesterol group compared to the control, high cholesterol +OPP and high cholesterol+ curcumin groups ($p<0.05$) (figure 9). We also noticed a higher concentration of dead neurons in the CA1 zone of the hippocampus. A significant decrease in healthy neurons was observed in the high cholesterol group (figure 10). The high cholesterol group contained the highest area of beta amyloid plaque deposition (figure 11).

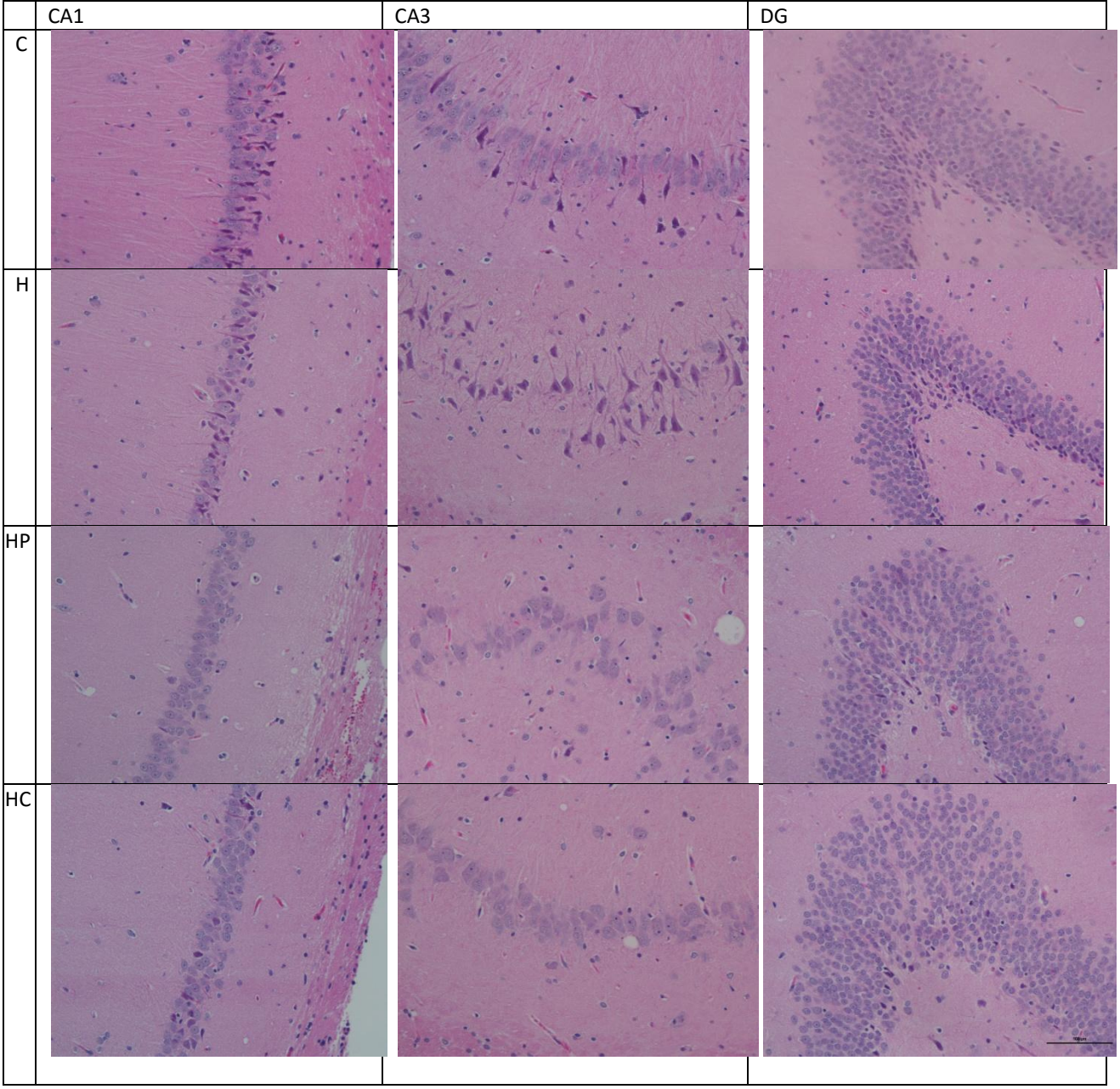

Figure 9: **Dead Neurons.**

This figure shows the presence of dead neurons (pyramidal shaped, intensely colored) in the hippocampal areas; CA1, CA3 and DG of the animal groups; C= Control, H=High Cholesterol, HP= High Cholesterol+ OPP, HC= High Cholesterol+ Curcumin

The Congo red stained slides detected extracellular plaque formation, predominantly in the high cholesterol group as observed through the microscope. Plaque deposition in the control group was negligible (figure 12). High cholesterol diet induced plaque was visualized in the high cholesterol group, high cholesterol + OPP group and high + curcumin group (figure 13, 14 and 15).

In addition, the congo red stained slides showed that the high cholesterol group had the highest average plaque load (% hippocampus area) compared to other two supplemented high cholesterol diet (Figure 16 and 17). ELISA was performed to identify the amyloid peptide and quantify the beta amyloid plaque that was observed (Figure 18).

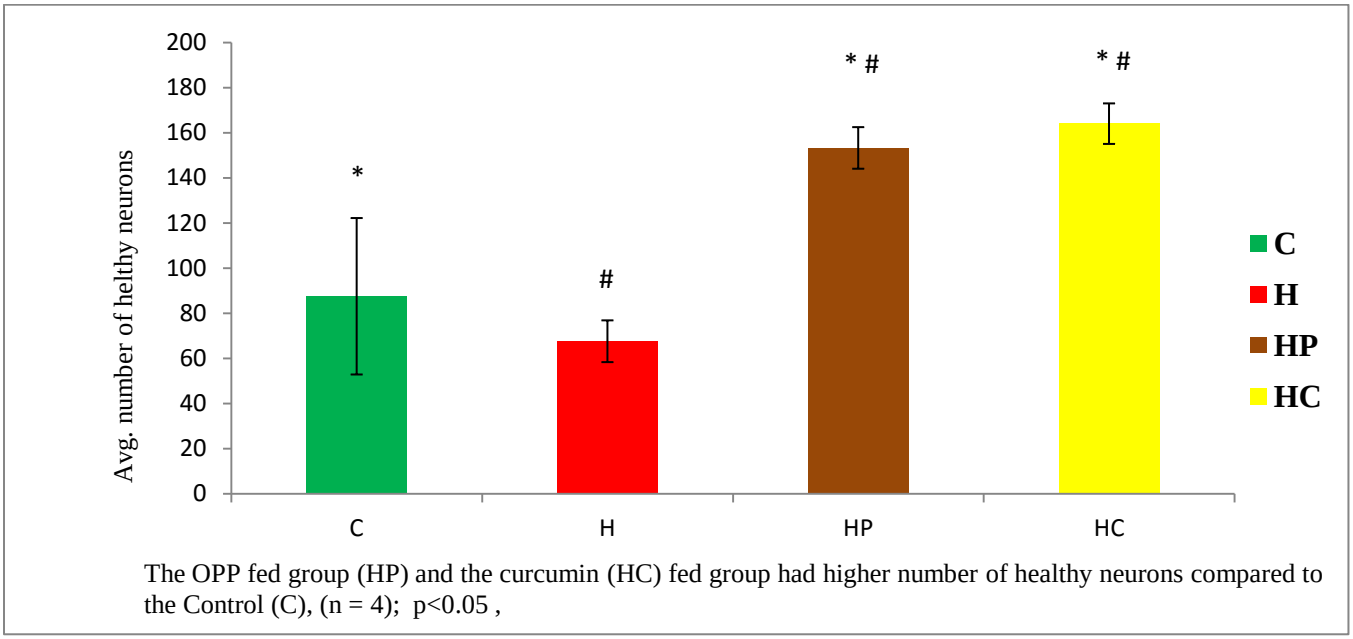

Figure 10: Number of healthy neuron s in the hippocampus

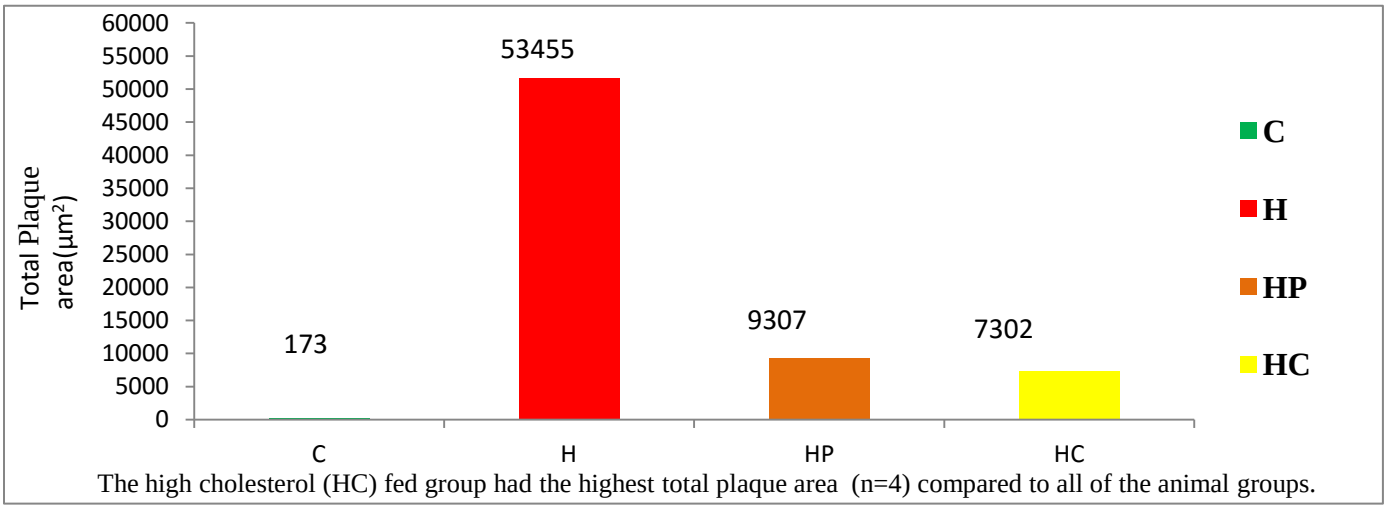

Figure 11: Sum of the area of individual plaque concentration in each group

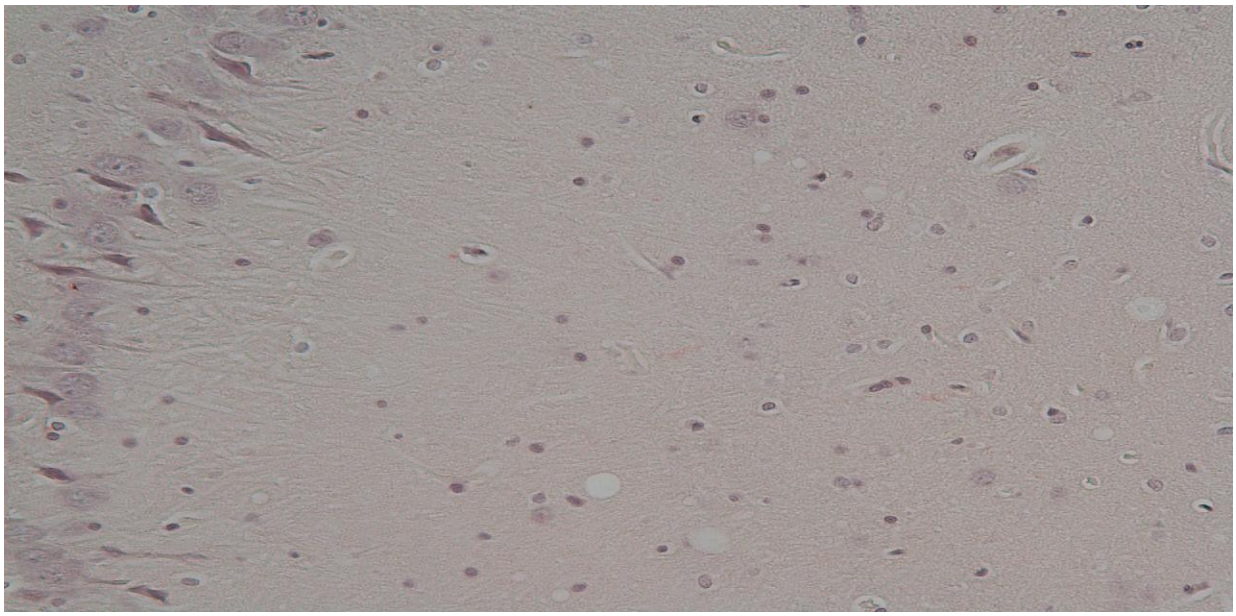

Figure 12: Congo Red stained slide of the Control group (C) revealing negligible traces of amyloid plaques.

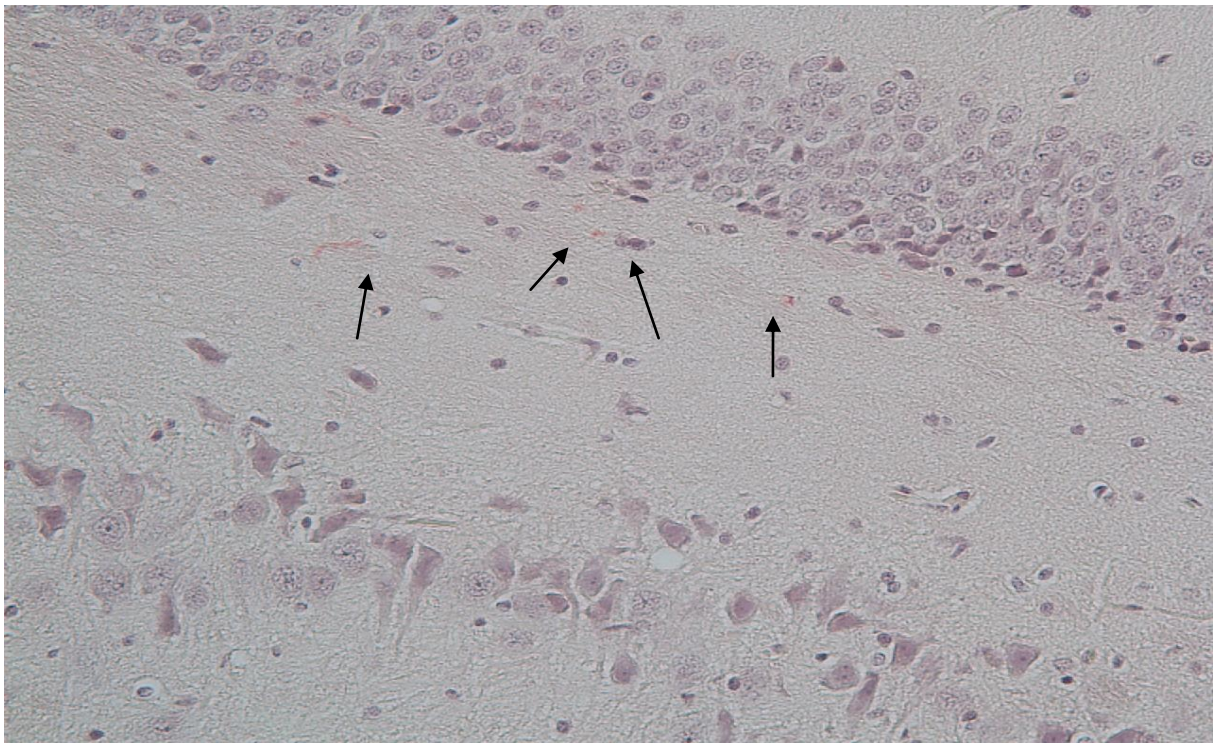

Figure 13: Congo Red stained slide of the High Cholesterol group (H) showing traces of amyloid deposition, indicated by the arrows.

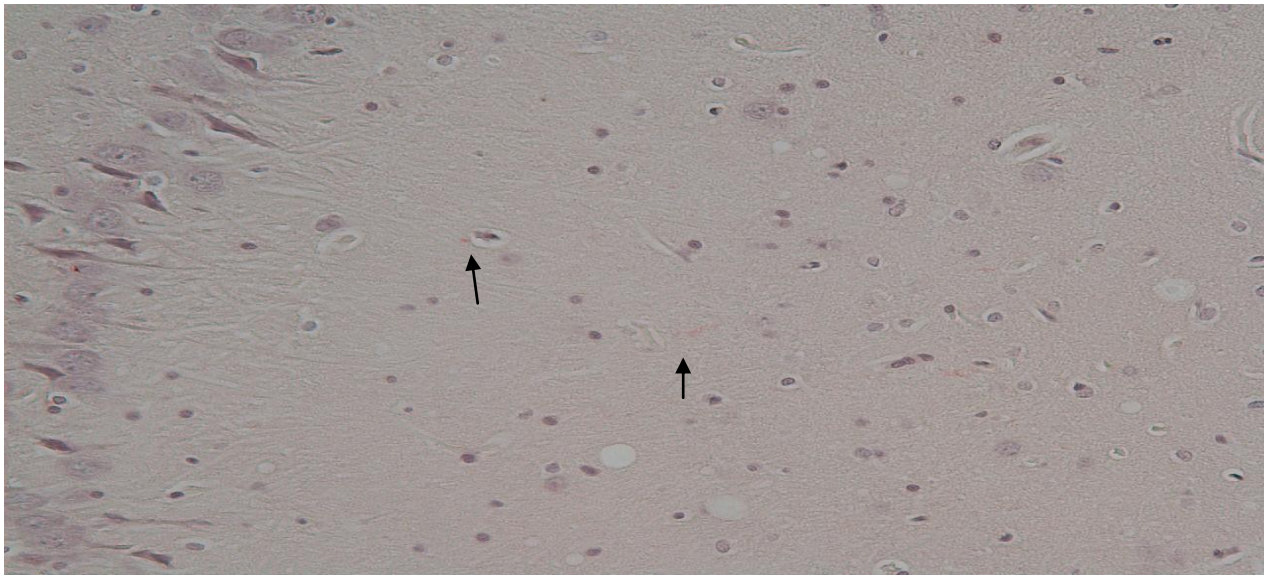

Figure 14: Congo Red stained slide of the High Cholesterol + OPP group (HF) showing few traces of amyloid deposition, indicated by the arrows.

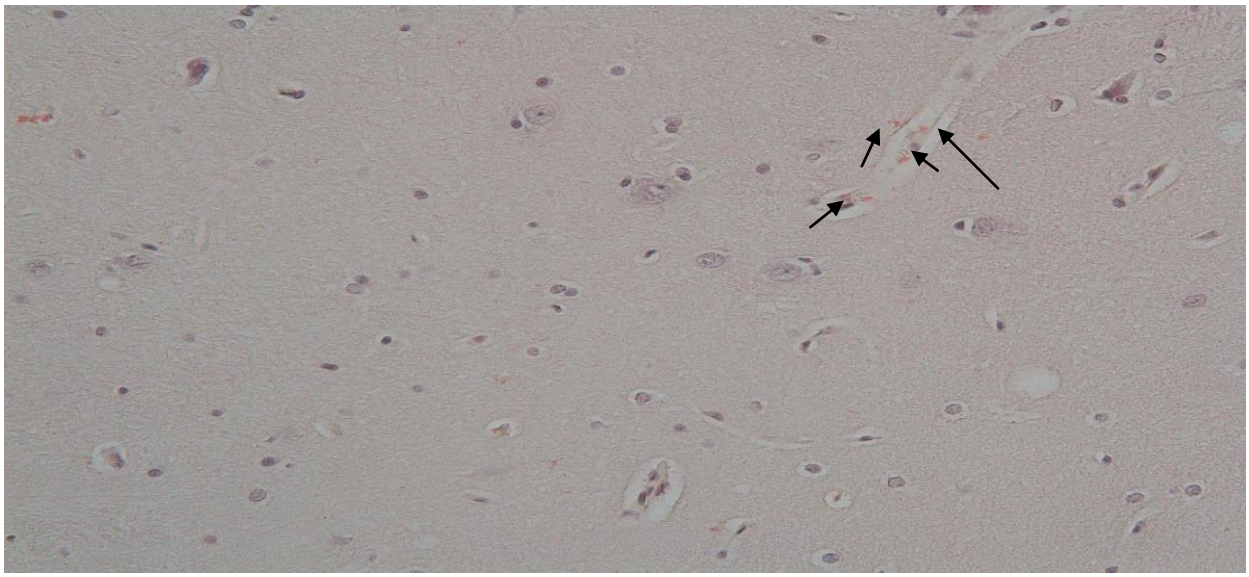

Figure 15: Congo Red stained slide of High Cholesterol + curcumin group (HC) showing few traces of amyloid plaques, indicated by the arrows.

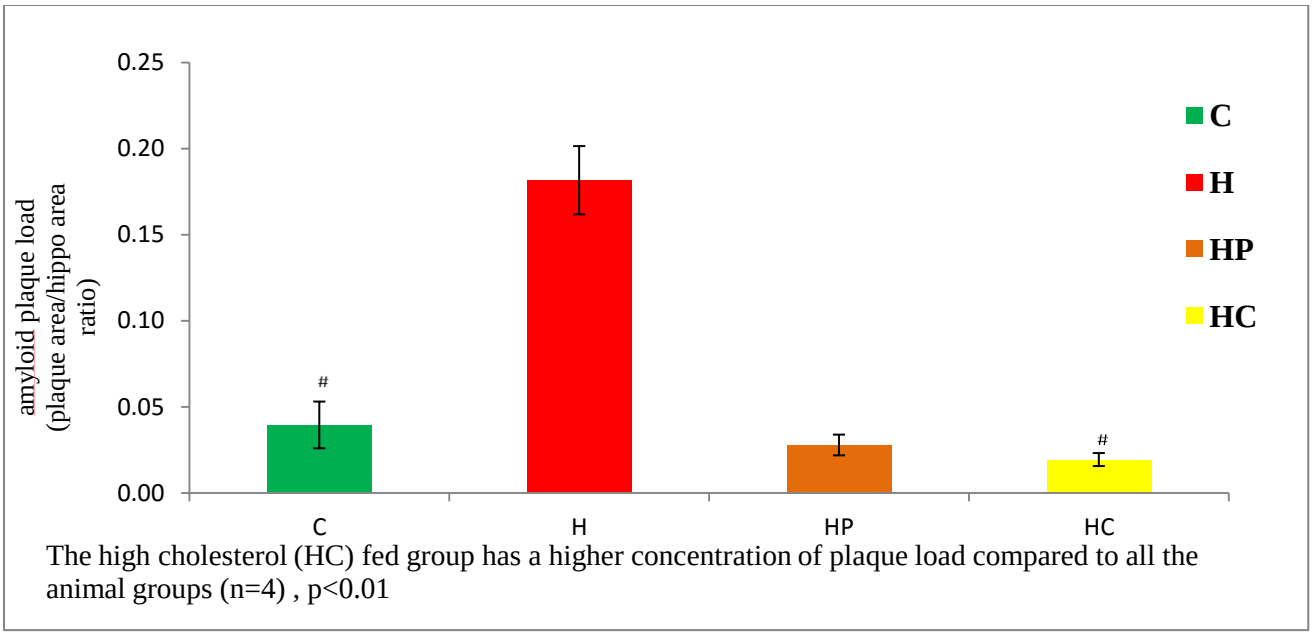

Figure 16: Amyloid plaque load

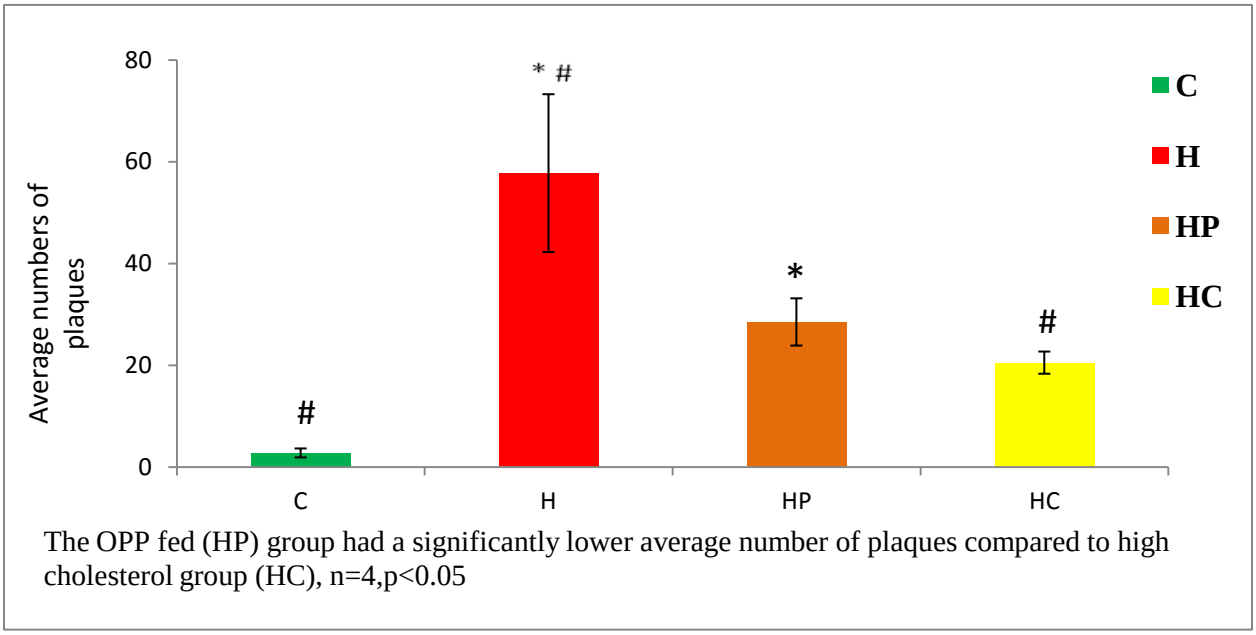

Figure 17: Average number of plaques.

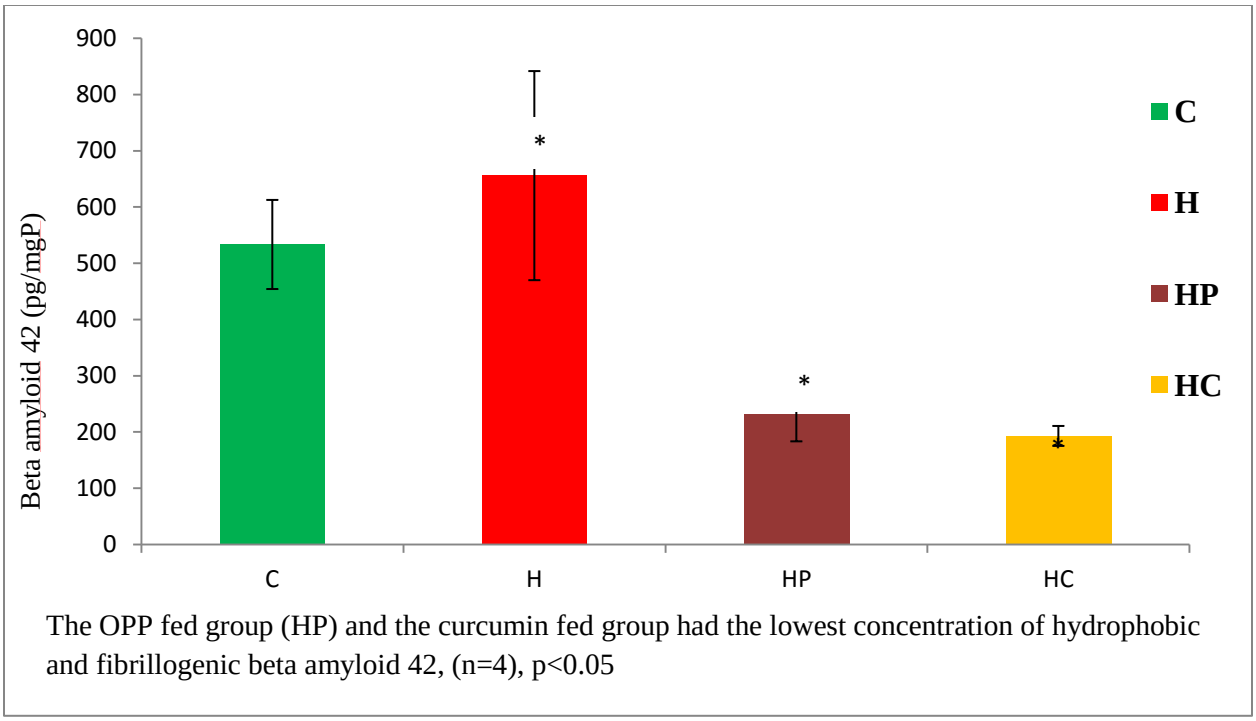

Figure 18: The concentration of β-amyloid 42 quantified via ELISA

CHAPTER 4

4.1 DISCUSSION

This study is part of a broader experiment being conducted by a PhD student focusing on the invivo effect of OPP on atherogenic diet induced rat models (figure 19). The goal of my study is to show histological evidence of the impact that a diet containing OPP will have on beta amyloid deposition in the hippocampus of aged Brown Norway rat model induced by a high fat diet. The results of our study is closely linked to that of Tara L Spires et al and West MJ et al were it was observed that selective damage seemed domicile in the CA1 zone compared to the CA3 zone. In our study dead and degenerated neurons were noticeable in the control and high cholesterol only group as depicted by the absorption of the dark blue dye vie H and E staining ; which histologically represents a diseased or dead neuron [41][42]. Congo red stain is a diagnostic tool for amyloidosis, it functions by binding to amyloid fibrils which can subsequently be observed under a microscope [43][44]. Congo red staining enabled visualization of extracellular amyloid deposition in the hippocampus.

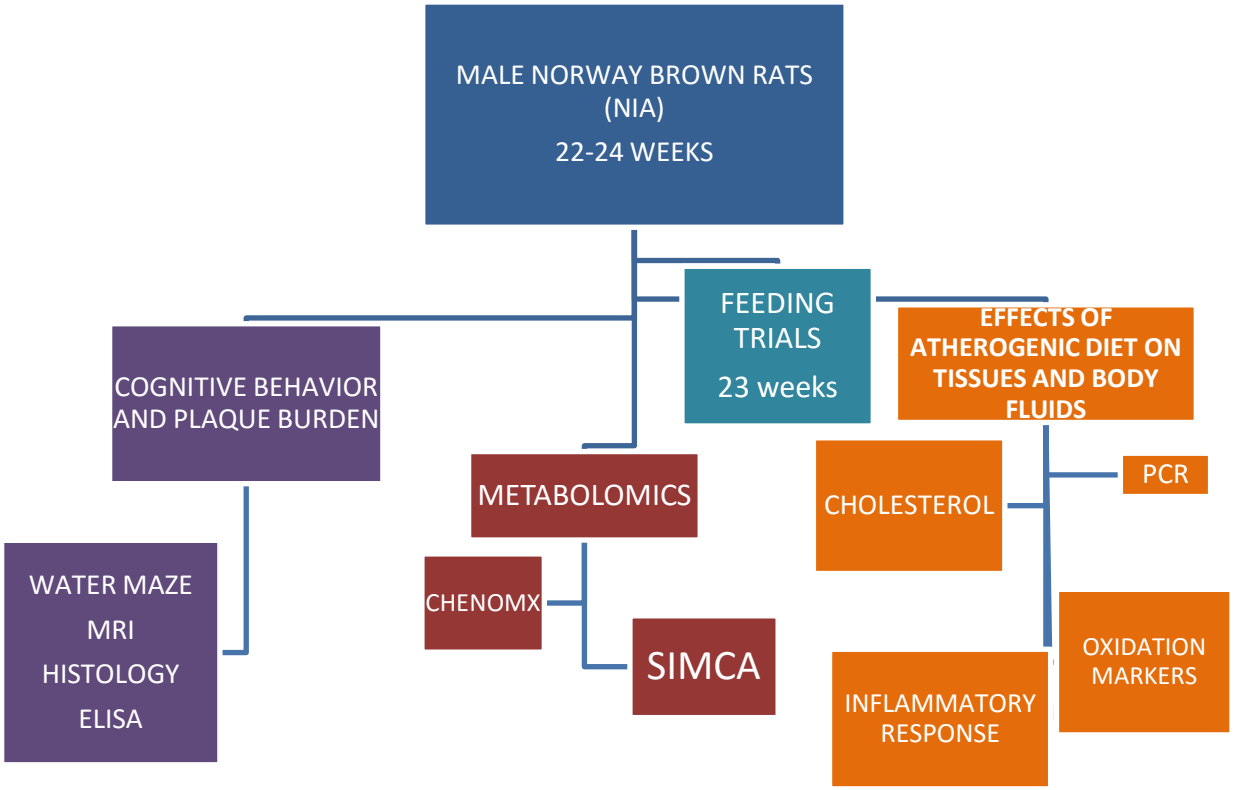

Figure 19: LAYOUT OF STUDY

In our study the control group showed minimal presence of amyloid deposition, this should not be the case because aging should impact the normal metabolism of beta amyloid peptides. Therefore, there should have been some accumulation of beta amyloid plaques in the hippocampus in the control group. This is an exception. It is likely that viewing the slides under the microscope failed to capture the plaque load in the control group because the ELISA conducted revealed plaque load in the control group. The ELISA proved to be a more sensitive and specific test due to its ability to reveal the hydrophobic beta amyloid peptide load undetected by Congo red staining. It could also be possible that the amyloid plaques observed were strictly

due to intake of the high cholesterol diet. Other epidemiology studies have revealed that ingestion of diets high in fat and cholesterol can be linked increased cases of dementia. In these studies the form of fat responsible for the presentation of AD tended to be saturated in nature [45]. Linoleic acid which is a form of omega-6-polyunsaturated fat was not linked to AD. Although the mechanism in which high cholesterol diet impacts cognitive function is yet to be known, it has been proposed by other extensive research that activation of protein kinase C and phosphorylation of protein F by high unsaturated fat lead to higher memory storage as a result of increased synaptic plasticity. In addition other studies have shown that cholesterol lowering drugs decreased the deposition of beta amyloid and othe AD related disease states. [46][47]. In our study, high fat diet had successfully induced the AD-like pathologies both behaviorally (i.e. Learning /memory deficits) and histologically (i.e. Aβ plaque formation in hippocampus). In addition, aging might also serve as a factor by accelerating the AD pathology, given the fact that all of our rats arrived at 22-24 month of age. So the elevated peripheral hypercholesterolemia and a higher liver and brain cholesterol observed by my colleagues working on this project could accelerate memory and brain damage, which confirm the opinion that high cholesterol diet is one of the risk factors that cause AD-like brain metabolic changes. In the current study,the aged rats amplified the effects of the diet in the model. It can be clearly put that our diet treatment effectively created hypercholesterolemia condition in the Brown Norway rat model and this effect was synergistically accelerated by aging effect. The test botanical, OPP clearly diminished not only the beta amyloid peptides but was neuro-protective since histologically more healthy neurons were observed in the rats that ingested this phenolic substance. The results seen in the rats fed OPP plus high cholesterol were similar to that of the curcumin fed group which served as our positive control. Curcumin was well suited as our positive control since literature has

reported its ability to prevent Aß fibril plaque formation and protect the neurons from being attacked by Aß oligomers and fibrils [48]. Curcumin has been widely studied for its clinical benefits as an anti-inflammatory agent, an anti-amyloidogenic, an antioxidationt and an anticarcinogen [49]. In previous studies, curcumin has been successful in suppressing the pro-apotoptic action of vasoactive peptide endothelin-1 (ET-1) which is increased in the human brain tissue presented in AD pathology [50]. It's mechanism of action facilitating this inhibition of ET-1 is still being researched. The anti-inflammatory, anti-oxidant and anti-amyloidogenic properties of curcumin have also been tested in a study performed in transgenic mice, Tg2576 that were provided a curcumin diet for 6 months [51]. It was observed that there was a significant reduction in IL-1β, GFAP (an astroglia marker), plaque load , soluble and insoluble beta amyloid peptides. Based on the aforementioned studies, we utilized curcumin as a positive control to test the neuroprotective properties of OPP. There is limited data on the effect of OPP in AD but it has been reported to possess anti-diabetic, anti-carcinogenic and antioxidant properties [52]. To test for possible cardioprotective effects of OPP, Sambanthamurthi et al. carried out in vitro LDL oxidation studies and they found that OPP inhibited the Cu-mediated oxidation of human LDL. It was concluded that OPP have an antioxidant protective role against free radical damage, which represents a new source of phenolic bioactives [49]. In order to test its neuro-protective effects, Leow et al. fed the mice model; BALB/c with an OPP infused diet and then investigated the effects of OPP on cognitive and motor functions. It was observed that better cognitive function and spatial learning was achieved when tested in a water maze. That study proposed that OPP can improve the neuron damage by up-regulating the genes involved in the regulation of the neurotrophic factor and meanwhile, down-regulating the genes involved in inflammation [50]. The main limitation of this study was the challenges faced with the microscope used to visualize

the congo red stained species and the reality that more research needs to be conducted using OPP. In addition, it is quite clear that we are yet to conduct transcriptomic analysis on the brains and then identify the gene expression changes. This will generate clues to help explain how OPP confers these neuro-protective effects. This study was meant to be exploratory rather than confirmatory , there are currently no extensive studies to demonstrate the effects of OPP on the high fat induced AD animals.

4.2 Conclusion

Data from the histological analysis and ELISA in this study did demonstrate that high fat diet induced beta amyloid deposition in the hippocampus. The neurotoxic species, Aβ42, was clearly prominent in the high cholesterol group and noticeably reduced in the rats fed high cholesterol plus OPP and curcumin respectively. OPP has significant neuro-protective properties as it effectively improved the spatial learning abilities of rats on high cholesterol diet. These improvements correlate to its neuron protective effect and its ability to lower amyloid load in hippocampus. Based on our study results, OPP can improve cognitive impairment, reduce neuron loss and decrease beta amyloid deposition in the hippocampus. Findings from this study has provided evidence for more research to geared towards investigating genetic regulation pathways and other innovative strategies to address the impact of OPP in AD models. It may also be noted that since late onset AD accounts for about 87% of this form of dementia, more studies can be conducted to investigate the impact of OPP on ApoEε4.

REFERENCES

1. Viswanathan A, Rocca WA, Tzourio C. Vascular risk factors and dementia: How to move forward? Neurology 2009;72:368–74
2. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition. Washington, D.C.: American Psychiatric Press, 1994.
3. Types of Dementia (2013). Cleveland Clinic. Retrieved October 13, 2013, http://my.clevelandclinic.org/disorders/dementia/hic_types_of_dementia.aspx
4. Maurer K., Maurer U. (2003). Alzheimer: The Life of a Physician and Career of a Disease. New York: Columbia University Press. ISBN 0-231-11896-1.
5. Murphy S. L., Xu J. Q., Kochanek K. D. National Vital Statistics Reports. Vol. 4. Hyattsville, Md, USA: National Center for Health Statistics; 2013. Deaths: final data for 2010
6. Prevalence of dementia in the United States: the aging, demographics, and memory study. Plassman BL, Langa KM, Fisher GG, Heeringa SG, Weir DR, Ofstedal MB, Burke JR, Hurd MD, Potter GG, Rodgers WL, Steffens DC, Willis RJ, Wallace RB Neuroepidemiology. 2007; 29(1-2):125-32.
1. Natural products as a rich source of tau-targeting drugs for Alzheimer's disease Laurent Calcul,[1] Bo Zhang,[2] Umesh K Jinwal,[3] Chad A Dickey,[2] and Bill J Baker[1]
2. Cholinesterases: new roles in brain function and in Alzheimer's disease. Giacobini E[1] . Neurochem Res. 2003 Apr;28(3-4):515-22.
3. Int J Mol Sci. 2015 Nov 4;16(11):26417-51. doi: 10.3390/ijms161125961. Alzheimer's Disease: Mechanism and Approach to Cell Therapy.Amemori T1, Jendelova P2,3, Ruzicka J4, Urdzikova LM5, Sykova E6,7.
4. Phosphorylation affects the ability of tau protein to promote microtubule assembly. Lindwall G, Cole RD. J Biol Chem. 1984 Apr 25;259(8):5301-5.
5. Neurodegenerative tauopathies. Lee VM[1], Goedert M, Trojanowski JQ. Annu Rev Neurosci. 2001;24:1121-59

6. Science. 1998 Dec 4;282(5395):1914-7. Mutation-specific functional impairments in distinct tau isoforms of hereditary FTDP-17. Hong M[1], Zhukareva V, Vogelsberg-Ragaglia V, Wszolek Z, Reed L, Miller BI, Geschwind DH, Bird TD, McKeel D, Goate A, Morris JC, Wilhelmsen KC,Schellenberg GD, Trojanowski JQ, Lee VM
7. Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, Beyreuther K. Amyloid plaque core protein in Alzheimer disease and Down syndrome. Proc Natl Acad Sci U S A. 1985;82:4245–4249.
8. Gamma-secretase is a membrane protein complex comprised of presenilin, nicastrin, Aph-1, and Pen-2.Kimberly WT, LaVoie MJ, Ostaszewski BL, Ye W, Wolfe MS, Selkoe DJ
   Proc Natl Acad Sci U S A. 2003 May 27; 100(11):6382-7.
9. Aβ43 is neurotoxic and primes aggregation of Aβ40 in vivo.
   Burnouf S, Gorsky MK, Dols J, Grönke S, Partridge L.,Acta Neuropathol. 2015 Jul;130(1):35-47. doi: 10.1007/s00401-015-1419-y. Epub 2015 Apr 11.
10. De Strooper, B. Proteases and proteolysis in Alzheimer disease: A multifactorial view on the disease process. Physiol. Rev. 2010, 90, 465–494
11. Alzheimer's Disease: Mechanism and Approach to Cell Therapy.Amemori T, Jendelova P, Ruzicka J,Urdzikova LM,Sykova E.IntJMol Sci. 2015 Nov 4;16(11):26417-51. doi: 10.3390/ijms161125961. Review
12. A disintegrin-metalloproteinase prevents amyloid plaque formation and hippocampal defects in an Alzheimer disease mouse model.
   Postina R, Schroeder A, Dewachter I, Bohl J, Schmitt U, Kojro E, Prinzen C, Endres K, Hiemke C, Blessing M, Flamez P, Dequenne A, Godaux E, van Leuven F, Fahrenholz F
   J Clin Invest. 2004 May; 113(10):1456-64.
13. Evidence for excitoprotective and intraneuronal calcium-regulating roles for secreted forms of the beta-amyloid precursor protein.

Mattson MP, Cheng B, Culwell AR, Esch FS, Lieberburg I, Rydel RE

Neuron. 1993 Feb; 10(2):243-54.

14. Cellular actions of beta-amyloid precursor protein and its soluble and fibrillogenic derivatives.

Mattson MP Physiol Rev. 1997 Oct; 77(4):1081-132.

15. Singh PP, Singh M, Mastana SS (2006). "APOE distribution in world populations with new data from India and the UK". Annals of Human Biology 33 (3): 279–308.

16. The genetics of Alzheimer's disease. Bertram L, Tanzi RE ; Cold Spring Harb Perspect Med. 2012 Oct; 2(10): a006296

17. Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy ; Chia-Chen Liu, Takahisa Kanekiyo, Huaxi Xu & Guojun Bu

Nature Reviews Neurology 9, 106-118 (February 2013)

18. Selfridge J.E., Lezi E., Lu J., Swerdlow R.H. Role of mitochondrial homeostasis and dynamics in Alzheimer's disease. Neurobiol. Dis. 2013;51:3–12. doi: 10.1016/j.nbd.2011.12.057.25.

19. Santos, C.R.A.; Cardoso, I.; Goncalves, I. Key enzymes and proteins in amyloid-β production and clearance. In Alzheimer's Disease Pathogenesis—Core Concepts, Shifting Paradigms and Therapeutic Targets; de la Monte, S., Ed.; InTech: Shanghai, China, 2011; pp. 53–86.

20. Citron M, Westaway D, Xia W, Carlson G, Diehl T, Levesque G, Johnson-Wood K, Lee M, Seubert P, Davis A, Kholodenko D, Motter R, Sherrington R, Perry B, Yao H, Strome R, Lieberburg I, Rommens J, Kim S, Schenk D, Fraser P, St George Hyslop P, Selkoe DJ (January 1997). "Mutant presenilins of Alzheimer's disease increase production of 42-residue amyloid beta-protein in both transfected cells and transgenic mice".Nat. Med. 3 (1): 67–72. doi:10.1038/nm0197-67.PMID 8986743.

21. Biomed Res Int. 2015; 2015:748212. doi: 10.1155/2015/748212. Epub 2015 Oct 15.Working Memory and Executive Function Decline across Normal Aging, Mild Cognitive Impairment, andAlzheimer's Disease.

Kirova AM[1], Bays RB[2], Lagalwar S[3].

22. Marksteiner J, Hinterhuber H, Humpel C. Cerebrospinal Fluid Biomarkers for Diagnosis of Alzheimer's Disease: Beta-amyloid(1–42), Tau, Phospho-tau-181 and Total Protein. Drugs of Today. 2007;43(6):423–31
23. De Meyer G, Shapiro F, Vanderstichele H, et al.Diagnosis-Independent Alzheimer Disease Biomarker Signature in Cognitively Normal Elderly People. Archives of Neurology. 2010;67(8):949–56
24. Sui X, Liu J, Yang X (2014). "Cerebrospinal fluid biomarkers of Alzheimer's disease". Neuroscience Bulletin (Review) 30 (2): 233–42
25. Mendez MF. The Accurate Diagnosis of Early-onset Dementia. International Journal of Psychiatry in Medicine. 2006;36(4):401–412.
26. Klafki HW, Staufenbiel M, Kornhuber J, Wiltfang J. Therapeutic Approaches to Alzheimer's Disease.Brain. 2006;129(Pt 11):2840–55.
27. Dementia: Quick Reference Guide (PDF). London: (UK) National Institute for Health and Clinical Excellence. November 2006. ISBN 1-84629-312-X.Archived (PDF) from the original on 27 February 2008. Retrieved 22 February 2008.
28. Schroeter ML, Stein T, Maslowski N, Neumann J.Neural Correlates of Alzheimer's Disease and Mild Cognitive Impairment: A Systematic and Quantitative Meta-nalysis involving 1,351 Patients. NeuroImage. 2009;47(4):1196–1206.
29. Rabins PV, Blacker D, Rovner BW, et al. American Psychiatric Association practice guideline for the treatment of patients with Alzheimer's disease and other dementias. The American Journal of Psychiatry. December 2007;164(12 Suppl):5–56.
30. Diet, cognition, and Alzheimer's disease: food for thought.
Otaegui-Arrazola A, Amiano P, Elbusto A, Urdaneta E, Martínez-Lage P.Eur J Nutr. 2014 Feb; 53(1):1-23. Epub 2013 Jul 27

31. Inhibitory activities of major anthraquinones and other constituents from Cassia obtusifolia against β-secretase and cholinesterases. Jung HA, Ali MY, Jung HJ, Jeong HO, Chung HY, Choi JS.J Ethnopharmacol. 2016 Jun 14. pii: S0378-8741(16)30396-8. doi: 10.1016/j.jep.2016.06.037
32. Doody RS, Stevens JC, Beck C, et al. Practice parameter: management of dementia (an evidence-based review). Report of the Quality Standards Subcommittee of the American Academy of Neurology.Neurology. 2001;56(9):1154–1166.
33. Pohanka M. Cholinesterases, a target of pharmacology and toxicology. Biomedical Papers of the Medical Faculty of the University Palacký, Olomouc, Czech Republic. 2011;155(3):219–229.
34. Nutrition and the risk of Alzheimer's disease. Biomed Res Int. 2013; 2013: 524820. Nan Hu, [1] Jin-Tai Yu, [1,2,*] Lin Tan, [1] Ying-Li Wang, [1] Lei Sun, [1] and Lan Tan ;Published online 2013 Jun 20. doi: 10.1155/2013/524820
35. Schroeder E. K., Kelsey N. A., Doyle J., Breed E., Bouchard R. J., Loucks F. A., et al. (2009). Green tea epigallocatechin 3-gallate accumulates in mitochondria and displays a selective antiapoptotic effect against inducers of mitochondrial oxidative stress in neurons. Antioxidants and Redox Signalling, 11(3), 469– 480.
36. Involvement of protein kinase C activation and cell survival/ cell cycle genes in green tea polyphenol (-)-epigallocatechin 3-gallate neuroprotective action.
    Levites Y, Amit T, Youdim MB, Mandel S
    J Biol Chem. 2002 Aug 23; 277(34):30574-8.
37. Polyphenolic Antioxidants and Neuronal Regeneration.
    Ataie A, Shadifar M, Ataee R.
    Basic Clin Neurosci. 2016 Apr;7(2):81-90. doi: 10.15412/J.BCN.03070201. Review.
38. Resveratrol-induced activation of the mitogen-activated protein kinases, ERK1 and ERK2, in human neuroblastoma SH-SY5Y cells.
    Miloso M, Bertelli AA, Nicolini G, Tredici G
    Neurosci Lett. 1999 Apr 2; 264(1-3):141-4

39. SIRT1 deacetylase protects against neurodegeneration in models for Alzheimer's disease and amyotrophic lateral sclerosis.

Kim D, Nguyen MD, Dobbin MM, Fischer A, Sananbenesi F, Rodgers JT, Delalle I, Baur JA, Sui G, Armour SM, Puigserver P, Sinclair DA, Tsai LH

EMBO J. 2007 Jul 11; 26(13):3169-79.

40. Antioxidant pathways in Alzheimer's disease: possibilities of intervention.Viña J[1], Lloret A, Giraldo E, Badia MC, Alonso MD. Curr Pharm Des. 2011 Dec;17(35):3861-4.

41. Curry consumption and cognitive function in the elderly. Ng TP, Chiam PC, Lee T, Chua HC, Lim L, Kua EH. Am J Epidemiol. 2006;164:898–906

42. Prasad S., Aggarwal B. B. Turmeric, the golden spice: from traditional medicine to modern medicine. In: Benzie I. F. F., Wachtel-Galor S., editors. Herbal Medicine: Biomolecular and Clinical Aspects. 2nd. chapter 13. Boca Raton, Fla, USA: CRC Press; 2011

43. Examining the potential clinical value of curcumin in the prevention and diagnosis of Alzheimer'sdisease.Goozee KG, Shah TM, Sohrabi HR, Rainey-Smith SR, Brown B, Verdile G, Martins RN Br J Nutr. 2016 Feb 14;115(3):449-65. doi: 10.1017/S0007114515004687. Epub 2015 Dec 1.

44. Neuroprotective effects of curcumin on endothelin-1 mediated cell death in hippocampal neurons. Stankowska DL, Krishnamoorthy VR, Ellis DZ, Krishnamoorthy RR. Nutr Neurosci. 2015 Dec 12 Neuroprotective role of polyphenolsSpagnuolo C, Napolitano M, Tedesco I, Moccia S, Milito A, Russo GL.Curr Top Med Chem. 2016 Feb 4.

45. Curcumin and Apigenin - novel and promising therapeutics against chronic neuro-inflammation in Alzheimer's disease.Venigalla M, Gyengesi E, Münch G. Erratum in: Neural Regen Res. 2015 Dec;10(12):2017.

46. Oil palm phenolics confer neuroprotective effects involving cognitive and motor functions in mice ; Soon-Sen Leow, Shamala Devi Sekaran, YewAi Tan, Kalyana Sundram, Ravigadevi Sambanthamurthi

Nutr Neurosci. 2013 September; 16(5): 207–217. doi: 10.1179/1476830512Y.0000000047

47. Reddy AC, Lokesh BR. Effect of dietary turmeric (Curcuma longa) on iron-induced lipid peroxidation in the rat liver. Food Chem Toxicol. 1994;32:279–283.

48. Sreejayan, Rao MN. Nitric oxide scavenging by curcuminoids. J Pharm Pharmacol. 1997;49:105–107.

49. Curcumin Promotes A-beta Fibrillation and Reduces Neurotoxicity in Transgenic DrosophilaI na Caesar, Maria Jonson, K. Peter R. Nilsson, Stefan Thor, Per Hammarström

PLoS One. 2012; 7(2): e31424. Published online 2012 February 13. doi: 10.1371/journal.pone.

50. Curcuminoids enhance amyloid-beta uptake by macrophages of Alzheimer's disease patients.

51. Zhang L, Fiala M, Cashman J, Sayre J, Espinosa A, Mahanian M, Zaghi J, Badmaev V, Graves MC, Bernard G, Rosenthal ,J Alzheimers Dis. 2006 Sep; 10(1):1-7.

52. Strimpakos AS, Sharma RA. Curcumin: preventive and therapeutic properties in laboratory studies and clinical trials. Antioxid Redox Signal. 2008;10:511–545

53. The up-regulation of BACE1 mediated by hypoxia and ischemic injury: role of oxidative stress and HIF1alpha.

Guglielmotto M, Aragno M, Autelli R, Giliberto L, Novo E, Colombatto S, Danni O, Parola M, Smith MA, Perry G, Tamagno E, Tabaton M

J Neurochem. 2009 Feb; 108(4):1045-56

54. Spires TL, Orne JD, SantaCruz K, Pitstick R, Carlson GA, Ashe KH, et al. Region-specific dissociation of neuronal loss and neurofibrillary pathology in a mouse model of tauopathy. Am J Pathol. 2006;168:1598–1607

55. West MJ, Kawas CH, Martin LJ, Troncoso JC. The CA1 region of the human hippocampus is a hot spot in Alzheimer's disease. Ann N Y Acad Sci. 2000;908:255–259.

56. Steensma, D. P. (2001). "Congo Red: Out of Africa?" (pdf). Archives of Pathology and Laboratory Medicine 125 (2): 250–252.doi:10.1043/0003-9985(2001)125<0250:CR>2.0.CO;2.

57. Dietary Fats and the Risk of Incident Alzheimer Disease FREE

58. Martha Clare Morris, ScD; Denis A. Evans, MD; Julia L. Bienias, ScD; Christine C. Tangney, PhD; David A. Bennett, MD; Neelum Aggarwal, MD; Julie Schneider, MD; Robert S. Wilson, PhD

59. Kalmijn SFeskens EJLauner LJKromhout D Polyunsaturated fatty acids, antioxidants, and cognitive function in very old men. Am J Epidemiol.1997;145:33-41.

60. Wong KLMurakami KRouttenberg A Dietary cis-fatty acids that increase protein F_1phosphorylation enhance spatial memory. Brain Res.1989;505:302-305.

61. Fassbender KSimons MBergmann C et al Simvastatin strongly reduces levels of Alzheimer's disease β-amyloid peptides Aβ42 and Aβ40 in vitro and in vivo. Proc Natl Acad Sci U S A.2001;98:5856-5861.

62. Antioxidative and Neuroprotective Effects of Curcumin in an Alzheimer's Disease Rat Model Co-Treated with Intracerebroventricular Streptozotocin and Subcutaneous D-Galactose.
Huang HC, Zheng BW, Guo Y, Zhao J, Zhao JY, Ma XW, Jiang ZF.
J Alzheimers Dis. 2016 Apr 5

63. Sambandan TG, Rha CK, Sinskey AJ, Sambanthamurthi R, Tan YA, Sundram K, Wahid MB (inventors) Malaysian Palm Oil Board (assignee) (2010) Composition comprising caffeoylshikimic acids, protocatechuic acid, hydroxytyrosol, hydroxybenzoic acid and their derivatives and method of preparation thereof. World patent application publication WO 2010137943

64. Leow SS, Sekaran SD, Sundram K, Tan YA, Sambanthamurthi R. Oil palm phenolics attenuate changes caused by an atherogenic diet in mice. Eur J Nutr. 2012; Published online DOI 10.1007/s00394-012-0346-0

ABSTRACT

HISTOLOGICAL EVIDENCE OF THE EFFECT OF OIL PALM PHENOLICS IN ATHEROGENIC DIET INDUCED RAT MODEL OF ALZHEIMER'S DISEASE

by

KENECHUKWU UCHENNA MONPLAISIR

August 2016

Advisor: Dr. Smiti Gupta

Major: Nutrition and Food Science

Degree: Master of Science

BACKGROUND: Alzheimer's disease (AD) is a neurodegenerative disease with the clinical presentation of memory loss and cognitive impairment. Alzheimer's disease pathology is the accumulation of beta amyloid plaques and neurofibrillary tangles.

METHOD: In this study atherogenic diet was used to induce AD in aged Brown Norway rats. The rats were assigned to the following four groups fed isocaloric diets; control group (C),high cholesterol diet (H), high cholesterol + oil palm phenolics group (HP), high cholesterol + curcumin group (HC). The impact of oil palm phenolics (OPP) on neuronal health and its effect on amyloid deposition was evaluated using histology and ELISA. The cognitive ability of the rats were tested using Morris water maze.

RESULTS: Our model was successful in facilitating the formation of extracellular beta amyloid plaques and causing neuronal loss. Histological findings revealed better neuronal health in the group of rats offered OPP in their diets compared to the high cholesterol only fed rats. The Morris water maze test showed better learning and cognition in rats fed the OPP diet.

CONCLUSION: OPP provided neuronal protection and decreased the deposition of beta amyloid plaques in the hippocampus of the rats.

AUTOBIOGRAPHICAL STATEMENT

Kenechukwu Uchenna Monplaisir was born in Detroit, Michigan on September 22, 1979. She received her early education in Nigeria, West Africa. In 2014 she earned her Bachelor of Science degree in both Pharmacy and Allied Health Sciences from the Eugene Applebaum College of Pharmacy at Wayne State University, Michigan. She had been practicing as a pharmacist for over 10 years then decided to embark on pursuing her passion of becoming a nutritionist in order help her patients achieve optimal health. She was accepted to pursue a Master of Science degree in Nutrition and Food Science at Wayne State University, Detroit, MI and would be completing her degree in August 2016. Upon completion of Masters, the author intends to practice as a nutrition specialist and health educator.