Investigation into the effect of Lemongrass on Beta Amyloid protein and its potential as a treatment to Alzheimer’s Disease

-Laura Hurst-
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1. Acknowledgements

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I would also like to show my appreciation to Anastasia Walker, who worked on a similar project, for her help in the upkeep of sometimes up to 15 flasks of cells. Her second opinions and observations of the cells were very insightful and her presence during the repetitive procedures of the project was certainly appreciated.

Finally, a special thank you to Dr John Dyer, for his guidance and support before and throughout the duration of this project. I am extremely grateful for all the hours Dr Dyer has spent helping me with this project, especially towards the final week of the project. This project would not have been a success without him.
2. Abstract

This project’s main purpose is to explore the potential neuroprotective effects of lemongrass (*Cymbopogon Citratus*) and how these effects can be utilised in the treatment of Alzheimer’s disease. The rates of this disease have greatly increased over the past few decades and so the development of new pharmaceuticals is increasingly important to society. To test the hypothesis of lemongrass having neuroprotective effects two well plates were set up with neuroblastoma cultures, one of which had beta amyloid protein (one of the key pathological markers of Alzheimer’s disease) added. Three different solutions of lemongrass essential oil were also added (0.1%, 0.5%, and 1%) as well as two control groups containing either F-12 Ham’s nutrient media only or 100% ethanol. The results of the experiment suggested that an increase in lemongrass solution reduced the concentration of cells per mm² but increased the viability of the cells in the amyloid beta protein plate. 0.5% lemongrass solution almost doubled the viability of the neuroblastoma from 37.04% in the media only control group to 68.61%. These results support both the Amyloid hypothesis and the hypothesis established for this project, and so it can be concluded that lemongrass has potential as a treatment to Alzheimer’s disease if further research is carried out.

3. Introduction

3.a. Alzheimer’s Disease – Background

Alzheimer’s disease is a neurodegenerative disease which results in the progressive decline of higher order cognitive function [1]. What this means is that Alzheimer’s causes the death of neurons over time in different areas of the brain, leading to trouble with complex brain functions such as memory, language, and the understanding/control of emotion.

Alzheimer’s disease is the most common cause of dementia [2]. A common misconception is that dementia and Alzheimer’s are synonymous; which isn’t quite accurate. Dementia describes a certain set of symptoms involving mental deterioration and can have many causes such as stroke – which leads to vascular dementia- and Pick’s disease – which is called frontotemporal dementia [3].

In the UK 835,000 people had dementia in 2014, two thirds of which were women [4]. The reason for the higher proportion of people with dementia being female was initially explained by the fact that women have a slightly higher life expectancy than men (83.0 years for females and 79.3 years for males) [5]. One of the biggest risk factors for developing Alzheimer’s disease is age [6]. Therefore, it seems logical that women, who live longer on average, are more likely to have Alzheimer’s. However, research has also shown that Alzheimer’s can start developing 10-15 years before the appearance of symptoms [7] therefore the few years extra a woman lives cannot fully explain the increased risk. Observations have been made, such as the presence of the ApoE4 gene (which has been firmly linked to Alzheimer’s) having a more profound effect on female carriers than their male counterparts [8]. Why this is the case is unknown, but suggestions have been made pointing to the potential protective role of oestrogen and how it’s decrease in levels after menopause may be a viable reason for the increased risk of older women [9] [10].

In 2006, 26.6 million people had Alzheimer’s disease [11]. By 2011, this value increased to 33.9 million and is expected to triple by 2050 [12]. If a drug or alternative form of intervention could halt the progression of Alzheimer’s disease for just a year, levels could drop by 9.2 million [11].
The estimated global cost of dementia was $604 billion (£497 billion) in 2010 [13]. In the UK alone the financial cost of dementia is £26.3 billion per annum, which would be enough to pay for every household in the country's annual energy bill [4].

From the graph, it is clear to see that deaths caused by many diseases such as stroke, heart disease and HIV have decreased over the years - thanks to excellent advances in technology and research. Sadly, the same cannot be said about Alzheimer’s disease, which has seen an immense 71% increase of people dying with the disease. This increase in rates can be attributed the ageing population [15] which means more people than ever before are at a high risk, and as our understanding of the disease has improved, it has led to an increase in the numbers of diagnoses made.

It is however, important to note that Alzheimer’s disease is not exclusive to the elderly. Early onset Alzheimer’s - which occurs in people under 65 years [16] - affects up to 1 in 20 sufferers of dementia [4].

With the increasing prevalence of dementia and Alzheimer’s disease, the importance of learning more and responding to the disease is being realised. Currently, there is no cure for Alzheimer’s and -except for familial Alzheimer’s, where a mutation in a gene is involved- the cause is unknown [17] [18] [16]. Drugs have been developed to slow the progression and symptoms are well documented, but, the diagnosis rate in England for dementia is only 48% [4]. Meaning over half of people with dementia and their families are not aware of it.

The symptoms of Alzheimer’s disease include memory loss, issues with speech, trouble with sleeping, increased agitation, confusion and an inability to perform gestures [4] [16] [19]. This makes Alzheimer’s difficult to diagnose as many of these symptoms can occur in either normal ageing, or in other mental diseases. It is believed that 10% to 30% of patients are misdiagnosed [18]. In fact, it isn’t until a patient’s autopsy that Alzheimer’s disease can be positively diagnosed, which isn’t exactly reassuring for patients [16].
3.b. Alzheimer’s Disease- Pathology

Alzheimer’s disease and its pathology was first described in 1906 by German neuropathologist Alois Alzheimer. For about 5 years he observed his patient: 51-year-old Auguste D. Auguste was admitted to the Frankfurt hospital in 1901 as she suffered an array of symptoms such as paranoia, memory-loss, aggressiveness, insomnia, aphasia (condition that affects the comprehension of and the ability to use language [20]), and auditory hallucinations [21] [22]. Cognitive decline was viewed as a normal event during aging by doctors at the time but Auguste’s good health and young age suggested a more complex reason for her symptoms. She would struggle to answer simple questions for her husband’s name and even her own surname. On her second day in Alzheimer’s psychiatric hospital, she famously told him that “I have lost myself”, a simple but melancholic quote that many sufferers and their families identify with today [16] [23].

In 1906, when Auguste D died, Alzheimer did an autopsy to look at her brain. It was there that he found Auguste’s brain to be very different from a healthy brain of someone her age. Amidst the brain cells Alzheimer found extracellular amyloid plagues and neurofibrillary tangles. He believed it was the abnormal presence of these that caused the cognitive symptoms Auguste D suffered with. [16] [21] [22].

Alzheimer presented his findings at the 37th Meeting of South-West German Psychiatrists in November 1906, but disappointingly for Alzheimer, his revelation of this mysterious disease was met with a rather lukewarm response [21] [22]. However, thanks to Alois Alzheimer’s initial discovery much research has been done on the disease and we now understand that the amyloid plaques and neurofibrillary tangles found by Alzheimer are key pathological markers in Alzheimer’s disease [7]. Auguste D is the first person to be diagnosed with what is now understood as early onset Alzheimer’s disease [16].

How these plaques and tangles occur and how they cause Alzheimer’s disease is not yet completely understood, however a lot of research over the past century has led to many suggestions and hypothesises being made on the mechanisms behind the formation of these abnormal lesions.

One of the well-known and influential hypothesises is called the ‘Amyloid Cascade Hypothesis’ which was proposed in 1991 by John Hardy and David Allsop [25].

This hypothesis suggests that the accumulation of beta amyloid protein (Aβ) into insoluble senile plaques is one of the main causes, or triggers, for the onset of Alzheimer’s disease [25] [26].

Beta amyloid, Aβ is formed when APP (amyloid precursor protein) is proteolyzed by enzymes known as β-secretase and γ-secretase at specific sites [27]. Firstly, the β-secretase cleaves the APP at the β site into two molecules of C99 and APPsβ. Following this the γ-secretase then cleaves the APPsβ into Aβ proteins [28]. The short, sticky Aβ then clump together into the senile plaques frequently found in the brains of people with Alzheimer’s disease.
One of the major genetic risk factors of Alzheimer’s disease is the possession of the ApoE4 allele of the ApoE (apolipoprotein) gene. This version of the gene has been found to not be as effective as the ApoE3 and the ApoE2 alleles at clearing the Aβ proteins from the brain [16] [8] [28]. This significant increase in risk of Alzheimer’s disease supports the Amyloid hypothesis as the APOE4 gene leads to the production and eventual aggregation of Aβ.

Usually it is another enzyme that proteolyzes APP. This enzyme is α-secretase, it cleaves the APP at a different site than β-secretase to produce C83 and APPsα. When the APPsα is cleaved by γ-secretase it will not form the neurotoxic Aβ proteins [28].

A lot of research has been done into the β-secretase enzyme in the hopes of developing a possible inhibitor to prevent it producing the APPsβ and consequently reduce the levels of Aβ. Unfortunately, not a lot is known about the enzyme other than its role in Alzheimer’s, but BACE (beta-site-APP-cleaving-enzyme) has been shown to both reduce levels of APPsα and increase β-secretase activity. So this could potentially be the same enzyme as the β-secretase [28].

The other oddity Alois Alzheimer found were what are known as neurofibrillary tangles. These are made of tau protein. Tau proteins are very important in neurones, they make up the microtubules running along the neurone from soma to axon. Their role is to provide stability, flexibility and act as a transport network for all the nutrients the cell needs. However, in Alzheimer’s disease, the tau proteins undergo a modification which leads to it being hyper-phosphorylated. All the tau proteins in the microtubule fall away and accumulate into the intracellular neurofibrillary tangles, leading to the death of the neurone. The neurofibrillary tangles are strongly associated with Alzheimer’s because, unlike Aβ proteins, the progression of the accumulation of tangles corresponds to the progression of symptoms [7] [29] [30].

Acetylcholine (ACh) was the first neurotransmitter discovered [31] [32]. It is found all over both the peripheral nervous system (PNS) and the central nervous system (CNS). The function of ACh is well-understood in the cholinergic neurons of the PNS such as in the neuromuscular junction, but not as much is known about its role in the CNS [33]. It is believed to be vital for cognitive processes such as learning and memory formation and a cholinergic projection has been found from the basalis of Meynert to the forebrain neocortex (neocortex is where many higher order cognitive functions occur). This projection is known to degenerate in cases of Alzheimer’s and the nucleus basalis of Meynert is affected in all cases of dementia [32] [16].

ACh is synthesised by the enzyme choline acetyltransferase (CAT) from acyl-CoA and choline. It is then hydrolysed by the enzyme acetylcholinesterase (AChE) after binding to receptors in the synaptic cleft into acetate and choline [32] [33]. AChE has a very high catalysis rate [31] [32], and its ability to regulate levels of ACh is extremely important. Without AChE, the ACh neurotransmitter would accumulate and constantly bind to the post synaptic receptors. When this occurs in the neuromuscular junction it leads to muscle spasms, paralysis and death by asphyxiation. Many AChE inhibitors have been developed as insecticides and even biological weapons such as Sarine for this reason [32] [34].

AChE inhibitors aren’t all bad, they are especially useful in the treatment of Alzheimer’s disease. In patients with Alzheimer’s the levels of ACh are significantly lower- up to a 90% decrease- than other healthy people [31] [35]. Developing AChE inhibitors to prevent the hydrolysis of this neurotransmitter in the synapse can help elevate the concentrations back to a normal level and so can help to alleviate some of the symptoms [35].
3.b.1. Drugs

There isn’t a cure yet for Alzheimer’s disease, current pharmaceuticals are only capable of slowing its progression and reducing some of the symptoms [17].

As mentioned previously, most of the drugs used to treat Alzheimer’s disease are AChE inhibitors. Donepezil, galantamine, rivastigmine, and tetrohydroaminoacridine (THA or tacrine) are all AChE inhibitors. Memantine is the exception, it regulates levels of a different neurotransmitter important for learning and memory called glutamate [36] [31].

Tacrine, donepezil and rivastigmine are approved and commonly used to treat the cognitive symptoms of Alzheimer’s disease in Europe [37]. Memantine (Ebixia), which can treat moderate to severe Alzheimer’s, is not an AChE inhibitor. Rather, it is a non-competitive inhibitor N-methyl-D-aspartate (NMDA) receptor antagonist. It helps to regulate levels of the neurotransmitter glutamate, which are too high and lead to neurodegeneration in people with Alzheimer’s disease [36] [38] [39].

None of these drugs are without their side effects. The AChE inhibitors frequently affect the gastrointestinal tract with symptoms of vomiting and altered bowel movements. Symptoms of memantine include headache, dizziness, bowel issues and somnolence (drowsiness) [36] [38]. THA (or tacrine) was the first drug approved for Alzheimer’s disease, but due to the side effects of increasing the levels of enzymes in the liver and thus damaging the liver- THA is currently unlicensed in the UK [40] [41].

3.b.2. Natural compounds

A lot is still left to be done in developing drugs to cure Alzheimer’s with minimal side effects. Natural compounds may be a solution. Plenty of drugs used today were developed from natural compounds, examples such as aspirin which is chemically similar to compounds in white willow [42], morphine derived from opium poppy [43] and many anti-cancer drugs [44]. In fact, ‘Up to 50% of approved drugs in the last 30 years are either directly or indirectly from natural products’ [45].

Curcumin (Curcuma Longa), found in the commonly used spice turmeric, has gained much attention recently for its potential in treating Alzheimer’s disease. It has a wide array of medicinal properties; anti-microbial, anti-inflammatory, anti-cancer and so has been used as a natural remedy in India for over 2500 years [46] [47]. Rates of Alzheimer’s disease in 70-79 year olds are 4.4 times lower in India than in the USA [46]. This has led many scientists to consider curcumin- which is prevalent in Asian diets- as a reason for this significant difference. Curcumin’s anti-cytotoxic nature is of particular interest, as it means curcumin
has the potential to clear and prevent the formations of senile beta-amyloid plaques from the brain [46].

Huperzine A is found in Chinese club moss (*huperzia serrata*) and has been commonly used in Chinese herbal medicine [49] [50]. A synthetic version of huperzine A; Huprine X, has been developed as a drug for Alzheimer’s disease since it has been shown to improve cognitive function in people with Alzheimer’s disease [49]. It too is an AChE inhibitor, with 1200 times the binding affinity of THA and 40 times the affinity of donepezil [50] [51].

3.b.III. Lemongrass, *Cymbopogon Citratus*

*Cymbopogon* is a genus of around 55 species. *Cymbopogon citratus* commonly referred to as lemongrass- originates from Sri Lanka and South India, but is a common herb in all South-East Asia and the Americas [53] [54]. Lemongrass has had many different uses in these regions both culinary and medicinally. The fragrant leaves are often used as a herb or spice as well as for making herbal teas. The citrus smell of the plant also means it is commonly used in perfumes and cosmetics. In India, the plant is more often used in aromatherapy [54]. The essential oil contains mostly citral, the aldehyde responsible for the plants lemony aroma [54] [55].

In natural medicine, lemongrass and another plant of the same genus called *Cymbopogon Schoenanthus*, otherwise known as camel grass, are used to treat a wide variety of ailments. Both have shown anti-bacterial properties and so the root of lemon grass is chewed in many parts of the world to prevent tooth decay [56]. The citral in lemongrass also has anti-nociceptive properties similar to that of morphine, and so has often been used for pain relief [56] [57]. Anxiolytic and anti-mutagenic properties of lemongrass have also peaked interest recently for treating anxiety and some genetic diseases such as cancer [54] [56].

The properties of most interest in terms of Alzheimer’s disease however, are the anti-oxidative, anti-inflammatory and anti-cholinesterase properties of *Cymbopogon Citratus/Schoenanthus* [54] [56] [57] [58].

Oxidative stress occurs when there is an imbalance in the levels of free radicals and antioxidants [59]. In Alzheimer’s, oxidative stress in the brain may occur and even affect the key pathological markers: amyloid plaques and neurofibrillary tangles. This stress damages the neurones and eventually can cause cell death [60].

There is also a relationship between inflammation and many neurodegenerative diseases including Alzheimer’s disease [56]. Brains of patients with Alzheimer’s are known to shrink to a much smaller size than a healthy brain [61] but before this shrinkage occurs, many regions of the brain become inflamed. Aβ plaques and hyper phosphorylated tau found in Alzheimer’s disease are the most likely culprits of this inflammation [62]. The inflammation then contributes to the further progression of the disease as it leads to mass cell degeneration in the brain [62] [63].

Curcumin also shares these anti-oxidative and anti-inflammatory properties which are possible explanations for how it- and potentially lemongrass- can treat Alzheimer’s disease. A big issue with using these however is ensuring that the compounds can cross the blood brain barrier into the brain as well as giving a big enough dose to even have an effect [46].
4. Methodology

**Hypothesis:** Lemongrass (*Cymbopogon citratus*) will protect the neurones in the affected brain of Alzheimer’s disease by preventing the cytotoxic effects of accumulated beta amyloid proteins.

For this project, neuroblastoma cells (seed culture SHSY-5Y) provided by the University of Liverpool were used. Neuroblastoma cells were preferred over ordinary neurones because of their cancerous properties. Since the neuroblastoma are more resistant to death and reproduce rapidly compared to healthy neurones. This makes cell culturing more feasible and the neuroblastoma can still hold an accurate representation of a model brain since they are neurones after all.

4.a. Aseptic Technique

As cell cultures (especially tissue cells) are highly affected or killed by any contaminants, it was of extreme importance to have a thorough aseptic technique throughout this project. The cells remained in a restricted area to reduce chances of uncontrollable contamination by people not involved in the project. If not in a sterile incubator, the neuroblastoma cells were in a class 2 microbiological cabinet. The biological cabinet keeps both the person working with the neuroblastoma safe (although the chances of the neuroblastoma doing harm are very small) and the neuroblastoma safe from external airborne contaminants. It does this by providing a constant airflow over the workspace which ensures any microbes stay up above the cells out of contact. To ensure the biosafety cabinet is sterile, once the monitor said, ‘airflow safe’ plenty of 70% ethanol solution was sprayed on all the surfaces and wiped with paper towel. When the cabinet is sterile, anything going inside also needed to be sterilised with 70% ethanol. This included all equipment, the flask containing the neuroblastoma, and the person working in the cabinet. Latex gloves and a clean isolated lab coat are sufficient, ensure that gloves are sprayed with the ethanol solution every time hands enter the biosafety cabinet. Keeping equipment inside the cabinet is ideal but confirm that there will be enough room to do work as well. When working in the cabinet, avoid sudden large movements and crossing arms as this can affect the internal airflow. The filter cap was kept on the flask of cells whenever possible and when pipetting in the flask avoid contact with any surfaces of the flask, longer pipettes (i.e. 5ml) are preferred rather than using multiple small pipette tips as opportunity for contamination will be reduced. Used flasks and pipette tips were discarded in bleach and waste liquid discarded in sodium hypochlorite (10,000ppm).

4.b. Culturing the Cells

If not being used, the neuroblastoma cells needed to be kept in a sterile incubator at 37°C and 5% CO₂. These conditions are to replicate the conditions which the neurones would naturally be found in within the human body.

Checked the cells under a light microscope every day, they should be adhered to the bottom of the flask and each cell should have dendritic projections from the soma. Checked for any contaminants in the culture and also checked if there were any dead cells by lightly tapping the flask, dead cells would float and may be in a different focal plane to the living neuroblastoma cells. If there’s a high number of dead cells contamination may have occurred or the nutrient media needs to be changed.

The nutrient media provided the cells with all the nutrients needed to survive, grow and reproduce. To make up the nutrient media added 50ml of foetal bovine serum, 5ml of Penicillin
Streptomycin mixture, 5ml of L-Glutamine and 5ml of non-essential amino acids to 500ml of F12-Ham’s nutrient rich media. The presence of the antibiotics should help kill any bacteria if the neuroblastoma culture did happen to become contaminated. The nutrient media was made in the biological safety cabinet and a portion divided into 50ml falcon tubes. This helped to avoid contamination occurring from the media. Stored the media in a refrigerator at 5°C labelled with the date.

The cells need fresh media every other day. For this project, the media was changed on Mondays, Wednesdays and Fridays. On Mondays and Wednesdays, when the cells would be left with the media for 48 hours they would receive 5ml of fresh nutrient media. On Fridays, they would be given 10ml of media to guarantee they had enough over the longer period of 72 hours.

The method for changing the nutrient media was very simple. Pre-warmed the falcon tube of nutrient rich media in the incubator containing the cells for 30 minutes. This was to make sure the cells and the media are at the same temperature to avoid the cells going into shock and consequently dying. Once warmed, sterilised and moved both the flask of cells and the falcon tube of media into the cabinet. Removed the flask’s lid and carefully aspirated the waste media, avoided touching the inside of the flask by aiming the pipette tip into one of the corners of the flask. Disposed of the waste media in the sodium hypochlorite. Then removed the lid of the media and pipetted the appropriate volume of fresh media into the flask. Placed lids back on as soon as possible, returning the flask’s lid takes priority, when lids were removed they were placed on the laminar flow bed so that the inside faced up- not in contact with any other surface. Then returned the flask of cells to the incubator and the nutrient media to the refrigerator.

4.c. Splitting the Cells

While in the flask, the neuroblastoma cells reproduce and less nutrients are available per cell, which eventually leads to many of the cells dying. To avoid this, the flask of cells was divided—or split—into multiple flasks. The cells needed to be split when the confluence was between 70-90%, meaning that 70-90% of the flask surface is occupied by cells when observed under a microscope.

The nutrient media as well as trypsin needed to be pre-warmed for 30 mins in the incubator first. The waste media can then be aspirated from the flask. Then added 100μl of trypsin per cm² of flask surface, the flasks used in this project were 25cm² and so 2500μl/2.5ml of trypsin was added. The cells had to be observed under the microscope. Trypsin is an enzyme and will cause the neurones to detach from the surface, when this occurred gently tapped or flicked the sides of the flask to guarantee as many cells had detached as possible. The trypsin needed to be neutralised after a maximum of 10 minutes, if left longer the trypsin would have started to hydrolyse the neurones. Neutralised the trypsin by adding an equal volume as trypsin of nutrient media to the flask (so for this project 2500μl/2.5ml of media) and then gently agitated the flask. Aspirated the solution of detached cells and placed in a centrifugation tube, centrifuged at 220 x g for 3 minutes. After centrifuging removed the supernatant, leaving a white pellet of the cells. Added 1ml of media and resuspended the cells by slowly pipetting up and down to break up the pellet. Then added a further ml of media for every extra flask (so for 4 flasks, added 3ml of media to the 1ml of cells suspended in media). Inverted the centrifugation tube to disperse the cells equally and then added 1ml of this to each
flask. Extra media was then added to each flask up to a total of either 5ml or 10ml depending on what day it was. All the flasks then needed to be returned to the incubator as soon as possible. On average a flask needed to be split into 4 every 4-7 days, however at the beginning of the project the cells grew rapidly and so flasks were occasionally split into 6.

4.d. Addition of Lemongrass Essential Oil

Lemongrass essential oil is insoluble, the nutrient media is water-based and so this caused an issue for the project. An emulsifier was required in order to get the lemongrass to interact with the cells rather than sitting above them as a separate layer. The oil was insoluble in acetone but was soluble in alcohols; therefore, ethanol was used to create the 1%, 0.5%, and 0.1% solutions of lemongrass. To make the 1% solution, 250μl of both lemongrass essential oil and pure ethanol were added to a falcon tube, swirled briefly, followed by the gradual addition of 24.5ml of the F-12 Ham’s nutrient media, swirling throughout. For the 0.5% solution 125μl of lemongrass and ethanol were added with 24.75ml of media and for the 0.1% solution 25μl of lemongrass and ethanol were added with 24.95ml of media. Well-plates containing the neuroblastoma cells then needed to be prepared to add the lemongrass solutions to. Followed the method for splitting the cells up to the point of adding 1ml of media to the centrifuged pellet of cells, agitated, and then added another 11ml of media to get a total volume of 12ml. Pipetted 1 ml of the cell-media solution into each well to fill 12 of the wells. The well plates used had 24 wells so 2 flasks of cells were required per well plate. The well-plates were then placed into the incubator for 24 hours to allow the cells to settle and adhere to the plate surface. Before adding the lemongrass, the solutions needed to be pre-warmed in the incubator with the cells. 1 ml of media and each lemongrass solution were then be pipetted into their respected rows (refer to diagram), also added 1ml of 100% ethanol to the 6th well of each row to have a comparative group of dead cells. Did the same for a second plate, this time adding 10μl of β amyloid protein suspended in media, the protein is biohazardous and so care needed to be taken and anything in direct contact with the protein (e.g. pipette tips) were safely disposed of. Incubated the plates for a further 24 hours and then counted the cells using a haemocytometer.

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Row A: F-12 Ham’s media only
Row B: 1% lemongrass solution
Row C: 0.5% lemongrass solution
Row D: 0.1% lemongrass solution
Column 6: 100% ethanol

[65]
5. Results

Table 1: Plate containing neuroblastoma cells and lemongrass solutions, beta amyloid proteins absent

<table>
<thead>
<tr>
<th>Solution (Lemongrass %)</th>
<th>Average no. Cells per mm²</th>
<th>[Cells] per mm²</th>
<th>Cell Viability %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2237.5</td>
<td>4.475 x10⁷</td>
<td>82.68</td>
</tr>
<tr>
<td>0.5</td>
<td>650</td>
<td>1.3 x10⁷</td>
<td>90.38</td>
</tr>
<tr>
<td>0.1</td>
<td>600</td>
<td>1.2 x10⁷</td>
<td>83.33</td>
</tr>
<tr>
<td>0</td>
<td>1975</td>
<td>3.95 x10⁷</td>
<td>84.18</td>
</tr>
<tr>
<td>100% Ethanol</td>
<td>550</td>
<td>1.1 x10⁷</td>
<td>15.91</td>
</tr>
</tbody>
</table>

Table 2: Plate containing neuroblastoma cells and lemongrass solutions, beta amyloid proteins present

<table>
<thead>
<tr>
<th>Solution (Lemongrass %)</th>
<th>Average no. Cells per mm²</th>
<th>[Cells] per mm²</th>
<th>Cell Viability %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>850</td>
<td>1.7 x10⁷</td>
<td>48.04</td>
</tr>
<tr>
<td>0.5</td>
<td>900</td>
<td>1.8 x10⁷</td>
<td>68.61</td>
</tr>
<tr>
<td>0.1</td>
<td>1091.6</td>
<td>2.18 x10⁷</td>
<td>58.03</td>
</tr>
<tr>
<td>0</td>
<td>1216.6</td>
<td>2.43 x10⁷</td>
<td>37.04</td>
</tr>
<tr>
<td>100% Ethanol</td>
<td>458.3</td>
<td>9.16 x10⁷</td>
<td>10.93</td>
</tr>
</tbody>
</table>

Figure 1: Average concentration of neuroblastoma cells x10⁷ per mm² with beta amyloid absent and present.
6. Discussion

The results of the experiment with lemongrass are very interesting. A first point of note is comparing the concentration of cells per mm² and cell viability of the 0% lemongrass solutions in both table 1 and table 2. The cell concentration in the plate without Aβ was $3.95 \times 10^7$ mm², but when Aβ proteins only were added the concentration decreased to $2.43 \times 10^7$ mm². The cell viability had a vast 47.14% decrease from 84.18% in the Aβ absent plate to 37.04% in the Aβ present plate. This supports the previously discussed beta-amyloid hypothesis because the results demonstrate increased neurodegeneration in the presence of Aβ proteins as stated by Hardy and Allsopp’s hypothesis.

The addition of lemongrass solution in the Aβ plate seems to have had a negative effect on the concentration of cells, it is not clear if this is due to the lemongrass or the Aβ as the concentration of cells in the Aβ free plate at the highest, 1%, solution had the highest concentration though this particular result may be anomalous as it doesn’t match the decrease in concentration at 0.1% and 0.5% lemongrass solution.

The change in viability of the cells is much more promising. The viability of both peak at 0.5% lemongrass solution. No significant difference is shown in viability between different lemongrass solutions in the Aβ plate, which is promising as it suggests that the lemongrass isn’t killing any of the neuroblastoma cells. In the Aβ present plate, the cell viability of all the wells with lemongrass added were higher than the wells containing media only, the highest viability of 68.61% at 0.5% lemongrass solution is nearly twice that of the 0% lemongrass solution at only 37.04% viability.

So, even though the lemongrass may have caused a decrease in the concentrations of neuroblastoma, it appears it has caused more of the cells actually present to survive. It is arguably more beneficial to have less neurons, but most of them be functional, than to have plenty of cells of which most are dead. The results showing the increase in viability in the Aβ present well plate is
evidence supporting the hypothesis used in this project that lemongrass has a neuroprotective effect as it appears to have prevented some of the cell death that occurs when lemongrass isn’t present.

Some surprising results also occurred in the project. The viability of the wells with 100% ethanol wasn’t 0% as expected. The viability was low at 15.91% and 10.93% but the fact that some of the neuroblastoma managed to survive in 100% ethanol was unexpected. This may be due to the fact that there was already 1ml of nutrient media in each well when the 1ml of ethanol was added. The ethanol will have become more diluted and so the cells were actually in a 50% ethanol solution, so over the 24 hours a small percent could have still survived. Another surprising finding was the appearance of the neuroblastoma cells, the cells dendritic extensions appeared shorter than normally observed. They were more circular in shape, but were definitely alive as they were adhered to the flask surface and were not stained blue by trypan blue when being counted using the haemocytometer.

Overall, these results and scientific literature suggest a definite potential in lemongrass as a treatment for Alzheimer’s disease. There are many other issues that this experiment didn’t cover though, such as the ability of lemongrass to cross the blood brain barrier and dosages needed to have an effect. Much more research using in vivo experiments with animal models and clinical trials on humans would have to be done to know for certain if lemongrass is a truly viable option for drug development. If this project was to be continued repeats of the experiments would have to be done in order to obtain more accurate and reliable averages, and a possible investigation into the effect of lemongrass on AChE to see if it could also work like the many current AChE inhibitor drugs would also be carried out.
7. Bibliography


8. Appendix

A1 – Aβ absent

A2 – Aβ absent

A3 – Aβ absent

A4 – Aβ absent

A5 – Aβ absent

A6 – Ethanol– Aβ absent
D1 – Aβ present

D2 – Aβ present

D3 – Aβ present

D4 – Aβ present

D5 – Aβ present

D6 – Ethanol – Aβ present