Anticancer Effects of Blueberry and Pomegranate Extracts on the AGS Gastric Adenocarcinoma Cell Line

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***Abstract* —** *There is an abundance of research correlating diets rich in fruits and vegetables to the reduction or prevention of chronic diseases such as cancer and cardiovascular disease. This study focuses on the cytotoxic effects of blueberry and pomegranate extracts on gastric adenocarcinoma cells in vitro and examines the synergistic effect of the combined extracts on tumor cell proliferation and protein expression. Our results show that both extracts exhibited a potent cytotoxic effect on the AGS gastric adenocarcinoma cells, with the greatest effect observed from the combined treatment, suggesting a synergy between the blueberry and pomegranate extracts on AGS cell proliferation.*

Keywords — adenocarcinoma, synergy, cytotoxic, antiproliferative, anticarcinogenic

Introduction

Today there is a plethora of information correlating diets rich in fruits and vegetables to the reduction or prevention of chronic diseases such as cardiovascular and neurodegenerative diseases, diabetes, obesity, and certain cancers. The disease-prevention properties of fruits and vegetables are attributed to the biological activities of the dietary fiber, vitamins, minerals and phytochemicals in the plants, however many studies suggest the protective effects of fruits and vegetables against chronic diseases are due in large part to the phytochemical content of the plants (Nichenametla & Taruscio, 2006). Experimentally, these plant compounds have been shown to exert a wide range of biological activities in both *in vitro* and *in vivo* systems, including antioxidant, anti-inflammatory and anticarcinogenic properties. In addition, certain plant compounds have been reported to have immunological, antiviral, antibacterial and estrogenic properties as well as antiproliferative and cytotoxic effects in human and animal tumor cell lines (Tuñon, Mediavilla, Campos, & Gallego, 2009).

The main aim of this ongoing research is to evaluate the *in vitro* effects of water extracts from blueberry and pomegranate on tumor cell viability and examine the synergistic effect of the combined extracts on tumor cell proliferation. Additionally, changes in gene expression are being monitored by examining changes in the protein profiles of the fruit- and berry-treated cells compared to untreated control cells.

Materials and Methods

Blueberry and pomegranate extracts were prepared from fresh fruits acquired from local markets to examine the cytotoxic or cytocidal effects of the extracts upon gastric adenocarcinoma cells. The AGS cell line was acquired from ATCC (ATCC® CRL-1739™) and cultured according to their recommended guidelines.

The fruit and berry extracts were prepared by blending 5 g of berries with 35 mL distilled water. The extract supernatants were sterilized through a 0.2 µm syringe filter and mixed with complete growth medium to give final concentrations of 5%, 15% and 25% blueberry, pomegranate, and blueberry/pomegranate extracts.

AGS cells were aliquoted in triplicate into a 96 well plate, yielding a final cell density of 2.5×10^4 cells per 100 µL per well of complete growth medium containing 0%, 5%, 15% and 25% blueberry, pomegranate, and blueberry/pomegranate extracts. The plate was incubated for 48 hours at 37°C in 5% CO_2. To measure cell viability, the CellTiter 96®Aq_{ueous} Non-Radioactive Cell Proliferation Assay was performed according to the manufacturer's protocol. Briefly, this is a colorimetric assay in which MTS, a tetrazolium compound, is converted into formazan, a product soluble in tissue culture medium, by dehydrogenase enzymes that are found in metabolically active cells. The absorbance of the formazan at 490 nm is directly proportional to the number of living cells in the sample.

Results

An *in vitro* assay was performed to detect the effects of blueberry and pomegranate extracts on the proliferation of the AGS gastric adenocarcinoma cell line. A synergistic effect between the blueberry and pomegranate extracts was also evaluated. The cells were cultured for 48 hours in the presence and absence of extract and their viabilities measured.

Changes in AGS cellular morphology, ability to adhere, and rate of proliferation were noted in all samples treated with the blueberry and pomegranate extracts (Figures 1 and 2 and Table 1). Both extracts exhibited an antiproliferative effect in a concentration-dependent manner. AGS samples treated with the blueberry extracts decreased in proliferation between 29.2% and 58.6% while samples treated with the pomegranate extracts decreased between 36.3% and 53.6% compared to untreated control samples. However, the largest antiproliferative effect was observed in AGS cells treated with a combination of blueberry and pomegranate extracts. This combination treatment resulted in a 72.4% decrease in proliferation at the highest concentration. Compared to the individual treatments with blueberry and pomegranate, this result suggests a possible synergism between the two fruits when combined together.

Conclusions

Numerous studies have demonstrated the anticancer properties of fruits and berries on tumor cell proliferation *in vitro* (Olsson, Gustavsson, Andersson , Nilsson, Duan, 2004), and the study from this laboratory supports these findings. Our results show that the blueberry and pomegranate extracts had a potent cytotoxic effect on the AGS cell line and contributed to the >50% reduction in proliferation observed in the treated cells. The greatest effect, however, was achieved using a combination of blueberry and pomegranate extracts, suggesting a possible synergism between the two fruits in producing an antiproliferative effect on AGS tumor cell growth *in vitro*.

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References

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Tables & Figures

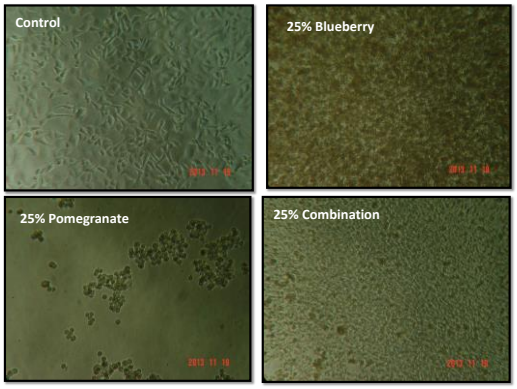

Figure 1. Morphological and antiproliferative effects of blueberry, pomegranate, and combination extract treatment on AGS cells.

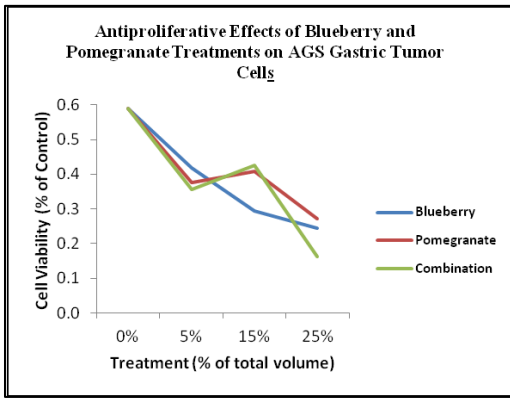

Figure 4. Antiproliferative effects of three concentrations of blueberry and pomegranate extracts on AGS cell proliferation.

Table 1. Percent decrease in AGS proliferation following extract treatment (B = Blueberry, P = Pomegranate, and C = Combination)

Treatment	B.	P.	C.
5%	-29.2	-36.3	-39.5
15%	-50.2	-30.8	-27.8
25%	-58.6	-53.6	-72.4