

THE INHIBITORY EFFECT OF *BIDENS BIPINNATA L.* EXTRACT ON U14 TUMOUR IN MICE

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Abstract

The objective of this paper was to study the in vitro and in vivo inhibitory effect of *Bidens bipinnata L.* extract on growth of cervical carcinoma U14 cells. MTT method was used to determine the inhibitory effect of *Bidens bipinnata L.* extract on U14 tumour cells, and the effects of *Bidens bipinnata L.* extract on inhibition rate of solid tumour and life prolongation rate of ascites tumour were observed through the establishment of two animal models of mouse cervical carcinoma U14 solid tumour and ascites tumour. In the in vitro MTT assay, the inhibition rate gradually increased with the increase of dose of *Bidens bipinnata L.* and the extension of time. Its inhibition rate was 70.44% at a concentration of 80µg/L. Solid tumour inhibition rates in the high- and low-dose groups and cisplatin group were 49.13%, 2.26% and 75.72% respectively; life prolongation rates in each ascites tumour group were 63.63%, 34.86% and 87.34% respectively. The *Bidens bipinnata L.* extract has a certain inhibitory effect on growth of mouse cervical carcinoma U14.

Keywords: *Bidens bipinnata L.* extract; U14; solid tumour; ascites tumour

Introduction

Bidens bipinnata L. is a plant in the genus *Bidens* of the family Asteraceae, which is also known as Popozhen, Guichacao, Mangchangcao, and Yinxianbao. It is produced in the northeast, north, east, southwest regions, as well as Shaanxi, Gansu provinces of China, and is grown on the roadsides, wastelands, hillsides and fields. *Bidens bipinnata L.* is initially recorded in a Chinese classical pharmaceutical book "Bencao Shiyi" as bitter in taste, lightly sweet, slightly cold, and non-toxic. Its main effects are heat-clearing and detoxifying, anti-diarrhoeal, stasis-eliminating and anti-swelling, and is mainly used in the treatment of malaria, diarrhoea, dysentery, hepatitis, acute nephritis, stomach-ache, traumatic injury, snake and insect bites, etc. (Wang et al., 2009).

As a traditional Chinese ancient medicine, *Bidens bipinnata L.* contains multiple chemical constituents, and has a wide range of biological activities and clinical efficacies. Its main chemical constituents include flavonoids, enynes, coumarins, organic acids and their esters, triterpenes, sterols, volatile oils, as well as a few amino acids and trace elements (Yang et al., 2009; Zhang et al., 2004). It has been found in recent years that the *Bidens bipinnata L.* can be used for anti-oxidation, anti-bacteria, anti-inflammation, anti-malaria, anti-tumour, and for the treatment of colds, goitre, chronic pelvic inflammation, annex inflammation, uterine prolapse, and neurasthenia. It also has relatively good curative effects on primary hypertension, thrombosis prophylaxis and diabetes (Qian et al., 2003; Yi et al., 2009; Celia et al., 2004; Huang et al., 2012; Zhang et al., 2010; Tang et al., 2010).

Materials and Methods

Drugs and Reagents

Bidens bipinnata L. (AF-2012-BHF) decoction pieces (Jiangxi Nanhua Medicine Co., Ltd.), which was identified by Dr Sun JH; cisplatin (Kunming Guiyan Pharmaceutical Co., Ltd.)

Instruments

AE31 inverted phase contrast microscope (Motic); SW-CJ-IF clean bench (Suzhou Purification Equipment Factory); electronic balance (Beijing Sartorius Instrument & System Engineering Co., Ltd); haemocytometer (Shanghai Qiujiang Biochemical Instrument Factory)

Experimental Animals

Kunming mice, equal numbers of males and females, weighing 18~22 g, purchased from the Laboratory Animal Centre of

Xinxiang Medical College University (XX-09-006). All experimental procedures were approved by the Animal Research Ethics Committee of Xinxiang Medical College University

U14 Tumour Lines

Purchased from the KeyGEN Biotech Co., Ltd.

Preparation of *Bidens bipinnata* L. extract

50g of *Bidens bipinnata* L. powder was weighed, added with a 15-fold volume of 80% ethanol, and ultrasonically extracted three times with 30 min each time, followed by suction filtration. After the filtrates were combined, cryoconcentrated, and freeze dried, *Bidens bipinnata* L. extract powder was obtained.

Determination of effect of *Bidens bipinnata* L. extract on U14 cell proliferation by MTT assay

Cervical carcinoma U14 cells were cultured in DMEM medium containing 10% foetal bovine serum, and incubated in a constant temperature incubator at 37°C with 5% CO₂. Cells were collected in the logarithmic growth phase. The logarithmic growth phase U14 cells were diluted to a concentration of 1×10⁶ cells/ml and seeded in 96-well plates with the addition of different concentrations of *Bidens bipinnata* L. extracts (5, 10, 20, 40, 80µg/L). The one without addition of the drug was also prepared as the blank group. Cells were cultured for 24, 48, 72 h respectively, then added with MTT, and cultured for another 4 h. After that, culture medium was discarded; DMSO was added and the plates were shaken. Optical density (OD) value was measured at 570 nm with a microplate reader, and cell growth inhibition rate was calculated according to the formula. The calculation was repeated three times and averaged. The formula is as follows:

Cell inhibition rate = (1 – average OD value of experimental group / average OD value of control group) × 100%

Establishment of U14 tumour-bearing mice model

0.5 ml of well-grown mouse cervical carcinoma U14 cell suspension was injected into the peritoneal cavities of mice, and passaged in their peritoneal cavities. When the passaged mouse ascites turned white, ascites were collected and diluted to a 1×10⁷ cells/ml tumour cell suspension with sterile 0.9% saline. 32 Kunming mice were all inoculated in the right armpit with 0.2 ml of U14 mouse cervical carcinoma cell suspension having a concentration of 1×10⁷ cells/ml, and weighed immediately after inoculation.

Establishment of ascites tumour model

Under aseptic conditions, U14 tumour cells passaged in the Kunming mice were taken and 0.2 ml of U14 mouse cervical carcinoma cell suspension with a concentration of 1×10⁷ cells/ml was intraperitoneally injected in each mouse. Inoculation success rate was 100%.

Grouping of animal models

32 Kunming mice were randomly divided into four groups, with each group containing 8 mice. Four days later, after the tumours grew to the size of a rice grain, grouped administration was begun: model control group was administered by intraperitoneal injection of 0.2ml of sterile saline per mouse every other day, cisplatin group was administered by intraperitoneal injection of 3mg/ml cisplatin every other day, *Bidens bipinnata* L. extract high-dose group was intragastrically administered 10 g/kg *Bidens bipinnata* L. extract everyday, and *Bidens bipinnata* L. extract low-dose group intragastrically administered 5 g/kg *Bidens bipinnata* L. extract everyday. After 10 days of consecutive administration, models were successful. On the eleventh day after administration, the mice were sacrificed by cervical dislocation, weighed, and tumour inhibition rate was calculated. The calculation formula is as follows:

Tumour inhibition rate (%) = (average tumour weight of control group - average tumour weight of administration group / average tumour weight of control group) × 100%

Determination of ascites tumour life prolongation rate

After discontinuation of administration, number of survival days of mice was observed, and life prolongation rate of mice was calculated. The calculation formula is as follows:

Life prolongation rate (%) = (mean survival days of administration group - mean survival days of control group) / mean survival days of control group × 100%.

Statistical Methods

All data were statistically analysed using one-way ANOVA in SPSS 13.0 statistical software.

Results

Determination of effect of *Bidens bipinnata L.* extract on proliferation of cervical carcinoma U14 cells by MTT assay

Bidens bipinnata L. extract significantly inhibited the proliferation of cervical carcinoma U14 cells in a dose- and time-dependent manner. Within the same time, inhibitory effect was gradually enhanced with the increase of dose; at the same dose, inhibitory effect was gradually increased with the extension of time, as shown in Figure1

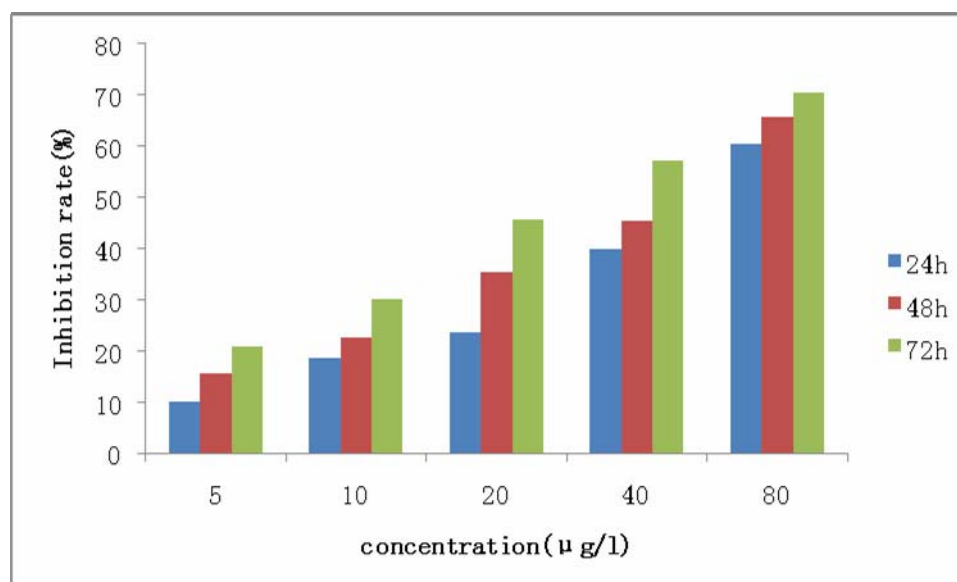


Figure 1: Extract on proliferation of cervical carcinoma U14 cells

Effect of *Bidens bipinnata L.* extract on tumour inhibition rate in U14 tumour-bearing mice

As can be seen from Table 1, compared with control group, *Bidens bipinnata L.* dose groups all had different degrees of inhibitory effects on U14 solid tumour. Tumour weights of high- and low-dose groups were significantly lower than that of the control group. The effect was obvious and the difference was statistically significant.

Table 1: Effect of *Bidens bipinnata L.* extract on tumour inhibition rate in U14 tumour-bearing mice ($\bar{X} \pm S$, n=10)

Group	Dose (g/kg)	Initial weight(g)	Final weight(g)	Tumour weight(g)	Tumour inhibition rate (%)
Control group	-	20.12±0.74	26.72±1.03	1.73±0.13	-
Cisplatin group	0.3	19.89±0.56	23.84±1.15	0.42±0.25**	75.72
<i>Bidens bipinnata L.</i> high-dose group	10	21.42±0.84	24.57±1.41	0.88±0.16**	49.13
<i>Bidens bipinnata L.</i> low-dose group	5	20.34±0.62	25.14±1.37	1.26±0.19*	27.16

Note: comparison with the control group, *P<0.05, **P<0.01

Effect of *Bidens bipinnata L.* extract on life prolongation rate

As can be seen from Table 2, mice in the two *Bidens bipinnata L.* extract dose groups show a life prolongation rate of 34.86% and 63.62% respectively. The statistical analysis revealed significant difference (P<0.01) for the two *Bidens bipinnata L.* extract dose groups and cisplatin group when compared with the control group.

Table 2: Effect of *Bidens bipinnata L.* extract on life prolongation rate ($\bar{X} \pm S$, n=10)

Group	Dose (g/kg)	Survival days (d)	Life prolongation rate (%)
Control group	-	12.48±1.34	-
Cisplatin group	0.3	23.38±1.09	87.34
<i>Bidens bipinnata L.</i> high-dose group	10	20.42±1.58	63.62
<i>Bidens bipinnata L.</i> low-dose group	5	16.83±1.42	34.86

Discussion

Traditional Chinese Medicine has advantages such as abundant resources, and small toxic and side effects, which have gradually become a focus of attention at home and abroad. The use of traditional Chinese medicine for anti-tumour therapy is also one of current research focuses. *Bidens bipinnata* L. contains a variety of chemical constituents and possesses a wide range of pharmacological activities (Feng et al., 2007). Besides, *Bidens bipinnata* L. has a higher amino acid content, which can be used as the raw material for extraction of amino acids. It has higher flavonoid content as well, and possesses antimicrobial effects (Wang et al., 2009). *Bidens bipinnata* L. extract has certain protective effect against liver injury (Li et al., 2008; Tang et al., 2006). The inhibition of tumour growth speed or tumour volume shrinkage is an important indicator for evaluating the efficacy of anti-tumour therapy of antineoplastic drugs. In this experiment, tumour growth inhibition rate was determined by MTT assay. When drug concentration was 80µg/L, the inhibition rate was 70.44%, indicating that the *Bidens bipinnata* L. extract has certain inhibitory effect on tumour growth speed. Solid tumour and ascites tumour experiments showed the inhibition rates of 49.13% and 63.63% in the high-dose group respectively, indicating that the *Bidens bipinnata* L. extract can inhibit the growth of tumour volume. Therefore, *Bidens bipinnata* L. extract can enhance immune function, and has certain anti-cancer effects, but the exact mechanism still needs further investigation.

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