Research Article

The effect of cranberry fruit extract on alpha -synuclein protein expression using immunostaining techniques

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ABSTRACT

Aim: The aim of the study was to identify the neuroprotective effect of cranberry fruit extract by reducing the release of this biomarker by immunostaining techniques. **Objective:** The present study is to find out the alpha-lipoic acid cranberry fruit extract on alpha-synuclein protein expression using immunostaining techniques. **Materials and Methods:** The neuroblastoma SH-SY5Y cells were cultured and treated with various concentrations of cranberry fruit extract and were incubated with primary antibody alpha-synuclein and the antigen-antibody activity was visualized under a light microscope, and the results were quantified using image analysis software. **Results:** The study infers that when cranberry extract is added to the neurotoxic cell lines, the antigen-antibody reaction is interfered and as the concentration of cranberry fruit extract increases the corresponding decrease in the expression of the alpha-synuclein protein can be seen. **Conclusion:** Cranberry fruit extract proves to effectively reduce the expression of alpha-synuclein protein in neurotoxic cell lines so further research must be conducted in this field to discover the useful effects of cranberry so that it can be used as a neuroprotective agent in medicines to treat neurodegenerative disorders.

KEY WORDS: Cranberry extract, Neuroblastoma cells, Neuroprotective

INTRODUCTION

Cranberry extract offers a host of antioxidants and nutrients that help fight infections and boost your overall health.[1] Cranberries are already popular as juice and fruit cocktails; however, in medical terms, they are commonly used to treat urinary complications.[2] Cranberry extract might also play a role in stomach ulcer treatment. Due to the multiple vitamins and minerals present in cranberries, they can make a healthy addition to a balanced diet.[3]

Cranberry is a type of evergreen shrub that grows in wet areas, such as bogs or wetlands. Cranberry is native to northeastern and north-central parts of the United States. The shrub has small, dark green leaves, pink flowers, and dark red fruit that are egg-shaped. Cranberry is most commonly used for the prevention and treatment of urinary tract infections (UTIs).[2,4] Cranberry is also used for kidney stones, neurogenic bladder (a bladder disease), to deodorize urine in people with difficulty controlling urination, to prevent urine catheters from becoming blocked, and to heal skin around surgical openings in the stomach that is used to eliminate urine.[5]

Cranberry is used cranberry to increase urine flow, kill germs, and reduce fever. Cranberry is also used for type 2 diabetes, chronic fatigue syndrome, enlarged prostate, common colds, flu, heart disease, memory, metabolic syndrome, and ulcers caused by *Helicobacter pylori*, scurvy, inflammation of the lining around the lung (pleurisy), and cancer.[6]

In foods, cranberry fruit is used in cranberry juice, cranberry juice cocktail, jelly, and sauce. Some researchers think that some of the chemicals in cranberries keep bacteria from sticking to the cells that line the urinary tract where they can multiply. Cranberry, however, does not seem to have the ability to release bacteria which are already stuck to these cells. This may explain why cranberry is possibly effective in preventing UTIs, but possibly ineffective in treating them.[7] Cranberry, as well as many other fruits and vegetables, contains significant amounts of salicylic acid, which is an important ingredient in aspirin. Drinking cranberry

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juice regularly increases the amount of salicylic acid in the body. Salicylic acid can reduce swelling, prevent blood clots, and can have antitumor effects.[8]

The North American cranberry and its products have been widely used for their antimicrobial, antimutagenic, antiangiogenic, and antioxidant properties. Alpha-synuclein protein in highly expressed in neurodegenerative disorders,[9] so this project aims to find out the neuroprotective effect of cranberry fruit extract by reducing the release of the biomarker (alpha-synuclein) by immunostaining techniques.

MATERIALS AND METHODS

The dry cranberry (Delmonte dried cranberries) was collected from field Fresh Food Private Limited, Haryana, India. The collected specimen was authenticated in National Institute of Siddha, Tambaram, Chennai.

Preparation of the Extract

About 15 g of dried fruit fine powder of cranberries were extracted with 150 ml ethanol (75%) for 1 min using an Ultra Turrax mixer (13,000 rpm) and soaked overnight at room temperature. The sample was then filtered through Whatman No.1 paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rotavator at 40°C to a constant weight and then dissolved in respective solvents. Cranberry ethanolic extract was freshly dissolved in 1 ml of saline for this *in vitro* work.

Cell Lines

SH-SY5Y human neuroblastoma cell lines were procuredfrom National Center for Cell Sciences, Pune, India. The cell lines were properly maintained and used for the study.

Cell Culture

SHSY5Y cells were cultured in minimal essential medium with Earle's salts supplemented with 10% fetal bovine serum, 1% non essential amino acids, 2 mM L-glutamine, and 1% Penicillin/Streptomycin, solution (PEST). The cells were plated at a density of 500 cells/mm^2 in 96 well plates overnight in the culture medium. The medium was replaced with the differentiation medium (Dulbecco's Modified Eagle's Medium with Ham's F12 medium [1:1], 1% N_2 supplement, and 1% PEST) containing 1 µM RA. The cells were differentiated for 3–6 days. Half of the medium per well was changed every 48 h.

Cells Treatment

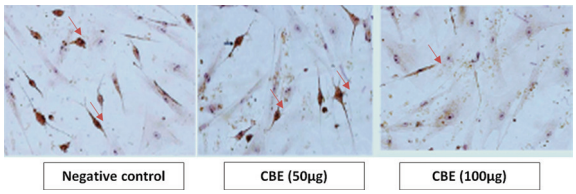
The cells were pretreated with different concentrations of extract for 4 h and then incubated with MPP^+ (1 mM) for 2 h. The effective dose of sample extract was used to identify potential neuroprotective effects against MPP^+ toxicity.

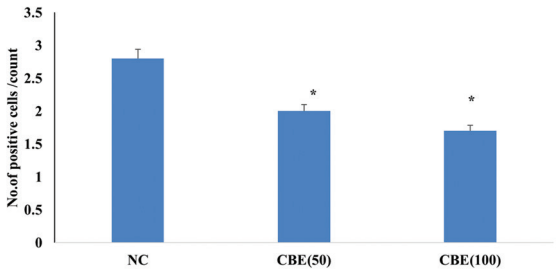
Immunostaining Techniques for Alpha-Synuclein Expression

The treated and untreated cell lines were cryoprotected in 30% sucrose, embedded in a tissue freezing medium with liquid nitrogen, and cut into frozen sections (3–5 µm) using a cryostat. Sections were stored under antifreeze buffer. Parallel free-floating sections were subjected to endogenous peroxidase quenched with 1% H_2O_2 in phosphate-buffered saline (PBS), followed by treatment with blocking buffer (5% normal chicken serum in PBS and 0.3% Triton X-100 for overnight at 4°C) and incubated with primary antibody alpha-synuclein. After washing with PBS, tissues were incubated with a biotinylated goat anti-mouse secondary antibody. The tissues were subsequently exposed to an avidin-biotin-peroxidase complex for 2 h. The peroxidase activity was visualized using a stable alpha-synuclein positive cells (%) diaminobenzidine solution. All immunoreactions were observed using a compound light microscope and these results were quantified using the Image analysis J 1.46 software.

RESULTS

Results were expressed as mean ± standard deviation. Statistical significance was determined by one-way analysis of variance and *post hoc* least significant difference test. $P < 0.05$ was considered significant.





Results are expressed as mean ± standard deviation *$P < 0.05$ significantly different as compared with negative control.

DISCUSSION

Synucleins are abundant brain proteins, the physiological functions of which are poorly understood. The synuclein family consists of three members: α-, β-, and γ-synuclein. α- and β-synuclein is concentrated in nerve terminals, whereas γ-synuclein seems to be present throughout nerve cells.[10]

α-synuclein is present in Lewy bodies, the main neuropathological characteristic of Parkinson's disease (PD). This observation has led to the proposal that α-synuclein might be involved directly in the pathogenic processes that underlie PD and several other diseases; the identification of α-synuclein mutations leading to the appearance of genetic forms of PD in two different families has provided support for this idea.[11]

The presence of α-synuclein in Lewy bodies has also been observed in other diseases, including dementia with Lewy bodies and multiple system atrophy.[12] Other conditions, such as Alzheimer's disease, the parkinsonism-dementia complex of Guam, and Hallervorden–Spatz Disease, can be accompanied by the formation of α-synuclein-positive Lewy bodies; however, it is not an invariant feature of these diseases.

The mechanism by which α-synuclein accumulation leads to neurodegeneration is not completely understood. A leading hypothesis is that the ordered assembly of α-synuclein into filaments, which seems to be induced by oxidative stress, is both necessary and sufficient to cause degeneration. However, alternative ideas have not been ruled out, and it is possible that oligomeric α-synuclein molecules are toxic.[13]

Gu *et al.*[14] in his similar study stated that treatment with mulberry extract inhibited the upregulation of α-synuclein and ubiquitin, well known as the composition of Lewy bodies in the substantia nigra and striatum of the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine/p mice. Taken together, these data suggest that mulberry extract may have therapeutic potential for preventing Pandey *et al.*[15] in his similar study suggest that curcumin inhibits α-synuclein oligomerization into higher molecular weight aggregates and therefore should be further explored as a potential therapeutic compound for PD and related disorders. From the present study, we could infer that cranberry fruit extract proves effectively to inhibit alpha-synuclein protein expression and the level of inhibition increases as the concentration of the extract is increased from 50 µg to 100 µg, further research must be initiated with varying concentrations of cranberry extract to discover its medical effects and its application to human science.

CONCLUSION

Neuroprotection refers to the relative preservation of neuronal structure and/or function. It is a widely explored treatment option for many central nervous system disorders including neurodegenerative diseases, stroke, traumatic brain injury, spinal cord injury, and acute management of neurotoxin consumption. Neuroprotection aims to prevent or slow disease progression and secondary injuries by halting or at least slowing the loss of neurons. Cranberry fruit extract proves to effectively reduce the expression of alpha-synuclein protein in neurotoxic cell lines so further research must be conducted in this field to discover the useful effects of cranberry so that it can be used as a neuroprotective agent in medicines to treat neurodegenerative disorders.

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