Effect of Anthocyanins from Purple Corn (*Zea mays L.*) on DF-1 Cells

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Abstract. This study investigates the composition and content of anthocyanins, which are from the purple corn (*Zea mays L.*) bracts. The content of purple corn anthocyanins (PCA) is 17.13%. Results of the identification of PCA showed that the most prominent of the all components was cyanidin type. DF-1 cells were treated with different concentrations of PCA at different time point. We observed that 50 μg/mL PCA was the optimal concentration, which had no cytotoxicity to DF-1 cells. The situation of cell growth was evaluated by optical microscope, cell counter, and MTT assays.

Introduction

Purple corn is a kind of anthocyanins food material, showing purple color, regardless of its bracts or grain. The color of purple corn is due to its anthocyanins [1]. Anthocyanins have been reported to have many biological functions, such as antioxidant, antimicrobial, antiobesity, and anticancer activities [2]. Chicken embryo fibroblasts have normal fibroblastic morphology and which are free of endogenous sequences related to avian sarcoma and leukosis viruses [3]. DF-1 is a continuous cell line of chicken embryo fibroblasts and it serves as a research model of avian leukosis. Purple corn is a kind of chicken feed. However, the effect of PCA to DF-1 cells has not been investigated yet. This work is to find out the optimal concentration of PCA to DF-1 cells living. Moreover, cells activity was explored by MTT assay.

Materials and Methods

Reagents and chemicals. Purple corn bracts and DF-1 cells line were supplied by Beijing Academy of Agriculture and Forestry Sciences. Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS) and Penicillin-Streptomycin Solution (100x) were supplemented by Gibco, (Shanghai, China); 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma (Shanghai, China). Trypsin-EDTA solution was produced from Wuhan Fine Biotechnology Company (Wuhan, China), dimethyl sulfoxide (DMSO) was purchased from Solarbio (beijing, China).

The clean workbench was from Suzhou Aetna air Technology Company (Suzhu, China); The CO_2 constant temperature incubator was obtained from Thermo Scientific Company (Boston, Massachusetts, USA). The microplate reader SpectraMax i3 was purchased from Molecular Devices Company (USA). The Cell counter was purchased from Wuzhou Eastern Company (Beijing, China). The 3K15 centrifugal machine was from Sigma Company (Shanghai, China).

Preparation of total anthocyanins from the purple corn bracts. The dried purple corn bracts were extracted with 60% (v/v) ethanol acidified with hydrochloric acid until pH = 3.0 at 50 °C for 120 min. The ratio of purple corn and extracted solution was 1:65 (v/v). The supernatants were evaporated to dryness at 45 °C with a rotary evaporator. It was filtered through a macroporous resin to remove the

sugars, organic acids, phenolic acids and flavonoids, respectively. Finally, the filtrate was dried in the freeze-drying machines to get anthocyanins powder, stored at -80°C. The concentration of anthocyanins was detected by pH differential method.

The recovery of DF-1 cell. Preheat the DMEM supplemented with 15% FBS, sodium penicillin(100 U/mL) and streptomycin (100 μg/mL). The cell cryopreservation tube was taken out from the liquid nitrogen and it was shaken in the warm water at 37 °C rapidly, ice dissolved quickly, cells were centrifuged at 1000r for 5min, the supernatant was discarded, the preheated medium was added and cells were aspirated by gently pipetting. All the cells were seeded into cell culture flasks(25 mm^2) . The cells were maintained at 37 °C in the atmosphere of 5% CO_2 and 95% O_2, the cells were allowed to attach for 24h.

The subculturing of DF-1Cells. The culture medium was removed and discarded, rinsed the cell layer with 5.0 mL PBS to remove all traces of serum. Then, 2.0 mL Trypsin-EDTA solution was added to flask and cells were observed under an inverted microscope until cell layer was dispersed. After that, 6.0 mL complete growth medium was added and cells were aspirated by gently pipetting. Finally, the cells were incubated at 37°C.

Effect of PCA for DF-1 cell growth. The PCA powder was diluted by 0.01 mol/L PBS until the concentration was 12.5 mg/mL, and filtered by a 0.22 μm syringe filter. The DF-1 cells were cultured by DMEM containing 10% FBS and antibiotics, they were seeded in 96-well plates at a density of 5.79×10^5 cells/well at 37°C with 5% CO_2. The culture medium was removed until forming monolayer, and it was replaced with DMEM containing 2% FBS. Cells in each well were treated with PCA (50, 75, 100, 150 μg/mL) for 12, 24, 36, 48, 60, 72, 96, 120 and 144h. Cells were recorded with cell counter at the same time. Three replicates for each concentration, set the cell control and repeat controls.

Results criteria as follows： "+++++" represents the best cell growth, cell has good refraction, and form a complete monolayer; "++++" indicates the cells grow well, form a monolayer; "+++" indicates cell growth in general, it cannot form a monolayer, there are much voids and few cell; "++" indicates cell growth poor, it can't present fundamental cellular morphology, a small amount of them adherent on the wall of cell flask; "+" indicates cell growth is poor, there is no basic cell morphology, a small amount of adherent; "-" indicates poor cell growth, all the cells have been broken, incomplete or poorly defined.

Cell viability (MTT assay). To evaluate the cytotoxicity of PCA, cell viability assay was performed by the same procedure as described previously[4]. DF-1 cells grew in DMEM medium containing 10% FBS, streptomycin (100 μg/mL) and sodium penicillin(100 U/mL). At the logarithmic growth phase, the cells were trypsinized and resuspended in complete growth medium. Briefly, 1.14×10^5cells suspended in DMEM medium (200 μl) containing 10% FBS were seeded in 96-well plates and treated with 0, 50, 75, 100, 150 μg/mL of PCA. After incubation for 24, 48, 72 and 96 h, MTT (0.5 mg/mL) was added to each well, the cells were incubated at 37°C for an additional 4 hours. Formazan was dissolved in dimethyl sulfoxide (DMSO) and add to each well. The 96 wells plates were agitated for 10 min at medium speed. Finally, absorbance was measured by multifunctional microplate reader (Molecular Devices company,USA) at 490 nm[5].

Statistical analysis. All experiments in this paper were repeated at least three times.Data are expressed as the mean±SD. The statistical analyses were done with the software origin 8.0.

Results and Discussion

HPLC measurement of PCA. The identification of PCA composition results were shown in Table 1. It is reported that the PCA belong to cyanidin, peonidin, pelargonidin or delphinidin aglycone, were bound with glucose or rhamonside, with glucose acylated with malonyl group[5]. According to the statistics in Table 1, it is obvious that cyanidin type is the main content of purple corn bracts extract, at about 63.20%, the figure of pelargonidin tpye is about 11.19%, the percentage of peonidin type is about 8.041%. The lowest among all the categories is the delphinidin type, is about 7.931%.

Table 1 HPLC-DAD results, proportion of compound type in PCA

No.	Compound type	Proportion (%)
1	Cyanidin type	63.20
2	Pelargonidin tpye	11.19
3	Peonidin type	8.041
4	Delphinidin type	7.931

Analysis of the effect of PCA on DF-1 cell growth. The PCA of different concentrations affected on DF-1 monolayer growth significantly. The results showed in Table 2. When the cells were cutured before 24h, the higher the concentration of the anthocyanins was, the better the DF-1 cells grew. After 24h, the optimal concentration for cell viability was 50 μg/mL. Cells were stained with Trypan blue and recorded viability by cell counter (Table 3), the results were similar to Table 2. The results criteria as previously described.

Table 2 Effect of different concentrations of PCA on DF-1 cell growth

Time (h)	Concentration (μg/mL)				Control
	50	75	100	150	
12	+++++	+++++	+++++	+++++	+++++
24	+++++	+++++	+++++	+++++	+++++
36	++++	++++	++++	+++	++++
48	++++	++++	++++	+++	++++
60	+++	+++	+++	++	+++
72	+++	+++	+++	++	+++
96	+++	++	++	++	+++
120	++	+	+	+	++
144	+	-	-	-	+

Table 3 Effect of different concentrations of PCA on DF-1 cell viability (%)

Time (h)	Concentration (μg/mL)				Control
	50	75	100	150	
24	95.41±1.259	95.7±2.013	96.24±1.547	96.92±1.933	98.81±1.541
48	93.84±1.870	94.53±1.424	89.95±2.371	94.09±0.971	96.77±1.834
72	91.57±1.838	83.66±2.602	84.87±1.751	80.70±2.243	89.77±3.927
96	86.11±1.703	79.84±1.459	80.00±1.839	76.83±1.309	83.15±3.305
120	96.88±2.970	93.55±2.284	93.94±1.775	92.93±1.231	84.62±4.505
144	86.25±0.806	85.00±1.768	78.69±1.036	85.92±1.534	68.04±2.283

Data are expressed as means±SD, n=3. Data in parenthesis represent the percentage (%) of the cell viability.

In order to verify the correctness of the results, we determined to treat DF-1 cells with various concentrations of PCA by MTT assay. The methods as previously described. The results were shown in Fig.1. Compared to the controls, the higher the concentration of PCA was, the better promoting effect to the cell grew, within 24 h. The treatment of 50 μg/mL PCA was similar to the controls, which had no cytotoxicity to DF-1 cells. When the concentration of PCA over 50 μg/mL, it showed a strong inhibitory effect for cells growth.

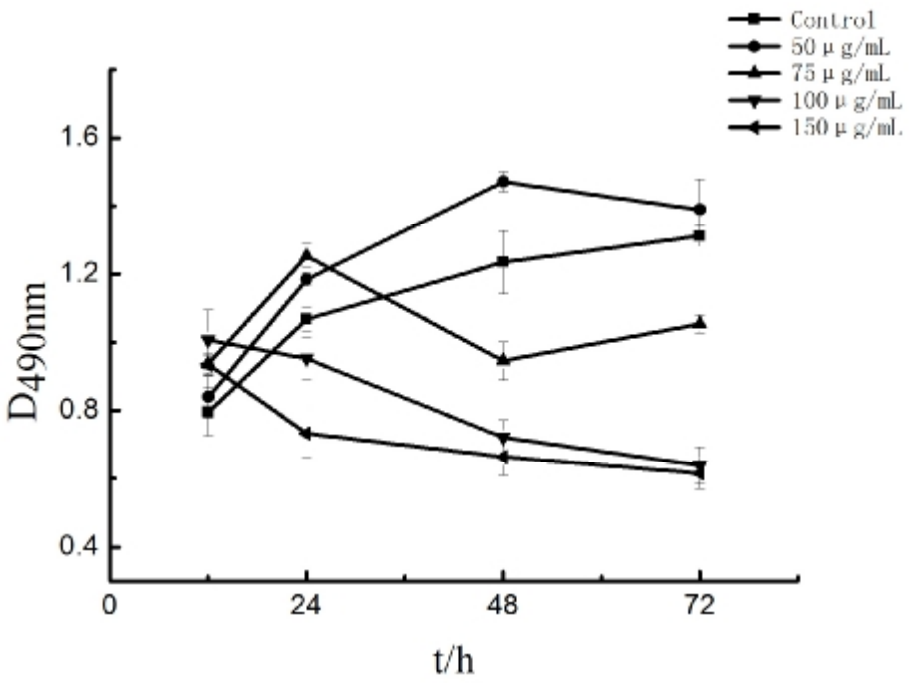

Fig.1-Effect of PCA for DF-1 cell growth by MTT assay

Summary

In summary, the results suggest that cyanidin type is the major compound in PCA. The optimal concentration of PCA for cell viability is 50 μg/mL, when the concentration of PCA below 50 μg/mL, it promotes DF-1 cells growth. On the contrary, it inhibits the cell growth. These will provide data support for the research of DF-1 cells in the future.

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References

[1] Xiaoyan Zhao, Chao Zhang, Claudia Guigas , Yue Ma, Margarita Corrales, Bernhard Tauscher, Xiaosong Hu, Composition, antimicrobial activity, and antiproliferative capacity of anthocyanin extracts of purple corn (Zea mays L.) from China, European Food Research and Technology. 228(2009) 759-765

[2] Katsube N, Iwashita K, Tsushida T, Yamaki K, Kobori M, J Agric Food Chem 51(2003) 68–75

[3] Martin Himly, Douglas N. Foster,Ivan Bottoli, Jason S. Iacovoni. Peter K.Vogt, The DF1 Chicken Fibroblast Cell Line: Transformation Induced by Diverse Oncogenes and Cell Death Resulting from Infection by Avian Leukosis Viruses. Virology. 2(1998) 295-304

[4] Martin, A., Morcillo, N., Lemus, D., et al., 2005. Multicenter study of MTT and resazurin assays for testing susceptibility to firstline anti-tuberculosis drugs. Int. J. Tuberc. Lung Dis. 9, 901-06.

[5] Dan Wang, Yongdong Lei, Yue Ma, Li Zhang, Xiaoyan Zhao, Inhibition of ALV-A-induced apoptosis in DF-1 cells via inactivation of nuclear transcription factor κB by anthocyanins from purple corn (*Zea mays L.*)Journal of Functional Foods. 10(2014) 274-282.