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by

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ABSTRACT

NEUROPROTECTIVE EFFECT OF BASIL LEAF
EXTRACT ON HIPPOCAMPAL CELL
LINE EXPOSED TO ETHANOL

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Basil plant (*Ocimum basilicum*) is a common part of the South Asian diet, and it is known to act as an antioxidant after consumption. To understand the potential of basil to relieve oxidative stress and to assess its neuroprotective effect, mouse hippocampal cell line (HT-22) were exposed to ethanol and to basil extracts simultaneously. The toxicity of the hippocampal cells was reduced significantly with the addition of the basil extract, and significantly higher cell viability was detected as compared to the ethanol treated control cells with no basil extract. These data suggest that basil can act as a neuroprotective agent with potential against oxidative stress-induced cell death, potentially leading to aid in neurological disorders that undergo oxidative stress.

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CHAPTER 1

INTRODUCTION

Many neurodegenerative diseases exist such as Alzheimer's, Parkinson's and Huntington's disease. Alzheimer's disease can lead to memory loss, particularly in the elderly. Parkinson's disease leads to loss in motor function due to degradation of motor neurons, and Huntington's disease essentially reduces the critical thinking skills of a person with this problem. Researchers observing the features of the neurons associated with these diseases found that the neurons have an excessive amount of oxidative stress (Aruna and Sivaramakrishnan, 1992). Oxidative stress can be dangerous to the human body because a large quantity of free radicals (reactive oxygen missing an electron) can easily react with other compounds (Barnham *et al*., 2004). This causes a series of imbalances in cell communication that interrupt functions within the cell, ultimately leading to neuronal degeneration. Loss of memory can be an effect of this interruption. In order to decrease the amount of oxidative stress in the body, antioxidants are used.

Antioxidants are chemicals that block the effects of free radicals, preventing the imbalance from occurring (Gülçin *et al.*, 2007). Normally, the human body makes its own antioxidants; however, under abnormal conditions not enough antioxidants are present within the body to reduce the excessive amount of oxidative stress caused by disease. A good source of antioxidants is from plants since they need the chemicals to prevent their own cells from having an aggregate amount of reactive oxygen species (Javandmardi, 2003). Many plants are obtained, and their phenolic compounds are observed to determine

if they contain useful antioxidants. One plant that appears to have useful compounds is basil.

Basil is a common plant found in the south-central part of Asia. It is used to improve the health of many people. It is known to improve head colds, warts, and inflammation (Mittler and Ron, 2002). Basil also appears to affect three enzymes associated with diabetes mellitus, allowing it to be a potential aid in preventing people from getting diabetes mellitus (Holm and Yvonne, 1999). It is also know to obtain phenolic compounds that are capable of behaving as antioxidants. A few phenolic compounds that make up basil are rosmarinic acid, ursolic acid and trolox (Vats *et al.*, 2003). Because of these properties, it is expected that basil has the potential to block oxidative stress in neurons and increase cell survival.

CHAPTER 2

MATERIALS AND METHODS

Basil leaves were placed in a mortar and pestle and crushed until the juice came out from the plant. The extract was collected into 1.5 mL vials. Then, one vial was diluted to 50% concentration with deionized water until 5 serial dilutions were made for each experiment. The rest of the vials were stored in the -20 ˚C freezer until further use.

As this occurred, a specific cell line, HT-22 cells, which came from the hippocampus of mice, was cultured with Dulbecco's Modified Eagle's Medium (DMEM) and prepared to use. The DMEM contained 10% FBS, 2 mM of L-glutamine, 100 units/mL penicillin, and 0.1 mg/mL streptomycin. The cells were maintained in a 37 ˚C humidified incubator with 5% carbon dioxide and 95% air.

After the basil was extracted and the cells were ready to be used, 80 µL of the cells were seeded into a 96-well plate with 50% confluence so the cells could grow quickly. After 24 hours of the cells being incubated, 20µL of the extract was added to the cells that were not the control, allowing those cells to have an antioxidant to potentially stay protected from the ethanol. The antioxidant should have behaved as a reducing agent to prevent the excess oxidation from creating a chain reaction that would have destroyed the cells. Immediately after, 500mM of ethanol, which was prepared prior and diluted with DMEM media, was added to those cells to create oxidative stress that was expected to harm the cells, and Parafilm was placed over the wells to prevent the evaporation of the ethanol. After another 24 hours of incubating the cells, an MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-

Diphenyltetrazolium Bromide) assay was performed to determine the cell viability after each treatment.

3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was measured and mixed with phosphate buffer saline using 5 mg/mL. Then, 20μL of that solution was added into the wells on the treated cells. The plate with the cells was then placed back into the incubator for 3-4 hours to allow the cells to reduce the MTT into a substance known as formazan. Afterwards, the plate was taken out, the MTT solution removed carefully from the wells, and 100μL of dimethyl sulfoxide was added into the wells. This dissolved the formazan to create a colored solution. The cells that released more color were the more viable cells. The plate shook for about 15 minutes so the colored solution was completely formed, and then the plate was placed in a spectrophotometer to allow the absorbance of the color to be read. A greater absorbance indicated more purple solution, which represented more living cells. Those values were then used to calculate the cell viability in each well and comparisons were made with the control using a student t-test to determine if the extract helped allow the cells to survive or not, and if a special molecular mechanism occurred.

CHAPTER 3

RESULTS

Basil, a plant that appears to contain useful compounds and provide health benefits, was crushed with a mortar and pestle and diluted to obtain varying concentrations of the extract. The different concentrations were added over mouse hippocampal cells treated with ethanol. The wells containing more concentrated extracts exhibited a darker purple color after dimethyl sulfoxide (DMSO) was added (Figure 3.1). The control group in the first row with no extract added, appeared to have a lighter purple color (Figure 3.1).

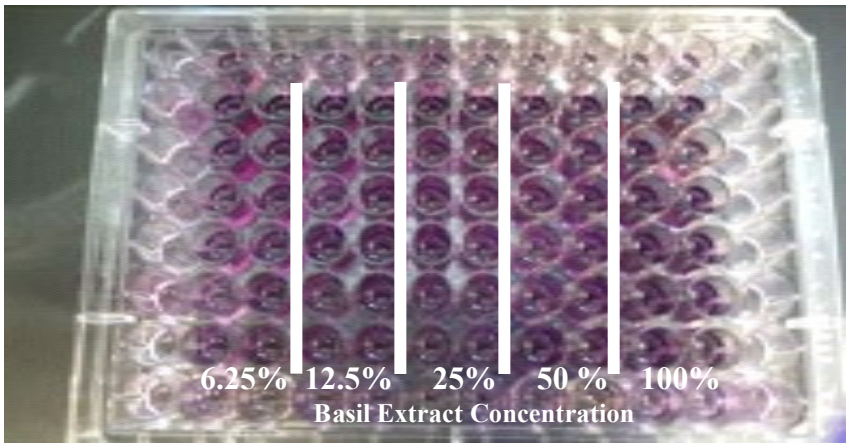

Figure 3.1: 96-well plate after addition of MTT and DMSO

When the 96-well plate was placed in a spectrophotometer, a greater average of absorbance was observed for the cells exposed to higher extract concentrations than with the control cells treated only by ethanol (Table 3.1). Standard error bars did not overlap between the concentrations of basil extract from 25% to 100% and the ethanol controls (Figure 3.2).

Table 3.1: Average and standard deviation of cell survival after addition of ethanol and basil extract

Basil Extract Concentration (%)	100	100	50	50	25	25	12.5	12.5	6.25	6.25
Basil + EtOH Mean	87.11093	100	61.10023	42.58048	35.86726	39.11102	26.53703	29.27958	31.04754	31.1472
EtOH Mean	17.10961	19.26886	20.63633	20.70071	21.2757	21.87365	23.17263	22.11303	22.63625	22.65035
Basil + EtOH Standard Deviation	33.96054	64.95774	59.54753	20.72935	20.6686	40.177	14.27002	28.88897	7.466391	4.505089

The percent of survival for cells treated with ethanol was consistent ranging from about 17% to 23%. The survival of the cells exposed to basil extract was greatest in cells treated with 100% concentrated basil extract. Cells exposed to 50% and 25% of the extract also had a high amount of cell survival when compared to concentrations of extract below 25%. The cells with the smallest concentration of basil extract experienced the least percent of cell survival (Figure 3.2). There were replicates performed for each basil concentration, and they each gave approximately similar percent survival of the cells.

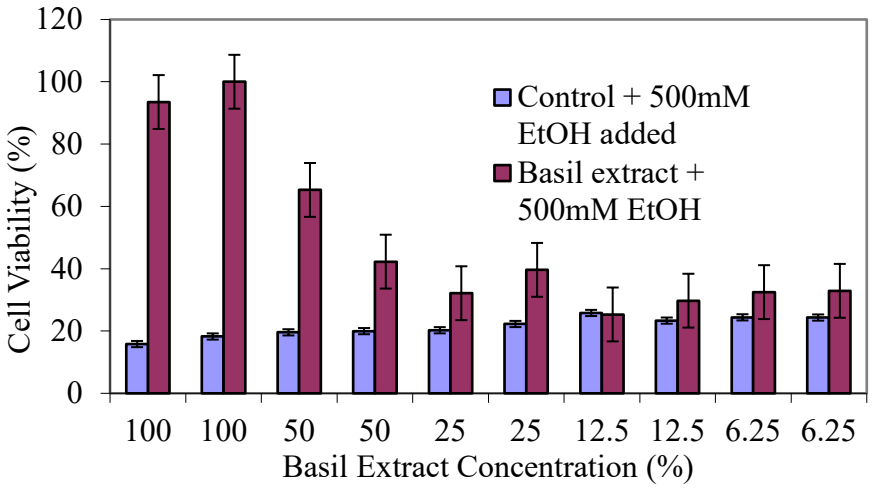

Figure 3.2: Average cell survival after addition of ethanol and basil extract

CHAPTER 4

DISCUSSION

Basil contains phenolic compounds that behave as antioxidants (Vats *et al.*, 2003). Antioxidants can reduce free radicals in a cell, decreasing the amount of oxidative stress present. It was expected that basil extract would decrease oxidative stress, allow for more cell survival and provide neuroprotection to neurons. After observing the average absorbance of cells treated with ethanol and basil extract, it appears basil does possess the capability of decreasing oxidative stress in neurons.

Basil extract was added to cells treated with ethanol and subjected to an MTT (3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay the following day. This involved addition of MTT to the cells, followed by analysis by spectrophotometry. The cells protected by 100% concentrated basil extract formed the darkest purple color solution when dimethyl sulfoxide was added into the well containing the cells. This indicated that more cells survived because they were capable of creating more formazan; a solution formed by living cells as the result of redox reactions that becomes purple and soluble after the addition of DMSO (Gerlier and Thomasset, 1986). As the concentrations decreased, a light shade of purple was observed, and the control wells with cells exposed to only ethanol

reflected a pale purple solution (Figure 3.1). Fewer cells survived in the control group and many were weakened, preventing the cells from producing a large amount of formazan.

The 96-well plate was placed into a spectrophotometer and absorbance values were recorded. The average absorbance values were greater in cells that had a higher concentration of basil (Table 3.1), indicating that the cells exposed to higher concentrations of the basil extract allowed for greater cell survival. The control cells exposed to ethanol alone had the smallest average absorbance readings because fewer cells survived. No protection was provided to the cells that had no exposure to basil, so more free radicals were present in the control cells. That caused more disruption in cell signaling, and it led to more cell death (Freeman and Crapo, 1982).

When the control cells with ethanol only were compared to the cells exposed to the extract, a student t-test was performed, and it was observed that there was a significant difference in the cell survival of the cells (Figure 3.2). Significantly more cells exposed to at least 25% basil extract survived when compared to control cells, which had very little cell survival, ranging from about 17% to 23%.

Observation of the cell survival percentage indicates that basil extract can increase cell viability and provide neuroprotection to the mouse hippocampal cells by reducing the amount of oxidative stress on the cell induced by ethanol. This could potentially alleviate damage caused by neurodegenerative diseases through reducing the amount of free radicals and permitting greater cell signaling. The results indicate that basil extract could potentially become a therapeutic aid in minimizing the damage done by neurodegenerative disorders.

To observe which compounds of basil aid in reducing oxidative stress in neurons, it is important to test the potential of the phenolic compounds that make up basil. The phenolic compounds will be isolated and added to neurons induced by the ethanol to

determine if neuroprotection is occurring. Then, the cells treated with the compounds will have histone proteins extracted, and a Western blot will be performed to determine if gene expression of neurons is affected by basil plant.

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BIOGRAPHICAL INFORMATION

Nadine Shihabeddin is a student studying at the University of Texas at Arlington. She will graduate in May 2017 with a double major in Biology and Microbiology and a minor in Biochemistry. She is interested in attending medical school and becoming a Pediatrician. Nadine also finds great interest in doing research during her free time.

She joined Dr. Subhrangsu Mandal's research lab as a sophomore, and began working on her own projects. Her first experiment was observing the neuroprotective effect of basil extract. Afterwards, she planned to perform Western Blot to observe if any protein change occurred with the cells exposed to basil. Nadine is interested in assessing the potential neuroprotective effect of other compounds as well.

Nadine would like to continue doing research as a hobby while she pursues her route in medicine. Her goal is to find ways to make the life of people easier, and she believes medicine and research in neuroprotection can provide that opportunity.