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ANTI-HEPATOCELLULAR CARCINOMA: EFFECT OF BIOACTIVE COMPONENTS OF SPERANSKIA TUBERCULATA (BUNGE) BAILL

Abstact. *Chinese herbal medicine has gradually been widely used in the clinical treatment of cancer patients. This paper's research starting point is the Chinese herbal medicine Speranskia Tuberculata (Bunge) Baill. First, the biologically active parts are extracted and separated. Then, the crude extracts of different polarities are used for cell experiments to explore their positive role in preventing and treating hepatocellular carcinoma. It is determined that the use of ethyl acetate allows obtaining extracts with maximum therapeutic effect.*

Keywords: *Hepatocellular carcinoma; Chinese herbal medicine; Speranskia Tuberculata (Bunge) Baill; Crude extracts.*

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АНТИГЕПАТОЦЕЛЮЛЯРНА КАРЦИНОМА: ВПЛИВ БІОАКТИВНИХ КОМПОНЕНТІВ SPERANSKIA TUBERCULATA (BUNGE) BAILL

Анотація. *Китайська фітотерапія поступово набула широкого застосування в клінічному лікуванні онкологічних хворих. У цій статті досліджено китайський фітотерапевтичний засіб Speranskia Tuberculata (Bunge) Baill. Спочатку екстрагують і відокремлюють його біологічно активні частини, а потім неочищені екстракти різної полярності використовують для експериментів на клітинах, щоб вивчити його позитивну роль у профілактиці та лікуванні гепатоцелюлярної карциноми. Визначено, що використання етилацетату дозволяє отримати екстракти з максимальним терапевтичним ефектом.*

Ключові слова: *гепатоцелюлярна карцинома; китайська фітотерапія; Speranskia Tuberculata (Bunge) Baill; неочищені екстракти.*

Introduction. Hepatocellular carcinoma is a common clinical disease, and due to the influence of many factors, it poses more and more severe threats to human health. In particular, cancer diseases have a rapid onset, rapid onset, and poor prognosis, so it is essential to take timely, effective, safe and reliable treatment for patients from the very beginning [2]. When treating cancer patients, the primary methods were western medicine and surgical treatment, such as radical mastectomy, chemotherapy and radiotherapy. Although this treatment method can achieve a particular effect, its safety is not high. For example, various adverse reactions and complications often occur after chemotherapy, radiotherapy, and surgery, significantly impacting patients' bodies. Because of these shortcomings of western medicine, Chinese medicine has begun to receive more attention in treating hepatocellular carcinoma. Primarily through Chinese herbal medicine treatment, safe and effective treatment effects can be achieved. Chinese herbal medicine is very safe [3] and will not cause severe complications and adverse reactions. Therefore, Chinese herbal medicine has been used more and more widely in the treatment of hepatocellular carcinoma.

Currently, there are several studies have proven that some herbal medicines have been shown to slow down the clinical signs and symptoms in patients with hepatocellular carcinoma. For example, ursolic acid UA, an extract of Hedyotis diffusa, significantly inhibited the growth of R-HepG2 cells in a time- and dose-dependent manner by a mechanism mediated by cell cycle blockade and induction of apoptosis [4]. Chrysanthemum extract effectively attenuated the mitosis effects of isoprenaline (ISO) on HepG2 and MH-CC97H cells by inhibiting the sub β2 receptor agonism of ISO on tumour cells thereby blocking the activation of MAPL/ERK1/2 signalling pathway [5]. Meanwhile, the poor prognosis of hepatocellular carcinoma patients is mainly related to the distal metastasis of malignant tumour cells. Oleanic acid and Ursolic Acid from Hedyotis diffusa extracts were able to inhibit the ability of Huh7 and HepG2 cells to proliferate and metastasise tumours and to regulate liver function, glucose metabolism and migration-related receptors [6]. Wogonin was able to inhibit MH-CC97L and PLC/PRF/5 cell migration by a mechanism related to the inhibition of MMP-9 activity [7]. The ethanolic extract of Dendranthema Indicum inhibited the metastasis of MH-CC97H cells by inhibiting MMP-2 and MMP-9 expression and promoting TIMP-1 and TIMP-2 expression [8]. These studies have shown that Hedyotis diffusa, Scutellaria baicalensis Georgi, and Dendranthema Indicum could inhibit the metastasis of hepatocellular carcinoma cells.

Speranskia tuberculata (Bunge) Baill is a kind of Euphorbiaceae Phyllanthus plant containing various nutrients, including polysaccharides, vitamins and amino acids, and a variety of drug structural components, including polyphenols, flavonoids, sterols, alkaloids and trace elements. Speranskia tuberculata (Bunge) Baill has the effects of resolving phlegm, relieving cough, strengthening the stomach, clearing heat and detoxification, and is widely used in antioxidant, lipid-lowering and anti-inflammatory aspects. In recent years, the bioactive components of Speranskia tuberculata (Bunge) Baill have been widely studied, but there are few studies on antitumor, anti-atherosclerosis and cardiovascular diseases. This paper mainly explored the crude extracts of Speranskia tuberculata (Bunge) Baill in treating and preventing hepatocellular carcinoma.

Problem statement. In this paper, the anti-hepatocarcinoma efficacy of Speranskia Tuberculata (Bunge) Baill extracts was studied by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cytotoxicity tests.

Experimental. For experiments, the Speranskia Tuberculata (Bunge) Baill were first powdered. The powder was sequentially extracted using a Soxhlet apparatus with petroleum ether, ethyl acetate, n-butanol and finally deionised water. Each extract was concentrated under reduced pressure, vacuum dried (-20ºC), then weighed to calculate yield. The extracts were dissolved in dimethyl sulfoxide (DMSO; Sigma – Aldrich) for storage at a 10-100 mg/ml concentration and used at a 1:1000 dilution ratio. The powder -solvent ratio was 1:10 w/v. DMSO (0.1% final concentration) was used as vehicle control.

Carcinoma cell culture.

1) Cell types: HepG2.

2) Medium types: HepG2cells were cultured in DMEM + 10 % FBS medium.

3) Culture conditions: 10cm diameter cell culture dish, cell culture box is set as follows: constant temperature 37°C, 5% CO_2 concentration; according to the cell growth condition, exchange fluid or passage every two or every other day.

Experimental steps.

HepG2 cells were spread in a 96-cell plate, diluted to $0.5\text{-}1\times10^4$ / well with DMEM + 10 % FBS, and cultured overnight (37°C, 5% CO_2). The cells were cultured for 24 h. When the cells are entirely adherent, the sample is added to stimulate the cells. 12.5, 25, 37.5, 50 and 62.5 (μg/ml) of four polar extracts were added, and the culture system was 100 μL per well. Three replicate wells were set in each group, with the control wells (only cells and DMEM +

10% FBS medium). 100µl PBS solution was added to the outermost hole. Remove the medium containing the sample, add the appropriate amount of PBS to clean two times, then add 100 µL medium containing 0.5% MTT (5 mg / mL) per well, continue to culture for 3–4 h.

The experimental principle is as follows. MTT can react with succinate dehydrogenase in living cells mitochondria to form blue-purple crystalline formazan, insoluble in water and deposited in living cells. The blue-violet formazan has a particular absorption peak at 492 nm after being dissolved in DMSO. When the number of collected cells was within a specific range, the number of living cells was proportional to the absorbance at 570 nm after the dissolution of formazan.

Research results. MTT cytotoxicity test was used to detect whether the crude extracts of different polarities have killing effects on HepG2 cells. HepG2 cells were stimulated with different types and concentrations of polar crude extracts. All experiments were repeated three times, and the obtained data were analysed by GraphPad Prism software. The t-test was used for comparison among groups, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Crude extracts from the polar fraction of petroleum ether (Fig. 1a) and water (Fig. 1b) had no significant effect on the viability of the cells at different concentrations.

When the sample concentration was greater than 50 µg/mL, the crude extract of the n-butanol polar fraction slightly inhibited the HepG2 cells (Fig. 1c). The ethyl acetate polar fraction (Fig. 1d) showed significant killing on HepG2 cells.

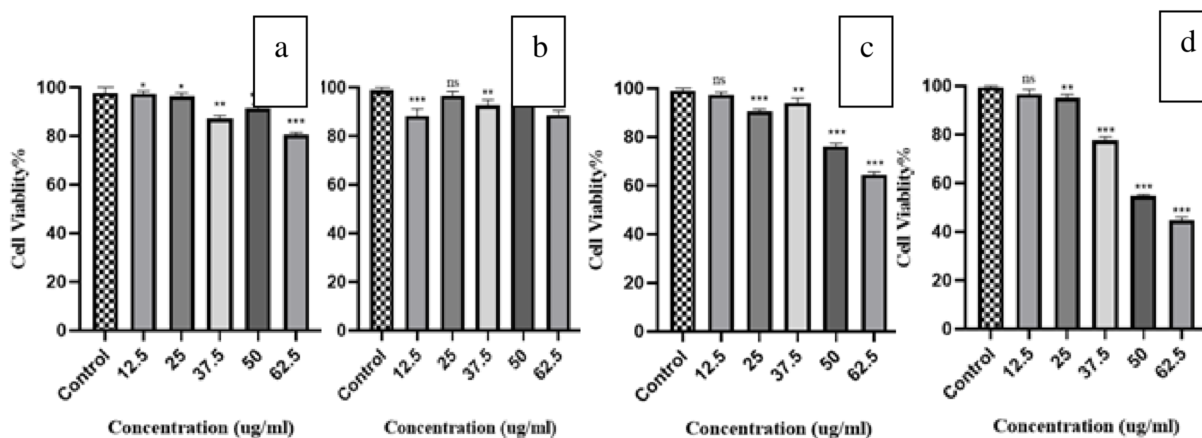

Figure 1. **The effects of 4 polar crude extracts on HEPG2 cells**: a is the crude extract of petroleum ether polarity; b – water; c – n-butanol; d – ethyl acetate. The t-test was used for comparison among groups: * $p < 0.05$, ** $p < 0.01$, *** p 0.001, all of the concentration groups were compared with the control group

Then we tested the IC_{50} (concentration of drug required for 50% inhibition) of the HepG2 cells. We again performed three sets of MTT cytotoxicity experiments that set the concentration gradient to 12.5, 25, 37.5, 62.5, 50, 75, 100 (µg/ml). The other experimental conditions remained unchanged, determining the IC_{50} of HepG2 cells (Fig. 2). The obtained data were analysed using GraphPad Prism software. The IC_{50} of the HepG2 cells is 64.41 ± 0.28.

The scratch method studied the effects of different ethyl acetate extracts on the cell migration of tumour cells. The experimental results were used to calculate and analyse the cell healing rate after 24 h of administration and determine whether the tested extracts promoted cell migration or repair. The healing rate can be calculated to reflect the migration or repairability of the selected test sample to five tumour cells.

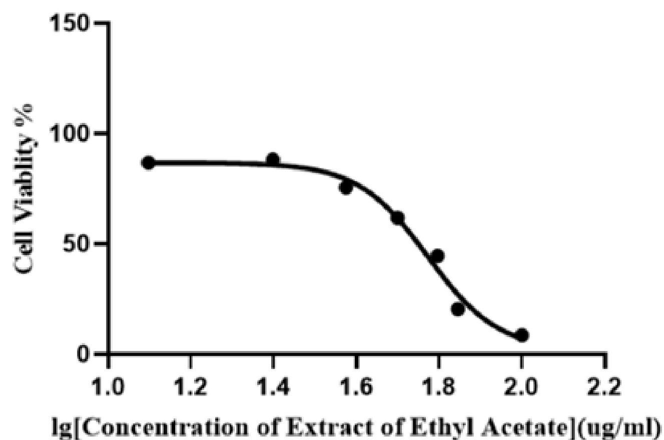

Figure 2. **IC_{50} of ethyl acetate extracts on HepG2 cells**

The measured experimental data are shown in Fig. 3. When the crude extract of the polar fraction of ethyl acetate was added at a concentration of 1.25 µg/mL, it did not practically affect the healing ability of HepG2 cells, with a healing rate of 32.27%. The inhibition of the healing rate is only 3.47% compared to the control sample.

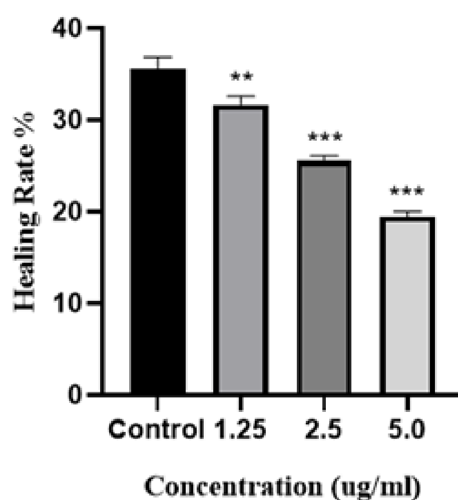

Figure 3. **The results of HepG2 Cell Scratch Test of the crude extract of ethyl acetate polarity**.The t-test was used for comparison among groups,* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, all of the concentration groups were compared with the control group

When the crude extract was added at a concentration of 2.5 µg/mL, it had a more apparent inhibitory effect on the healing of HepG2 cells, with a healing rate of 25.29 % and an inhibitory healing rate of more than 10%.

When the concentration of the crude extract was 5 µg/mL, the healing rate of HepG2 cells was significantly inhibited, with a healing rate of only 19.17 % and an inhibited healing rate of 16.57%.

Further, the healing rate of HepG2 cells decreased at higher concentrations of the crude extract of the polar fraction of ethyl acetate in the range of 1.25 µg/mL to 5 µg/mL. The crude extract of the polar fraction of ethyl acetate had a significant inhibitory effect on the growth of HepG2 cells.

Conclusions. In the present experiment, the crude extracts of different polar parts of the Speranskia Tuberculata (Bunge) Baill were extracted and separated. Four crude extracts with different polarities were obtained: petroleum ether, ethyl acetate, n-butanol and water. These four crude extracts were then screened for anticancer activity by using the MTT cytotoxicity test. It was concluded that the polar crude extracts of ethyl acetate had a significant ability to kill HepG2 cells. In contrast, the three polar crude extracts of petroleum ether, n-butanol and water did not have the powerful inhibitory ability on HepG2 cells.

References

1. Bray F., Ferlay J., Soerjomatarm I. et al. Global cancer statistics 2018: GLPBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018. 68 (6): 394-424. doi: 10.3322/caac.21492.
2. Feng R. M., Zong Y. N., Cao S. M. et al. Current cancer situation in China: good or bad news from the 2018 Global Cancer Statistics? *Cancer Commun (Loud)*. 2019. 39 (1): 22. doi: 10.1186/s40880-019-0368-6.
3. Siegel R. L., Miller K. D., Jemal A. Cancer statistics, 2020. *CA Cancer J Clin*. 2020. 70 (1): 7–30. doi: 10.3322/caac.21590.
4. Zongguo Yang, Xian Liao, Yunfei Lu, Qingnian Xu, Bozong Tang, Xiaorong Chen, and Yongchun Yu Add-On therapy with traditional Chinese medicine improves outcomes and reduces adverse events in hepatocellular carcinoma: A meta-analysis of randomised controlled trials. *Evidence-Based Complementary and Alternative Medicine*. 2017. doi: 10.1155/2017/3428253.
5. Xun Li, Guoyan Yang, Xinxue Li, Yan Zhang, Jingli Yang, Jiu Chang, Xiaoxuan Sun, Xiaoyun Zhou, Yu Guo, Yue Xu, Jianping Liu, Alan Bensoussan. Traditional Chinese medicine in cancer care: a review of controlled clinical studies published in Chinese. *PLoS One*. 2013. 8(4): e60338. doi: 10.1371/journal.pone.0060338.
6. Shi Z., Song T., Wang Y. *et al.* A systematic review and meta-analysis of traditional insect Chinese medicines combined chemotherapy for non-surgical hepatocellular carcinoma. *Sci Rep*. 2017. 7 (1): 4355. doi: 10.1038/s41598-017-04351-y.
7. Xi S. Y., Minuk G. Y. Role of traditional Chinese medicine in the management of patients with hepatocellular carcinoma. *World J Hepatol*. 2018. 10 (11): 799–806. doi: 10.4254/wjh.v10.i11.799.
8. Yuan Yue, Yumin Yang, Lei Shi. Suppression of human hepatocellular cancer cell proliferation by Brucea Javanica oil-loaded liposomes via induction of apoptosis. *Arch Med Sci*. 2015. 11 (4): 856–862. doi: 10.5114/aoms.2015.53306.