



Full Length Article

Effect of Broccoli Extracts on Proliferation Inhibition and Apoptosis in the Colon SW620 Cells

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Abstract

Isothiocyanate in the broccoli has proved to improve the organism immunity and anti-cancer ability. However, a high cost for extraction and purification of isothiocyanate limits a wide application of this natural compound. The colon disease is a common cancer resulting in a high death rate in human. In this report, an inexpensive method was used to produce the broccoli crude extracts (BRE) and comprehensively evaluated effects of different BRE concentrations on proliferation inhibition and apoptosis in the colon SW620 cells. The results showed that BRE was able to significantly suppress proliferation of the cancer cells ($P < 0.01$). The growth curve further demonstrated that the cancer cell growth was significantly inhibited by BRE. The colon cells SW620 incubated by the IC_{50} BRE showed a shrink and smaller shape and a cracking status indicating that these cells were under apoptosis. Two genes, *p53* and *caspase-3*, were induced by qRT-PCR to be highly expressed in the SW620 cells treated by the IC_{50} BRE, further demonstrating that BRE induced the cancer cells to apoptosis. © 2019 Friends Science Publishers

Keywords: Isothiocyanate; Broccoli crude extracts (BRE); Proliferation; Cell apoptosis; *p53*; *caspase-3*; qRT-PCR

Introduction

Broccoli, also named as ‘blue cauliflower’, belongs to a species of Brassica and is an annual or biennial, edible vegetable (Ferruzza *et al.*, 2016). This yummy vegetable shows a potential to improve the organism immunity and anti-cancer ability (Leenders *et al.*, 2013; Braakhuis *et al.*, 2016). Previous studies indicated that isothiocyanate (ITCS), a hydrolysate product of sulfur glucoside, prevalently in the broccoli showed an anti-cancer activity (Cheng *et al.*, 2016). This natural compound is also able to induce cell apoptosis of the HeLa cell, the hepatocarcinoma cell and embryo kidney cell and other human cell lines (Vasanthi *et al.*, 2009; Sestili and Fimognari, 2015). But the mechanisms for these phenomena were unclear. And the signal pathways of ITCS for induction of apoptosis may be different among the different cell lines.

Previously, researchers focused on evaluation of ITCS as the anti-cancer active ingredient in broccoli (Chinembiri *et al.*, 2014; Singh *et al.*, 2014; Traka *et al.*, 2014). There were few reports concerning evaluation of bio-activities of other natural compounds in broccoli (Nile and Park, 2014; Medina *et al.*, 2015). The additive or accumulative functions of multiple ingredients for anti-cancer activity have not yet been explored in broccoli. In addition, extraction and

purification of ITCS from broccoli is currently very costly in the technique view. Considered shortages in the previous studies, this research is aimed to comprehensively evaluate effects of the mixture extracts of broccoli on prevention of proliferation of colon cancer cell ‘SW620’ and induction of cell apoptosis using the well developed cell biology technologies. This study will provide a strong theoretical basis for development of the anti-cancer food from broccoli, and a practical basis for extension of broccoli as the healthcare food in the future.

Materials and Methods

Plant Materials and Reagents

The ripening plants of the hybrid broccoli (*Brassica oleracea* L. cv Yanxiu) was obtained from Jiangsu Runqing Shentai Co. Ltd. (Zhenjiang, China). The colon cancer cells of Strain SW620 were obtained from Shanghai Tumor Institute, Shanghai Jiaotong University (Shanghai, China). DMEM high sugar medium, fetal cattle serum and 0.25% pancreatic enzyme were bought from Gibico, Thermo Fisher Scientific, U.S.A. Four methyl even nitrogen effect salt and RNA extraction kit were bought from Bio Basic Inc., Canada. RT-PCR and

qRT-PCR kits were bought from TaKaRa, China. Other reagents were bought from Sigma (U.S.A.).

Preparation of Crude

The fresh broccoli was cleanly washed and naturally dried. The broccoli flower balls were sliced. 200 g of the sliced broccoli were smashed with two volumes of dd H₂O. The solution was hydrolysed at 60°C, then filtered. The supernatant was dried in a vacuum freezing dryer and was re-extracted and centrifuged twice using methanol. The supernatant as the crude extracts of broccoli was dried in a rotary evaporator. The gradual concentrations of the crude extract solutions were made as listed in Table 1 using 1.0 mg·mL⁻¹ PBS buffer. The extract solutions stored at 4°C for a short time till use (El-Houri *et al.*, 2014).

SW620 Cells Proliferation Detection

The 4×10⁴ mL⁻¹ cells were added in the 96-well plate. The cells were incubated in a carbon dioxide chamber with conditions of 5.0% CO₂ and 37°C. After the cells was sticky to the plate well wall (~24 h), 20 μL of the extract solutions was added to each well of the plate with three replicates for each concentration of S1–S7 in Table 1. 20 μL of the PBS buffer was added as the control. The cells were further incubated for 16 h in the carbon dioxide chamber with conditions of 5.0% CO₂ and 37°C. After removal of the supernatant, 20 μL of the 5.0 mg·mL⁻¹ MTT solution was added to each well. The cells were incubated for an additional 4 h in the carbon dioxide chamber with the same conditions. After removal of the supernatant, 150 μL of DMSO was added to each well, then the plate was suspended for 10 min (Aravind *et al.*, 2015). The *D* value at 570 nm of the cell suspension was determined using DG5033A ELISA detector (Pribo Lab Inc., Singapore). The inhibition rate of the cell proliferation (IR) was estimated by the following formula:

$$IR = (1 - \frac{D \text{ value of the tested group}}{D \text{ value of the control}}) * 100$$

Half maximal inhibitory concentration (*IC*₅₀) represented the concentration of the broccoli crude extracts when the inhibition rate of the cell proliferation reached 50% (Hao *et al.*, 2015).

Growth Curve of the SW620 Cells

The 1.6×10⁵ mL⁻¹ cells were added in the 24-well plate with 400 μL for each well. After 12 h, the *IC*₅₀ extract solution was added to six wells and the control (PBS) solution was added to another six wells, with 80 μL for each well. The plate was inoculated for additional 12, 24, 36, 48 and 60 h in the carbon dioxide chamber with conditions of 5.0% CO₂ and 37°C. For these five time

Table 1: The crude extract solutions with the gradual concentrations

Concentration (mg/mL)	S1	S2	S3	S4	S5	S6	S7
	1	0.5	0.25	0.125	10 ⁻¹	10 ⁻²	10 ⁻³

points, 20 μL of the 0.25% trypsin was added to each well for digestion of 5 min. Then the cell solution was transferred to a 1.5 mL tube and centrifuged at 1500 rpm for 5 min. After removal of the supernatant, 20 μL of the PBS solution was added to each well and mixed well using pipette. The cell numbers were counted using Count Star Cell Counter (Ailite International Co. Ltd., China). The alive cell numbers of each well were counted every 12 h. The growth curve of the SW620 cells was plotted according to the observations above.

Morphological Features of the Cell Line SW620

Two plate wells were chosen where the SW620 cells grew well. The *IC*₅₀ extract solution and the PBS solution were added to these two wells, respectively, with 1.2 mL for each well. After incubation of the cells for 24 h in the carbon dioxide chamber with conditions of 5.0% CO₂ and 37°C. Morphological features of the cells were described under electronic microscope observation.

Characterization of Expression Patterns of *p53* and *caspase-3* genes by qRT-PCR

~100 mg fresh cells were collected after incubation of the cells with the *IC*₅₀ extract solution and the PBS solution for 24 h in the carbon dioxide chamber with conditions of 5.0% CO₂ and 37°C. Total RNA of the cells was isolated and cleaned using BBI RNA extraction kit following the instruction. Genomic DNA was removed by DNase digestion. Synthesis of cDNA was performed by this protocol: incubation of RNA at 65°C for 5 min, reverse transcription reaction with enzyme and buffer at 42°C for 2 h, then reaction termination at after 95°C for 1 min. cDNA was stored at 4°C until use.

Sequences of the *p53*, *caspase-3* and *β-actin* genes were obtained from NCBI data base. Primers used for qRT-PCR were designed using the online tool of Integrated DNA Technologies.

The primers were listed:

p53 gene, 5'-AAGACCCAGGTCCAGATGAAGCT-3' and 5'-GGAGTACGTGCAAGTCA CAGACT-3'.

caspase-3 gene, 5'-TCGGTCTGGTACAGATGTCGATG-3' and 5'-GTAAACCCGGGTAAGAATGTGCA-3'.

β-actin gene, 5'-GAGACCTTCAACACCCCAGCC-3' and 5'-GCGTACAGGTCTTTGCGGATG-3'.

SYBR was used for qRT-PCR following the protocol: 35 cycles for 94°C for 20 s, 58°C for 30 s, and 72°C for 5 s.

A formula of 2^{-δδCt} was used for comparison of the gene expression levels between the treated and the control cells.

Results

Inhibition of Proliferation of Cell Line SW620 by Broccoli Extracts

Compared with the control, proliferation of the cell SW620 was significantly suppressed by the broccoli extracts ($P < 0.01$) and the inhibition extent was increased by a higher concentration of the broccoli extracts. The concentration of IC_{50} was S5, a concentration of 0.1 mg·mL of the broccoli extracts (Fig. 1).

Growth Curve of SW620 Cells Affected by the IC_{50} Broccoli Extracts

Growth of SW620 cells in the IC_{50} broccoli extracts was significantly slower than that in the control cells (Fig. 2). The concentration of the SW620 cells incubated in the broccoli extracts was also lower than that in the control cells. The inhibition extent mounted as the incubation time prolonged (Fig. 2).

Morphological Features in the SW620 Cells Affected by the IC_{50} Broccoli Extracts

In most cases, the colon SW620 cells presented a fusiform or oval shape in the control (Fig. 1). However, the cells incubated by the IC_{50} broccoli extracts showed a shrink and smaller shape (Fig. 1). Some cells appeared a cracking status and produced numerous cracking pieces (Fig. 1), indicating these cells were under apoptosis.

Expression Patterns of $p53$ and $caspase-3$ Genes in the SW620 Cells

Two genes, $p53$ and $caspase-3$, proved to be inductively expressed to an extensive high level during the cell during apoptosis (Jost *et al.*, 1997; Hentze *et al.*, 2001). These two genes were induced to be highly expressed in the SW620 cells treated by the IC_{50} broccoli extracts (Fig. 4 and 5). Particularly, expression of the $caspase-3$ gene was highly induced to an extensive high level compared to the control (Fig. 5). This indicated that the broccoli extracts were able to simulate expression of the genes related to the cell apoptosis.

Discussion

The anti-cancer substances in broccoli proved a potential to inhibit the formation of rat thoracic tumors (Jahangir *et al.*, 2009; Lee *et al.*, 2013; Natella *et al.*, 2016). Broccoli and other cruciferous plants were able to inhibit growth of white blood and cancer cells in a certain extent (Herr and Buchler, 2010; Lippmann *et al.*, 2014). A large number of experiments demonstrated that the mechanism of ITCS as the tumor suppressor in broccoli was interpreted as "Phase

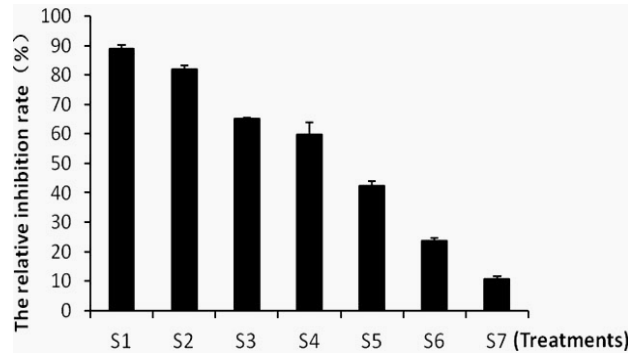


Fig. 1: Inhibition of the colon cell SW620 proliferation by the broccoli extracts

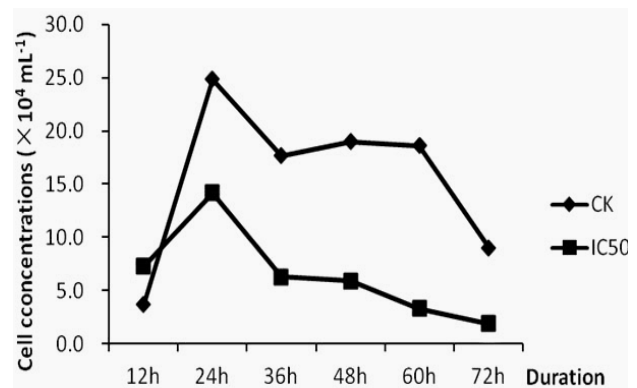


Fig. 2: Growth curve of the SW620 cells incubated in the IC_{50} Broccoli extract and the PBS buffer

Two Enzymes" (Nile and Park, 2014). More studies discovered that broccoli had effects on human cancer cells. ITCS was the major active ingredient in broccoli. Aforementioned, isolation and purification of ITCS is very expensive. Therefore, it needs further studies to explore inexpensive and efficient ways to develop values of the active ingredients in broccoli so that this vegetable will be more practical to our human health (Herr and Buchler, 2010; James *et al.*, 2012).

Apoptosis is an important process of the programmed cell death. Numerous factors involve regulation of this process, including the activator, apoptosis-promoting gene and tumor suppressor genes, *etc.* (Berube *et al.*, 2005; Roos and Kaina, 2006). Over-expression of the $p53$ and $caspase-3$ genes is conducive for suppression of proliferation of the cancer cells (Kim *et al.*, 2009; Zhong *et al.*, 2011). The human colon is a prevalent cancer resulting in a large quantity of death every year (Siegel *et al.*, 2016). Our study indicated that the broccoli extracts were able to initiate over-expression of the $p53$ and $caspase-3$ genes in the colon SW620 cells (Fig. 4 and 5). This may be the major factors resulting in a large quantity of apoptosis of the colon SW620 cells incubated by the broccoli extracts as we observed in Fig. 3.

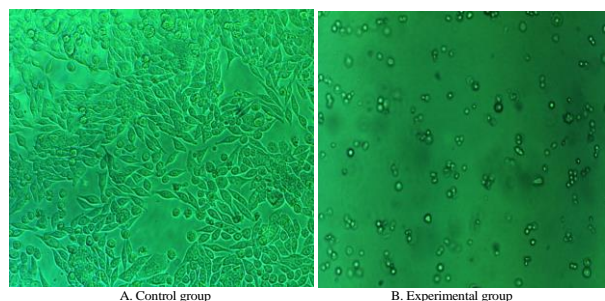


Fig. 3: Morphological observation of the SW620 cells dosed by Broccoli extract of IC_{50}

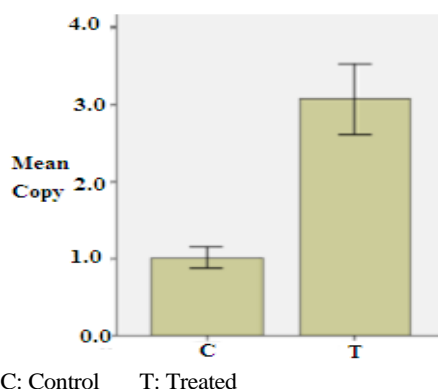


Fig. 4: Expression of the *p53* gene in the SW620 cells treated by the IC_{50} Broccoli extract and the control

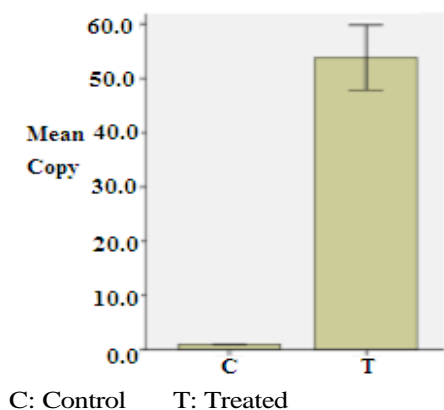


Fig. 5: Expression of the *caspase-3* gene in the SW620 cells treated by the IC_{50} Broccoli extract and the control

Conclusion

All evidences in this study constitute the conclusion that the broccoli extracts are able to suppress proliferation of the colon SW620 cells through initiation of the apoptosis process. The prominent feature of this study is that we used inexpensive chemicals and simple protocols for production of the broccoli extracts so that the cost of the processing is low. This may lead to rapid development of the broccoli extracts used as the natural anti-cancer reagent that benefits our human health in the

future. Remarkably, the inexpensive chemicals and simple protocols for production of the broccoli extracts was applied, so that the cost of the processing is lower. This may lead to a wide application of the broccoli extracts as the natural anti-cancer resources that benefits our human health in the future.

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References

- Aravind, S.R., M.M. Joseph, S.K. George, K.V. Dileep, S. Varghese, A. Rose-James, P. Balaram, C. Sadasivan and T.T. Sreelekha, 2015. TRAIL-based tumor sensitizing galactoxyloglucan, a novel entity for targeting apoptotic machinery. *Intl. J. Biochem. Cell Biol.*, 59: 153–166
- Berube, C., L.M. Boucher, W. Ma, A. Wakeham, L. Salmena, R. Hakem, W.C. Yeh, T.W. Mak and S. Benchimol, 2005. Apoptosis caused by p53-induced protein with death domain (PIDD) depends on the death adapter protein RAIDD. *Proc. Natl. Acad. Sci. USA*, 102: 14314–14320
- Braakhuis, A.J., P. Campion and K.S. Bishop, 2016. Reducing breast cancer recurrence: The role of dietary polyphenolics. *Nutrients*, 8: 1–15
- Cheng, Y.M., C.C. Tsai and Y.C. Hsu, 2016. Sulforaphane, a dietary isothiocyanate, induces G(2)/M arrest in cervical cancer cells through CyclinB1 downregulation and GADD45beta/CDC2 association. *Intl. J. Mol. Sci.*, 17: 1–13
- Chinembiri, T.N., L.H.D. Plessis, M. Gerber, J.H. Hamman and J.D. Plessis, 2014. Review of natural compounds for potential skin cancer treatment. *Molecules*, 19: 11679–11721
- El-Houri, R.B., D. Kotowska, L.C.B. Olsen, S. Bhattacharya, L.P. Christensen, K. Grevsen, N. Oksbjerg, N. Faergeman, K. Kristiansen and K.B. Christensen, 2014. Screening for bioactive metabolites in plant extracts modulating glucose uptake and fat accumulation. *Evid.-Based Complem. Altern. Med.*, 2014: 1–8
- Ferruzza, S., F. Natella, G. Ranaldi, C. Murgia, C. Rossi, K. Trost, F. Mattivi, M. Nardini, M. Maldini, A.M. Giusti, E. Moneta, C. Scaccini, Y. Sambuy, G. Morelli and S. Baima, 2016. Nutraceutical improvement increases the protective activity of broccoli sprout juice in a human intestinal cell model of gut inflammation. *Pharmaceuticals*, 201: 101–109
- Hao, Z., J. Qian and J. Yang, 2015. Shikonin induces apoptosis and inhibits migration of ovarian carcinoma cells by inhibiting the phosphorylation of Src and FAK. *Oncol. Lett.*, 9: 629–633
- Hentze, H., F. Schwoebel, S. Lund, M. Keel, W. Ertel, A. Wendel, M. Jaattela and M. Leist, 2001. *In vivo* and *in vitro* evidence for extracellular caspase activity released from apoptotic cells. *Biochem. Biophys. Res. Commun.*, 283: 1111–1117
- Herr, I. and M.W. Buchler, 2010. Dietary constituents of broccoli and other cruciferous vegetables: implications for prevention and therapy of cancer. *Cancer Treat. Rev.*, 36: 377–383
- Jahangir, M., H.K. Kim, Y.H. Choi and R. Verpoorte, 2009. Health-affecting compounds in Brassicaceae. *Compr. Rev. Food Sci. Food Saf.*, 8: 31–43
- James, D., S. Devaraj, P. Bellur, S. Lakkanna, J. Vicini and S. Boddupalli, 2012. Novel concepts of broccoli sulforaphanes and disease: induction of phase II antioxidant and detoxification enzymes by enhanced-glucoraphanin broccoli. *Nutr. Rev.*, 70: 654–665
- Jost, C.A., M.C. Marin and W.G.K. Jr, 1997. p73 is a human p53-related protein that can induce apoptosis. *Nature*, 389: 191–194
- Kim, E., A. Giese and W. Deppert, 2009. Wild-type p53 in cancer

- cells: when a guardian turns into a blackguard. *Biochem. Pharmacol.*, 77: 11–20
- Lee, S.G., J.H. Kim, M.J. Son, E.J. Lee, W.D. Park, J.B. Kim, S.P. Lee and I.S. Lee, 2013. Influence of extraction method on quality and functionality of broccoli juice. *Prev. Nutr. Food Sci.*, 18: 133–138
- Leenders, M., I. Sluijs, M.M. Ros, H.C. Boshuizen, P.D. Siersema, P. Ferrari, C. Weikert, A. Tjønneland, A. Olsen, M.C. Boutron-Ruault, F. Clavel-Chapelon, L. Nailler, B. Teucher, K. Li, H. Boeing, M.M. Bergmann, A. Trichopoulou, P. Lagiou, D. Trichopoulos, D. Palli, V. Pala, S. Panico, R. Tumino, C. Sacerdote, P.H. Peeters, C.H.V. Gils, E. Lund, D. Engeset, M.L. Redondo, A. Agudo, M.J. Sanchez, C. Navarro, E. Ardanaz, E. Sonestedt, U. Ericson, L.M. Nilsson, K.T. Khaw, N.J. Wareham, T.J. Key, F.L. Crowe, I. Romieu, M.J. Gunter, V. Gallo, K. Overvad, E. Riboli and H.B. Bueno-de-Mesquita, 2013. Fruit and vegetable consumption and mortality: European prospective investigation into cancer and nutrition. *Amer. J. Epidemiol.*, 178: 590–602
- Lippmann, D., C. Lehmann, S. Florian, G. Barknowitz, M. Haack, I. Mewis, M. Wiesner, M. Schreiner, H. Glatt, R. Brigelius-Flohe and A.P. Kipp, 2014. Glucosinolates from pak choi and broccoli induce enzymes and inhibit inflammation and colon cancer differently. *Food Funct.*, 5: 1073–1081
- Medina, S., R. Dominguez-Perles, D.A. Moreno, C. Garcia-Viguera, F. Ferreres, J.I. Gil and A. Gil-Izquierdo, 2015. The intake of broccoli sprouts modulates the inflammatory and vascular prostanoids but not the oxidative stress-related isoprostanes in healthy humans. *Food Chem.*, 173: 1187–1194
- Natella, F., M. Maldini, M. Nardini, E. Azzini, M.S. Foddai, A.M. Giusti, S. Baima, G. Morelli and C. Scaccini, 2016. Improvement of the nutraceutical quality of broccoli sprouts by elicitation. *Food Chem.*, 201: 101–109
- Nile, S.H. and S.W. Park, 2014. Edible berries: bioactive components and their effect on human health. *Nutrition*, 30: 134–144
- Roos, W.P. and B. Kaina, 2006. DNA damage-induced cell death by apoptosis. *Trends Mol. Med.*, 12: 440–450
- Sestili, P. and C. Fimognari, 2015. Cytotoxic and antitumor activity of sulforaphane: The role of reactive oxygen species. *Biomed. Res. Intl.*, 2015: 1–10
- Siegel, R.L., K.D. Miller and A. Jemal, 2016. Cancer statistics, 2016. *CA Cancer J. Clin.*, 66: 7–30
- Singh, K., S.L. Connors, E.A. Macklin, K.D. Smith, J.W. Fahey, P. Talalay and A.W. Zimmerman, 2014. Sulforaphane treatment of autism spectrum disorder (ASD). *Proc. Natl. Acad. Sci. USA*, 111: 15550–15555
- Traka, M.H., A. Melchini and R.F. Mithen, 2014. Sulforaphane and prostate cancer interception. *Drug Discov. Today*, 19: 1488–1492
- Vasanthi, H.R., S. Mukherjee and D.K. Das, 2009. Potential health benefits of broccoli- a chemico-biological overview. *Mini Rev. Med. Chem.*, 9: 749–759
- Zhong, Y.J., F. Shi, X.L. Zheng, Q. Wang, L. Yang, H. Sun, F. He, L. Zhang, Y. Lin, Y. Qin, L.C. Liao and X. Wang, 2011. Crocetin induces cytotoxicity and enhances vincristine-induced cancer cell death via p53-dependent and -independent mechanisms. *Acta Pharmacol. Sin.*, 32: 1529–1536

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