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STUDY ON IN VITRO ANTI-TUMOR ACTIVITY OF *BIDENS BIPINNATA L.* EXTRACT

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Abstract

We studied the *in vitro* anti-tumor activity of *Bidens Bipinnata L.* extract. MTT assay was used to investigate the inhibitory effect of different concentrations of the extracts on human hepatocellular carcinoma (HepG2) cell lines and human cervical carcinoma (Hela) cell lines, and the IC_{50} values were calculated. The *Bidens Bipinnata L.* extract had different degrees of inhibitory effects on these two cells, and when exposure time was 48 h, the inhibition rate reached its peak, with IC_{50} values of 14.80 μg/mL and 13.50 μg/mL respectively. The *Bidens Bipinnata L.* extract had a good inhibitory effect on human HepG2 cell lines and Hela cell lines, and thus has certain development prospects.

Keywords: *Bidens Bipinnata L.*, cell culture, anti-tumor, MTT

Introduction

Bidens Bipinnata L. belongs to the genus *Bidens*, and it is widely used in the treatment of malaria, diarrhea, dysentery, hepatitis, acute nephritis and other diseases. Chemical studies have found that it contains components such as alkaloids, tannins, saponins, flavonoids, volatile oils, tannins, bitter substance, and choline (Wu et al., 1998). In recent years, pharmacological activities of the *Bidens Bipinnata L.* extract have been widely studied, which were mainly focused on the prevention of liver injury (Tang et al., 2006; Hu et al., 2007), anti-hyperlipidemia (Huang et al., 2009), analgesia (Zhou et al., 2007), treatment of renal anemia (Li et al., 2009), anti-hypertension (Qian et al., 2003), hypoglycemia (Li et al., 2003), anti-oxidative damage (Yang et al., 2006), and anti-tumor (Zhang et al., 2010). In this paper, the inhibitory effect of the *Bidens Bipinnata L.* extract on different tumor cells were studied, thus providing a basis for further development and application of the *Bidens Bipinnata L.*

Materials

Drugs and reagents

Bidens Bipinnata L. was purchased from the Runtu Natural Plant Products Co., Ltd. in Anlong County, Guizhou Province. DMEM, 1640 medium (Beijing Borunlaite Science & Technology Co., Ltd.); fetal bovine serum (Beijing Biodee Biotechnology Co., Ltd.); trypsin (Jiangsu Yoke Chemical Co., Ltd.); MTT (Sigma); other reagents were domestically-made analytically pure ones.

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Apparatus

NUAIRETM US AUTOFLOW CO_2 incubator (NUAIRE, Germany); SW-CJ-IF Clean Bench (Suzhou Purification Equipment Factory); AE31 inverted phase contrast microscope (Motic); automatic microplate reader (Model 550, Bio-Rad, U.S.); stainless steel positive pressure filter (Haining Yatai Pharmaceutical Machinery Co., Ltd.); 96-well sterile culture plate (Costar, U.S.); rotary evaporator (RE-52AA) (Shanghai Yarong Biochemical Instrument Factory); HH-8 digital display thermostat water bath (Changzhou Guohua Electric Appliance Co., Ltd.); electronic balance (Beijing Sartorius Instrument System Co., Ltd.); vacuum drying oven (DZF-6020) (Shanghai Yiheng Scientific Instruments Co., Ltd.); blood cell counting chamber (Shanghai Qiujing Biochemical Instrument Factory)

Cell lines

Human HepG2 cell lines and Hela cell lines were both purchased from the Cancer Institute of Chinese Academy of Medical Sciences.

Experimental methods

Preparation of *Bidens Bipinnata L.* extract

Dried *Bidens Bipinnata L.* herb (voucher No.2012-3-11) was crushed and (300 g) was weighed, added with 70% ethanol 25 times its volume, and extracted for three times using ultrasonic extraction for 20 min each, the extracts were combined together, and after low temperature concentration, purified by polyamide resin, after low temperature concentration and freeze drying of the effluent, *Bidens Bipinnata L.* extract powder was obtained. When using, 5.0 mg of powder would be precisely weighed out and dissolved with 20 uL of DMSO, Aqueous solution of the extract were made at a fixed concentration, and were filtered in 0.22 μm membrane which would be diluted to 5.0 mg/mL with culture medium for later use.

Cell cultivation

After recovery, the human HepG2 cell lines and Hela cell lines were placed into the prepared DMEM medium or 1640 medium containing 10% fetal bovine serum, and cultured at 37℃ in air containing 5% CO_2, when the cells grew to about 80% of the sidewall, the old medium was discarded; the cells were digested with adequate amount of 0.25% trypsin, and the digestive fluid was discarded; culture medium was added, and the cells were passaged according to the proportion of 1:3, with one passage every three days, cells after recovery was regarded as the first generation cells, and the experiment was not be performed until the third generation cells were attained after the recovery.

Cell screening with MTT assay

Cell plating

Subculture period of the HepG2 Cell was 4~6 days while the period for Hela cell lines was 22~24 hours. Human HepG2 cell lines and Hela cell lines in the logarithmic growth phase were collected respectively, and added with phosphate buffered saline (PBS) to flush out the debris, after trypsin digestion, cells were counted by blood cell counting chamber, and the cell density was adjusted to 5×10^4 cells/mL. 100 μl of the cell suspension was added into each well of the 96-well microplate, and PBS was added to the wells around the plate to maintain the humidity within the plate. After addition, the culture plate was incubated in the CO_2 incubator (37℃, saturated humidity, 5% CO_2) for 12 h.

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Dosing

The drug was diluted to six different concentrations, namely 500, 250, 125, 62.5, 31.25, and 15.625 6 μg/mL, which were added to the pre-plated 96-well plate, with each well added with 20 μL of the drug, 5 parallel wells were prepared for each concentration of the drug, at last, the culture medium was replenished to 200 μL. The blank control group was directly added with the culture medium to 200 μL without the addition of the drug, they were incubated for 24 h, 48 h, and 72 h respectively.

Coloration and determination

The cell culture plate was removed and the drug-containing medium was aspirated, after addition of 100 μL of culture medium to each well, the wells were added with 20 μL of 5 mg/mL MTT solution, and cultured for 4 more hours, the culture medium was aspirated, 150 μL of DMSO was added to each well and gently agitated, after complete dissolution of the purple crystal powder, its absorbance (OD) value was determined at 490 nm wavelength using a microplate reader, inhibition rate was calculated according to the following formula, inhibition curve with logarithm of drug concentration as the abscissa and inhibition rate as the ordinate was plotted, and the IC_{50} value was calculated.

Cell growth inhibition rate (%) = ($OD_{\text{blank group}}$ - $OD_{\text{sample group)}}$ / $OD_{\text{blank group}}$ × 100%

Statistical methods

The experimental data was analyzed using the SPSS 13.0 software, and the two groups were compared using the t test, pairwise comparisons among several groups were performed using the one-way analysis of variance (ANOVA).

Results

Effect of *Bidens Bipinnata L.* extract on human hepg2 cell lines

The effect of *Bidens Bipinnata L.* extract on human HepG2 cell lines was as shown in Tables 1 and 2 and Figure 1. 1.5625 μg/mL~50 μg/mL concentrations of *Bidens Bipinnata L.* extracts inhibited the proliferation of human HepG2 cells after intervening for 24 h, 48 h, and 72 h. When the concentration was 50 μg/mL, the difference between the experimental group and control group was highly significant ($P<0.01$); comparisons among various experimental groups showed no significant difference. When the *Bidens Bipinnata L.* extract acted on the cells for 24 h, its inhibition rate did not reach the peak. However, inhibition rate was high for 48 h, but the inhibition rate increased slightly for 72 h with no significant difference with 48 h. Furthermore, lengthy time and lack of cell nutrition would also lead to the limited proliferation ability, thus, through comprehensive consideration, 48 h was selected as the optimum time of exposure. At 48 h, IC_{50} of *Bidens Bipinnata L.* extract on human HepG2 cell lines was calculated to be 14.80 μg/mL.

Effect of *Bidens Bipinnata L.* extract on hela cell lines

The effect of *Bidens Bipinnata L.* extract on Hela cell lines is shown in Tables 3 and 4 and Figure 2. 1.5625 μg/mL~50 μg/mL concentrations of *Bidens Bipinnata L.* extracts inhibited the proliferation of Hela cells to a certain degree after acting for 24 h, 48 h, and 72 h. From the perspective of drug concentration: when the concentration

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of the extract was 50 µg/mL, the difference between the experimental group and the control group was highly significant ($p<0.01$); comparisons among various experimental groups showed no significant difference. When the concentration of the extract was 25 µg/mL, the comparison between the experimental group and the blank control group showed significant difference ($P<0.05$). From the perspective of the time of drug exposure: when the *Bidens Bipinnata L.* extract acted on the cells for 24 h, its inhibition rate did not reach the peak, inhibition rate was higher at 48 h, the inhibition rate increased slightly for 72 h with no significant difference from 48 h. Furthermore, lengthy time and lack of cell nutrition would also lead to the limited proliferation ability, thus, through comprehensive consideration, 48 h was selected as the optimum time of exposure. At 48 h, IC50 of *Bidens Bipinnata L.* extract on Hela cell lines was calculated to be 13.50 µg/mL.

Table 1: Concentration and Time Effects of *Bidens Bipinnata L.* Extract on Human HepG2 Cell Lines

Group	Final concentration of the drug (µg/mL)	OD value 24h	48h	72h
Blank control group	-	0.446±0.014	0.775±0.023	0.819±0.041
Bidens Bipinnata L.	50	0.162±0.011**	0.132±0.007**	0.112±0.005**
extract group	25	0.234±0.018*	0.174±0.011*	0.154±0.011*
	12.5	0.337±0.028	0.466±0.032	0.354±0.025*
	6.25	0.407±0.027	0.657±0.021	0.668±0.032
	3.125	0.414±0.024	0.679±0.038	0.697±0.026
	1.5625	0.425±0.019	0.731±0.026	0.715±0.034

Comparison with the control group: "*" indicates $P<0.05$, "**" indicates $P<0.01$

Table 2: Inhibition Rate of *Bidens Bipinnata L.* Extract on Growth of Human HepG2 Cell Lines

Extract concentration (µg/mL)	Inhibition rate (%) 24h	48h	72h
50	63.68	82.97	86.32
25	47.53	77.55	81.20
12.5	24.44	39.87	56.78
6.25	8.74	15.22	18.43
3.125	7.17	12.39	14.90
1.5625	4.71	5.68	12.70

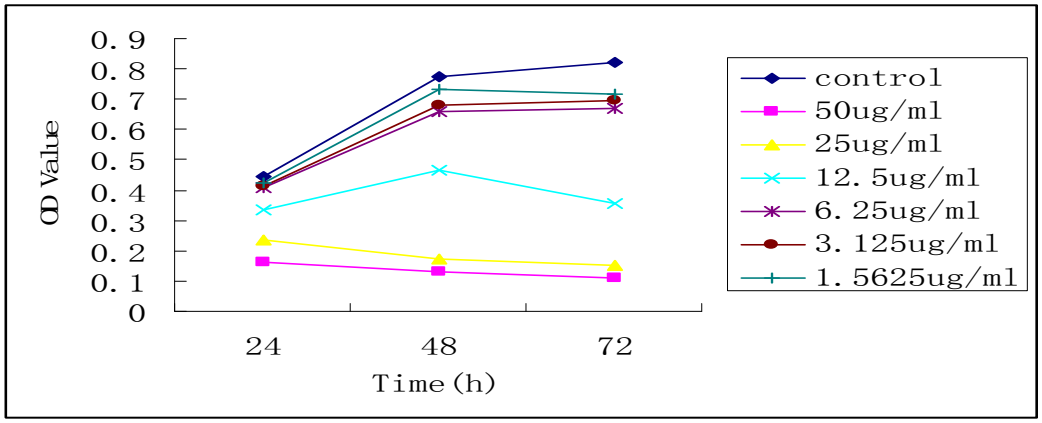

Figure 1: Concentration and Time Effects of *Bidens Bipinnata L.* Extract on Human HepG2 Cell Lines

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Table 3: Concentration and Time Effects of *Bidens Bipinnata L.* Extract on Hela Cell Lines

Group	Final concentration of the drug (μg/mL)	OD value 24h	48h	72h
Blank control group	-	0.459±0.015	0.785±0.021	0.807±0.035
Bidens Bipinnata L. extract group	50	0.143±0.012**	0.117±0.007**	0.105±0.006**
	25	0.221±0.018*	0.153±0.013*	0.143±0.013*
	12.5	0.341±0.025	0.426±0.033	0.355±0.024*
	6.25	0.392±0.031	0.631±0.027	0.649±0.042
	3.125	0.407±0.027	0.659±0.032	0.685±0.021
	1.5625	0.442±0.032	0.715±0.024	0.698±0.033

Comparison with the control group: "*" indicates $P<0.05$, "**" indicates $P<0.01$

Table 4: Inhibition rate of *Bidens Bipinnata L.* extract on growth of hela cell lines

Extract concentration (μg/mL)	Inhibition rate (%)		
50	68.85	85.10	86.99
25	51.85	80.51	82.28
12.5	25.71	45.73	56.01
6.25	14.60	19.62	19.58
3.125	11.33	16.05	15.12
1.5625	3.70	8.92	13.51

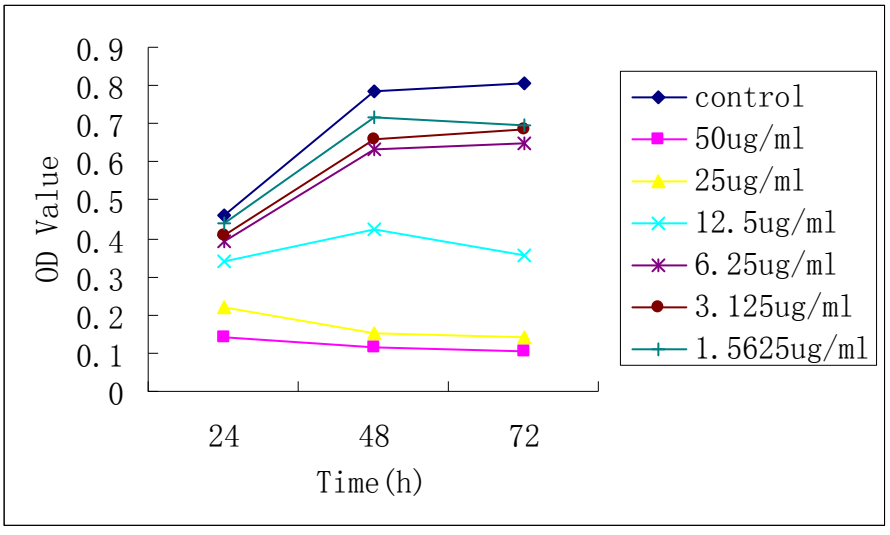

Figure 2: Concentration and Time Effects of *Bidens Bipinnata L.* Extract on Hela Cell Lines

Comparison between effects of *Bidens Bipinnata L.* extract on human hepg2 cell lines and on hela cell lines

The growth inhibition curves of the human HepG2 cell lines and Hela cell lines after 48 h incubation with *Bidens*

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Bipinnata L. extract are shown in Figure 3-3, with the increasing of the concentrations of the extract, the growth inhibition rates of the two cells also increased. When the concentration of the extract was greater than 25 μg/mL, the inhibition rate was stabilized. However, the inhibitory effect of the extract was stronger for human HepG2 cells than for Hela cells.

Discussion

Bidens Bipinnata L. was used in folk medicine for the treatment of various inflammations. Recently, it was reported in the literature that 70% ethanol extract of *Bidens Bipinnata L.* had an anti-tumor effect on the U14 tumor-bearing mice (Feng et al., 2007). In this paper, the extract of *Bidens Bipinnata L.* was taken as the study object, and MTT assay was used to screen its effect on human HepG2 cells and Hela cells. The results showed that the extract had different degrees of inhibition effects on both of these two tumor cells, and its effect reached its peak 48 h after administration, which provided a basis for finding out the monomer components with anti-tumor activity from *Bidens Bipinnata L.*

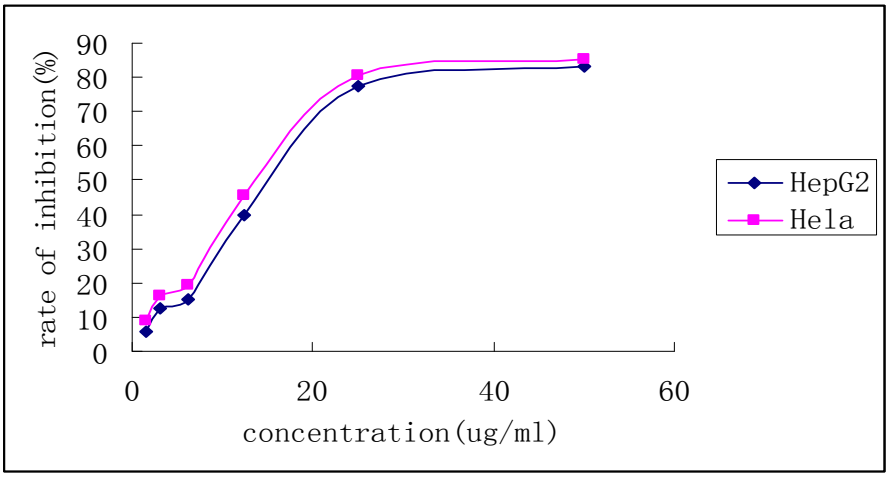

Figure 3: Growth Inhibition Curves of the Human HepG2 Cell Lines and Hela Cell Lines by *Bidens Bipinnata L.* Extract

Some reports claimed that the Cytopiloyne as a kind of ethanol extract has some effect on the modulation of T cell.(Chiang et al., 2007) and the extracts had obvious effect on A549 and U14 cell lines. So far, the inhibition of *Bidens Bipinnata* L. extracts on HepG2 was rarely found; however, there were many reports on its effects on inflammation, such as appendicitis (Li et al., 2007). Some reports said that the Cytopiloyne as a kind of ethanol extract has some effect on the modulation of T cell (Li et al., 2003).

In conclusion, the extracts has obvious effect on the cell lines of A549 and U14.

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