Study on Crude Flavonoids Extraction from Longan Leaves and its anti Human Lung Adenocareinoma Cell Line A549

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Abstract. The crude extracts of flavonoids were obtained from longan leaves via ultrasonic and purified with macroporous resin of AB-8. Taking rutin as the standard, the amount of flavonoids in crude extracts was measured by ultraviolet spectrophotometer. The concentration of flavonoids after extraction and purification was 0.775 μg/mL. Total flavonoids in the extractum accounted for 77.5%. Total flavonoids accounted for 77.49% of the dry weight of the longan leaves. MTT assay was applied to test the A549 cell activities treated by a series of concentrations of flavonoids (50, 100, 200, 400 and 800 μg/mL) for 24h, 48h and 72h, respectively. The morphological changes of A549 cells treated by 100, 200 and 400μg/mL flavonoids for 48h were observed by inverted microscope and fluorescence microscope, respectively. The results showed that the total flavonoids of longan leaves obviously inhibit the proliferation of A549 cells, with cell shrinkage, nuclear fragmentation and apoptosis occurred.

1. Introduction

Longan is well known as one of the medicine and food plants [1]. Eating longan helps to relieve fatigue and regulate sleep [2-4]. The flavonoids extracted from longan meat have been demonstrated to show good anti-tumor effect [5-7]. However, the anti-tumor effect of total flavonoids in longan leaves has not been explored yet. In this study, the total flavonoids from longan leaves was extracted and its effects on tumor cells were observed by MTT assay, inverted microscope and fluorescence microscope. The results are benefit for the discovery of new types of natural anti-tumor drugs.

2. Materials and Methods

2.1. Reagents and instruments

Fresh longan leaves, A549 cells, 95% Ethanol, Rutin, Model 680 Microplate Reader, Olympus IX70 inverted microscope AE31, Fluorescence microscope MR-205, AB-8 resin.

2.2. Experimental method

2.2.1. Extraction and purification 12 g longan leaf powder was added into 160 mL 95% of ethanol and then ultrasonic extraction for 2.5 h. The rotary evaporator was used to condense the filtrate to 5

mL. Macroporous resin AB-8 was applied to purify total flavonoids. The elute is 70% ethanol. Take 0.1 g of extractum and place it in a 100 mL volumetric flask, and dilute it to the mark with 60% ethanol to obtain a sample solution.

2.2.2. Quantitative flavonoids UV Spectrophotometry was applied to determine the quantities of flavonoids in extracts with rutin as standard.

2.2.3. Cell culture and MTT Assay A549 cells were collected for cell counting and then inoculated on 96-well culture plates. The number of cells per well was 4×10^3. The cells were incubated at 37 °C, 5% CO2 for 24 h. 100 μL of drug solution was added to each well, and the negative control group was added with serum-free culture medium. The positive drug was cisplatin at a concentration of 2 μg/mL. The dosage of the drug for MTT assays were 50 mg/mL, 100 mg/mL, 200 mg/mL, 400 mg/mL and 800 mg/mL, respectively. And the time for treatment were 24 h, 48 h and 72 h, respectively. 200 μL 5 mg/mL of MTT were added into each group and incubate cells at 37 °C for 4 h. Aspirate the supernatant and add 200 μL DMSO to dissolve the purple crystals. The OD value at 490 nm was measured on a microplate reader and IC50 values were calculated and analyzed.

2.2.4. Inverted microscope observation of A549 cell morphology Different concentration of crude flavonoids extracts of longan leaves (100 μg/mL, 200 μg/mL and 400 μg/mL) were used to treat A549 cells, respectively. The negative control group was DMEM medium, and the positive control group was 2 μg/mL cisplatin. After treatment for 48 h, observation of cell morphology was conducted by inverted microscope.

2.2.5. Fluorescence microscope observation of A549 cell morphology A549 cells were inoculated on a 12-well plate with density of 1×10^5 per well and incubate the cells for 24 h. Different concentration of total flavonoids (100 μg/mL, 200 μg/mL, and 400 μg/mL) were adopted to treat A549 cells for 48 h, respectively. The positive control group was treated with 2 μg/mL cisplatin for 48 h. The blank control group was not subjected to any treatments. Remove the medium supernatant and wash twice with PBS. Add 0.5 mL of fixative solution and incubate for 20 min at room temperature. Rinse each well at least twice with PBS to remove the fixative solution. Stain with 0.5 mL Hoechs for 5 min and then wash twice with PBS to remove staining solution. The observation of the cell morphology was conducted by the fluorescence microscope.

3. Results and Analysis

3.1. Extraction and Content Determination of Total Flavonoids from Longan Leaves

The absorptivity of rutin standard and tested samples are shown in Table 1. Rutin standard curve is shown in Figure 1. The results showed that the concentration of flavonoids in longan leaves was 0.0775 mg/mL. Total flavonoids accounted for 77.5% of the extract, and total flavonoids extract accounted for 77.49% of the raw materials.

Table 1. Absorbance of rutin standards and samples.

Concentration mg/mL	Absorbance
0.005	0.047
0.01	0.125
0.02	0.224
0.03	0.369
0.04	0.523
0.05	0.660
Tested sample 0.01	0.726

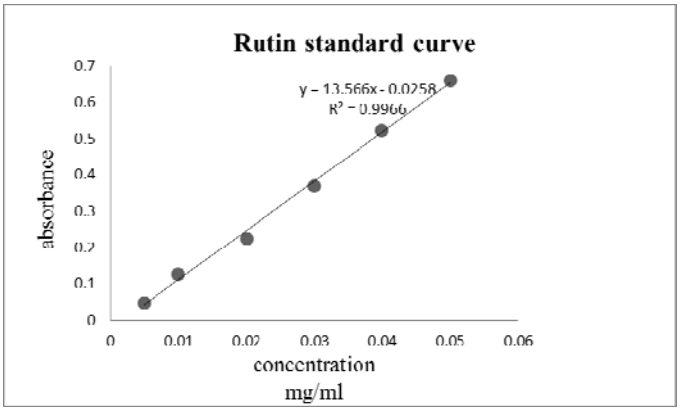

Figure 1. Rutin standard curve.

3.2. Inhibitory Effects of Total Flavonoid Extracts from Longan Leaves on A549 Cells

The relationships between the inhibition rate of A549 cells and the dosage and time of total flavonoids extracted from longan leaves were shown in Figure 2. 100 μg/mL, 200 μg/mL, 400 μg/mL and 800 μg/mL of crude flavonoids were used to treat A549 cells for 24, 48, and 72 h, respectively. Detection of inhibitory rate of cell proliferation was conducted by MTT assay. At a concentration of 400 μg/mL the proliferation of A549 cells was significantly inhibited, and the inhibition rates after 24 h, 48 h, and 72 h reached 28.12%, 63.33%, and 76.90%, respectively. Under the same treated concentration, the inhibition rate of tumor cells increased with increasing treatment time. Under the same time, the inhibition rate of tumor cell increased with the increase of the concentration.

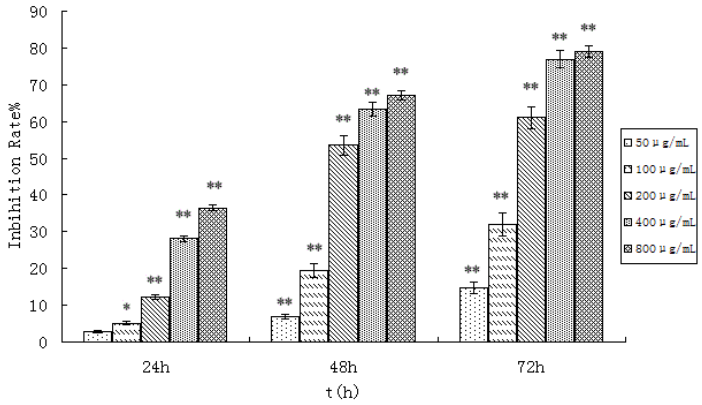

Figure 2. Effect of different treatment concentrations and time on the growth of A549 cells.

3.3. Observation of cell morphology after treatment of A549 cells with total flavonoids from longan leaves by inverted microscope

The morphology observations of A549 cell treated with different concentrations of flavonoids for 48 h were shown in Figure 3. The negative control group shows long spindle type with large number and high density of cells. When treated with 100 μg/mL flavonoids, the number of cells decreased compared to the negative control group, and part of the cells contracted into irregular oval shapes. When the dosage increased to 200 μg/mL, the cells shrinkage was severe. When the drug concentration reached 400 μg/mL, most of the cells shrinked into irregular round shapes. The results showed that total flavonoids extract from longan leaves inhibited the proliferation of A549 cells.

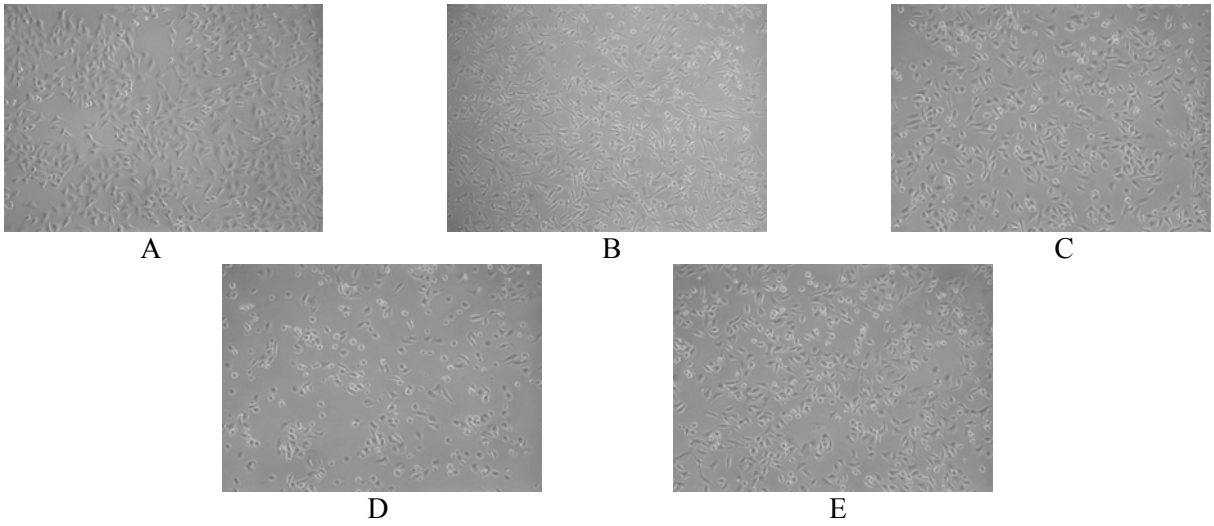

Figure 3. Observation of the cell morphology of A549 cells treated with different concentrations of flavonoids for 48 h by inverted microscope. A: Negative control DMEM, B: 100 μg/mL, C: 200 μg/mL, D: 400 μg/mL, E: Positive control cisplatin 2 μg/mL.

3.4. Observation of morphology of A549 cells treated with total flavonoids from longan leaves by fluorescence microscope

The morphology of A549 cells treated with different concentrations of total flavonoids of longan leaves for 48 h was shown in Fig 4. The results showed that along with the increasing concentration of flavonoids used to treat A549 cells, the living cells became less and less, and the apoptotic body appeared. These results demonstrated that the total flavonoids extracted from longan leaves are capable of inhibiting the proliferation of A549 tumor cells.

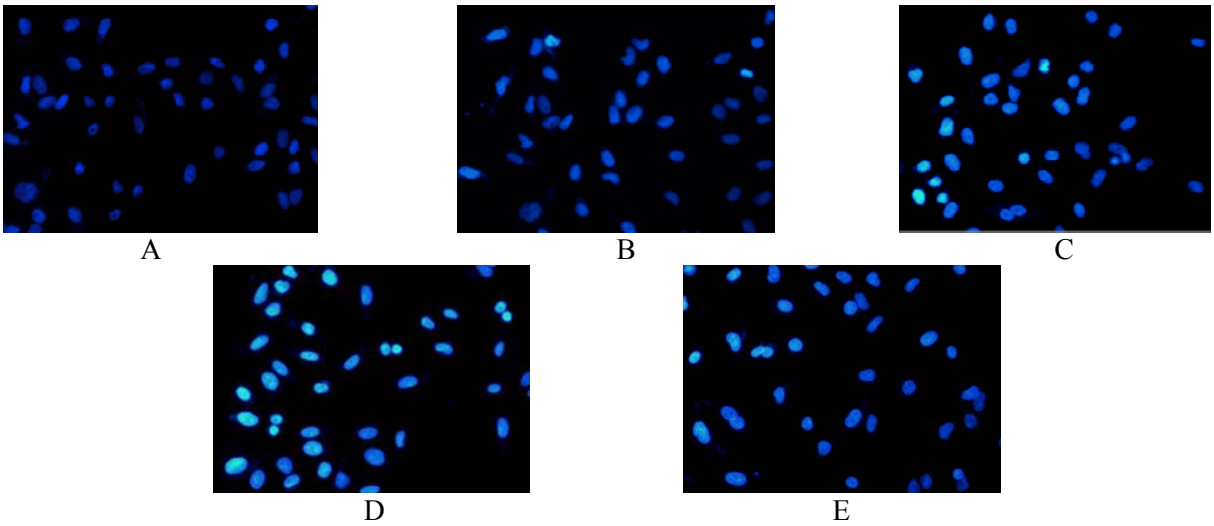

Figure 4. Observation of the cell morphology of A549 cells treated with different concentrations of flavonoids for 48 h by fluorescence microscope, A: Negative control DMEM, B: 100 μg/mL, C: 200 μg/mL, D: 400 μg/mL, E: Positive control cisplatin 2 μg/mL.

4. Conclusion

In this paper, longan leaves were used to extract the total flavonoids by ultrasonic method, and the inhibition of A549 tumor cell proliferation by total flavonoids were studied. The concentration of

flavonoids after extraction and purification was 0.0775 mg/mL. Total flavonoids accounted for 77.5% of the content of the extract. Total flavonoids accounted for 77.49% of the dry weight of the longan leaves. The results of cell inhibition assay showed that the total flavonoids extracted from longan leaves had inhibitory effects on A549 tumor cells. The cell inhibitory effect was significant when the cells were treated with flavonoids for 48 h. The inhibitory effect enhanced along with the increasing concentration of total flavonoids.

Acknowledgments
This work was financially supported by University Nursing Program for Young Scholars with Creative Talents in Heilongjiang Province (UNPYSCT-2017210), scientific research project of Harbin University of Commerce (17XN007) and Harbin University of Commerce doctoral research startup project (14LG06).

References
[1] Yuying Liu. Longan in China [J]. Chinese native products, 2000, 6 (2): 25-26.
[2] Changhe Cai, Xiaolang Tang, Aiyu Zhang. The therapeutic value of longan pulp and its development and application prospects[J]. food science, 2002, 23(8): 328-330.
[3] Zixing Ye, Jiankai Xu. Longan high yield technology question and answer [J]. Guangzhou: Guangdong Science and Technology Press, 2001: 56-58.
[4] Haifeng Lu, Jin Zhao, Suoyi Huang. Ultrasonic Extraction of Total Flavonoids from Longan Leaves and Identification [J]. Trace elements and health research, 2007, 24(4): 48-49.
[5] State Administration of Traditional Chinese Medicine Chinese Materia Medica Editorial Board. Chinese herb. Shanghai: Shanghai Science and Technology Press, 1999(13): 112.
[6] Jiangsu New Medical College. Dictionary of Chinese Medicine (Volume One) [M]. Shanghai: Shanghai Science and Technology Press, 2006: 879-880.
[7] Fujian Provincial Institute of Medicine. Fujian Journal of Traditional Chinese Medicine [M]. volume One. Fuzhou: Fujian People's Publishing House, 1979: 296-297.