Whitening Effect of an Active Ingredient from Fresh Peral

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Abstract: OBJECTIVE To indent the function of an active ingredient from fresh pearl. **METHODS** Pearl extract in different concentrations were added into the 96-well plates, which contained B16 melanoma cell. The cell viability, tyrosinase activity and melanin biosynthesis of the B16 melanoma cell were measured. **RESULTS** The effect of pearl extract on cell viability was better than that of arbutin and alpha hydroxy acid, for example, the value of cell viability conducted by 40 mg/L pearl extract was 88.83±2.69%, similar to the 10 mg/L of arbutin and higher than that of alpha hydroxy acid with the same concentration. In terms of melanin biosynthesis, pearl extract could significantly inhibit the formation of melanin. In addition, half amount of tyrosinase could be inactived by 80mg/L pearl extract. **CONCLUSION** Pearl extract is of high security, and has significant whitening effect, and can be used as new whitening raw materials.

Introduction

Skin whitening is one of the most important effects of skin care. Developments and applications of skin whitening agent promote the research of skin whitening mechanism. Currently, inhibition of skin melanin was found to be the crucial part. Melanin is induced by oxidation of tyrosine by tyrosinase, with various conditions, different oxides are formed, including dopa, dopaquinone, dopachrome, 5,6-Dihydroxy-1H-indole, indole-5,6-dione, and ultimately pigment granules. It was found early by Japanese researchers that, ultraviolet radiation stimulates the proliferation and cellular differentiation of melanoma cell, and improves tyrosinase activity, and finally facilitate the melanin biosynthesis. The inhibition of melanin can be divided in two parts, reducing the activity of tyrosinase and proliferation. In addition, tyrosine is oxidized with involvement of free radicals. Recombinant polypeptide of fibronectin CH50 was confirmed to reduce the invasiveness and proliferation of B16 melanoma cell.

Pearl was literate in an ancient book kaibao bencao as a medicine, it has tranquilizing effect, heat-clearing and yin-nourishing, promotes good eyesight and detoxify, and heals wounds. With the development of manual pearl (fresh water) and processing methods, pearl is used not only as jewelry, but also functional material to protect from myopia and aging, heal wounds and Cosmetology. Pearl was also proved to have protection on skin cells and have anti-inflammatory and antioxidant effect by Qian and Zhou. The proteins in pearl was considered to related to the functions, but barely proved in literatures. The objective of this research is to investigate the effect of pearl extract on melanoma cell and tyrosinase. The inhibition of melanin by pearl extract is proved by the two parts mentioned previously.

Materials and methods

Materials

Freeze-dried pearl extract was provided by Zhejiang Osm Group Deqing Bio-tech Co.Ltd, mainly containing pearl peptide. B16 melanoma cell was bought from Shanghai Institute of Biochemistry and Cell Biology.

Fetal calf serum FBS, 0.25% pancreatic enzymes (with 0.02% EDTA) and PBS buffer solution (pH=7.4) are from Hangzhou Keyi Bio-tech Co. Ltd. Culture medium (RPMI-1640), MTT, Triton

X-100 were bought from Sigma. DMSO is from Shanghai Chemical Reagent Co. and Triton X-100 is from Merck. Microplate reader (Bio-Rad US). Carbon dioxide incubator

Methods

Cell culture [1]**.** B16 melanoma cell in logarithmic growth status was rinsed by PBS buffer solution (Hangzhou Keyi Bio-tech Co. Ltd.), and digested using 0.25% pancreatic enzymes (Hangzhou Keyi Bio-tech Co. Ltd.), then counted the cell population. The passage cells were diluted to 5×10^4 CFU/mL by using 10% Fetal calf serum (Hangzhou Keyi Bio-tech Co. Ltd.), to make a single cell suspension. The suspension was added into 96-well plates, 200 μL for each, incubated in the condition of 37 °C and 5% CO_2 for 24 h, then the supernate was discarded and the solid was rinsed by PBS once again. Different concentrations of pearl extract (10, 20, 40, 80, 10 mg/L) and 160 μL of PBS were added in the solid respectively, then incubated in 37°C and 5% CO_2 for 24 h. The control group was free of pearl extract, and the blank group only contained 200 μL of PBS.

MTT method[2]**.** After 24h of incubation, 10 μL of 5 g/L MTT (Sigma, US) was add in every well, the supernatant was discarded after 4h, then add 100 μL of DMSO (Shanghai Chemical Reagent Co.), vibrated for 10 min, and the absorption value was detected by a microplate reader (Bio-Rad, US) at 490 nm. The survival rate of melanoma cells was calculated by the following equation 1.

$$\text{Survival rate} = \left(1 - \frac{\text{Average absorption}}{\text{Average absorption of control}}\right) \times 100\% \quad (1)$$

Microscopic inspection. The culture medium was removed after 24h and 48h of incubation, the cells were washed 2 times by using PBS buffer, stabilized by 4% of methanal for 15 min, then rinsed by deionized water for 3 times and added 0.1% of dopa solution to dye for 3.5h, then the cells were washed by PBS and deionized water again. After that, the cells were observed using a microscope at 100 times of magnification.

Content of melanoma [3]**.** To detect the content of melanoma in cells, after 24h of incubation, the cells were rinsed by PBS for 2 times, added in 1 mol/L of NaOH, and diluted to 0.2 mol/L by double-distilled water, then bathed in 80°C for 1h to dissolve the cells and detected the absorption by the microplate reader at 400 nm. The relative content of melanoma was calculated by the following equation 2 and 3.

$$\textbf{Content of melanoma} = \frac{\textbf{Absorption of sample}}{\textbf{Absorption of control}} \times 100\% \quad (2)$$

$$\textbf{The relative content of melanoma} = \frac{\textbf{Content of melanoma}}{\textbf{survival rate}} \quad (3)$$

Activation of tyrosinase [4]**.** The concentration of melanoma cells suspension was adjusted to 1×10^5/mL and added into the 96-well plate renewed the medium after 24h, and added 100μL of pearl extract at different concentrations (10-100 mg/L). After 48h of incubation, the supernatant was discarded and the cells were washed by PBS buffer for 2 times, add 1% of Triton X-100 to dissociate the cells and freeze in -80°C for 1h, then melted at room temperature to release the tyrosinase. After a pre-thermal treatment of 37°C, 1% of dopa solution was added and kept for 1h, then the absorption was detected at 490 nm, the activation of tyrosinase was calculated by the following equation 4.

$$\textbf{Activation of tyrosinase} = \frac{\textbf{Absorption of sample} - \textbf{Absorption of control}}{\textbf{Absorption of control}} \times 100\% \quad (4)$$

Statistical method. The data was presented by the combination of average and standard deviation, the variance analysis was finished by SPSS 18.0.

Results and conclusion

Effects on the B16 cell

Cell viability. The stimulation of cosmetic materials on body skin ought to be investigated before used as ingredients. Figure 1 compared the effects of pearl extract, arbutin, and alpha hydroxy acid on the viability of skin cells. Higher concentration of the samples induced more impact on cell viability. The pearl extract has the least influence, and the alpha hydroxy acid has the biggest

influence. In low concentrations, the skin cells using pearl extract kept relatively higher viability, and the one using alpha hydroxy acid lose more cell viability. Arbutin in low concentration promoted the reproduce of skin cell, with higher concentration, it gradually had negative influence. Pearl extract with 40 mg/L resulted in higher population of B16 cell than 10 mg/L of arbutin and alpha hydroxy acid, which means pearl extract, has safety advantages over arbutin and alpha hydroxy acid.

Table 1 Effects of pearl extract on the B16 cell survival rate

Concentration, mg/L	Pearl extract	Arbutin	Alpha hydroxy acid
10	96.22±3.13ab	88.72±1.42b	71.00±9.55b
15	93.35±2.48bc	80.38±5.48bc	69.71±6.18b
20	92.34±0.92bc	71.00±3.55cd	58.28±7.81bc
40	88.83±2.69bcd	65.21±1.56d	51.00±4.67c
80	87.92±5.64bcd	57.24±2.78e	39.71±6.79d
100	86.00±0.74d	48.24±5.15f	28.28±6.21d

Microscopic inspection. Figure 1 shows the effect of pearl extract on B16 melanoma cell. Figure 1(a) and figure 1(b) presented that pearl extract reduces pigmentation after 24h of cultivation as compared with control. After 48h of cultivation, the increasing population that using pearl extract was less than that of control. In general, pearl extract can effectively inhibit the reproduce of B16 melanoma cell and pigmentation.

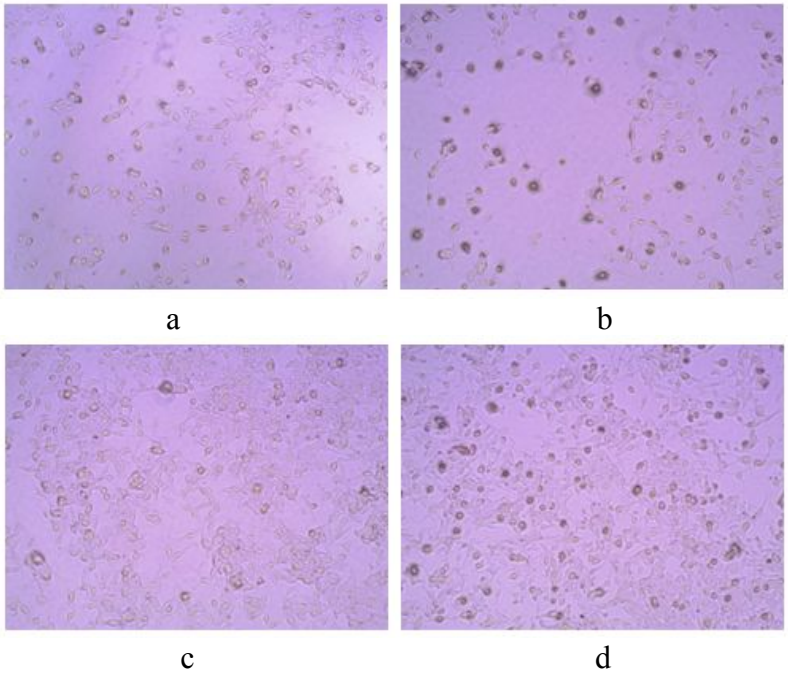

Fig.1 Effect of pearl extract on B16 melanoma cell
(a.24h , 40mg/L pearl extract; b 24h, blank; c. 48h, 40mg/L pearl extract; b. 48h Blank)

Effect of pearl extract on the content of melanoma

To compare the effects of three samples on the intracellular melanoma, we divided the relative melanoma content with the survival rate, which was showed in figure 2. It indicated that only pearl extract was capable to reduce the melanoma content in living cells significantly. The arbutin and alpha hydroxyl acid reduce the total melanoma content by destroying the cells. Obviously, pearl extract was better in both protection of skin cells and inhibition of melanoma.

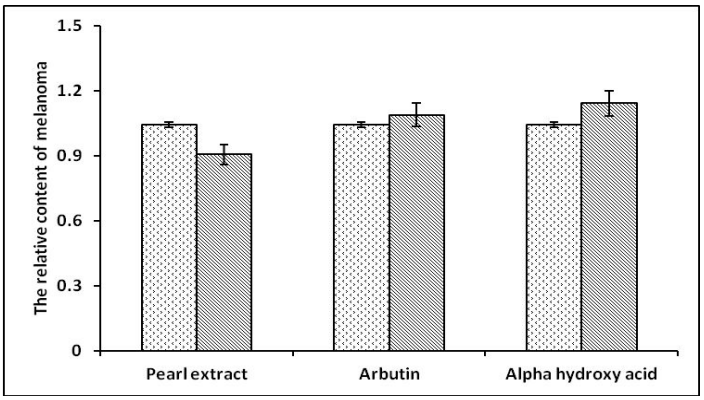

Fig.2 Effect of pearl extract on the content of melanoma

Effect of pearl extract on the tyrosinase activity

Pearl extract was proved to have significant inhibition effect on tyrosinase activity. With increasing of concentration of pearl extract, tyrosinase activity decreased from 87.2% to 41.36%. Half of the activity of tyrosinase was inhibited by 80 mg/L of pearl extract. However, 100 mg/L of arbutin can only reduce about 30% of the activity.

Table 2 Effect of pearl extract on the tyrosinase activity

Concentration mg/L	Pearl Extract	Arbutin
10	79.22±6.26a	89.67±2.62a
20	70.34±1.84b	84.91±7.58ab
40	61.83±5.38c	79.91±6.64bc
80	50.92±10.22d	76.61±6.26bc
100	41.36±4.88d	69.11±4.26c

Conclusion

The effects of pearl extract on reproduce of B16 melanoma cell, pigmentation and tyrosinase activity were investigated in this research. Compared with arbutin and alpha hydroxy acid, 40 mg/L of pearl extract resulted in higher survival rate of skin cell than 10 mg/L of arbutin and hydroxyl. The research indicated that pearl extract had better inhibition of pigmentation and reproduce, which was also proved by the microscopic examination. Pearl extract was demonstrated to reduce more tyrosinase activity than arbutin and alpha hydroxy acid. In general, pearl extract was proved to be a functional material that can be used in cosmetics. In the future, more clinical tests are needed to verify the effect of pearl extract.

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