

Full Length Research Paper

Antitumour effects of *Scutellaria barbata* ethanol extracts in mice transplanted with human hepatocellular carcinoma (HepG2) cells

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***Scutellaria barbata*, also known as “Banzhilian”, is one of the commonly used Chinese medicinal herbs. The objective of this study was to examine the antitumour activities of ethanol extract prepared from *S. barbata* in mice transplanted with human hepatocellular carcinoma (HepG2) cells lines, and to test proliferating cell nuclear antigen (PCNA), vascular endothelial growth factor (VEGF), CD31 in tumour. SP immunocytochemistry staining was employed to quantify these protein expression levels. The present results demonstrated that ethanol extract of *S. barbata* could significantly inhibit tumour growth in a dose-dependent manner. Levels of PCNA, VEGF and CD31 proteins expression decreased with increased concentration of ethanol extract of *S. barbata*. The effect is statistically significance when compared with control group.**

Key words: *S. barbata*, mice, human hepatocellular carcinoma (HepG2), cancer, vascular endothelial growth factor (VEGF).

INTRODUCTION

Primary liver cancer (or hepatocellular carcinoma, HCC) is the sixth most common cancer worldwide in terms of numbers of cases of 626,000, and the third most common cause of death from cancer (598,000 deaths annually) (Parkin et al., 2002). Since over 80% of deaths are in developing countries, liver cancer has been a major public health problem in these parts of the world. China is the area of the world most affected by liver cancer, with an age-standardized incidence rate of 37.9 per 100,000 for men, and of 14.2 per 100,000 (Parkin et al., 2002).

Traditional Chinese Medicine (TCM) is one of the world's most ancient herbal medicines and has been applied by TCM practitioners for thousands of years. *Scutellaria Barbata*, an important member of Chinese medicinal herbs, is derived from the dried whole plant of

S. barbata (Labiateae) and has been listed in the Pharmacopoeia of the People's Republic of China (Shao, 2010). The traditional Chinese medicinal herb “Banzhilian”, derived from the dry whole plant of *S. barbata* D. Don, is commonly used for the treatment of tumors, hepatitis, cirrhosis and other diseases (Wang et al., 1996). Modern pharmacological studies have showed that *S. Barbata* has the effects of antibacterial, anticancer, as well as antioxidative and so on (Sato et al., 2000; Yin et al., 2004; Goh et al., 2005; Shang et al., 2010). So it plays an increasingly important role in clinic for the treatment of urinary, ophthalmic, respiratory and digestion system disorders in China (Shang et al., 2010). In recent years, more than thirty flavonoids, over ten neoclerodane type diterpenoids, triterpene acids and sterol glucosides have been isolated, some of which exhibit interesting biological activities (Wang et al., 1996; Ducki, 1996; Kizu et al., 1997; Yu and Lei, 2004; Yin et al., 2004).

The present investigation focuses attention on the

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antitumor and anticarcinogenic activity of ethanol extract of *S. barbata* in mice transplanted with HepG2.

MATERIALS AND METHODS

Preparation of ethanol extract of *S. barbata*

The dried *S. barbata* were purchased from a local market in YinChuan City, China. Ethanol extracts were obtained as follows. In brief, 150 g of dried samples were extracted by refluxing with 10 volumes of ethanol for 60 min at 70°C. The extracts were filtered through a filter paper and the filtrates were dried. Extraction percentage of ethanol extract of *S. barbata* was 1.06%.

Determination of antitumor activities of the *S. barbata* ethanol extracts

One million human hepatocellular carcinoma (HepG2) cells were intraperitoneally injected in to four of male female Kunming mice (Group II to V; ten mice/group). Animals in Groups II to V received *S. barbata* extracts at a concentration of 50, 100, 150 and 200 mg/kg body weight, respectively, 24 h after tumour inoculation and continued for 15 days. Animals in Group I was kept as control, which received the vehicle. Diameter of the tumor was measured on the sixteenth day using vernier calipers and volume was calculated using the formula, $V = 4/3\pi r_1^2 \times r_2$, where V is volume, r_1 and r_2 represent the radii of the tumor at two different planes.

Immunocytochemistry

The protein expression of proliferating cell nuclear antigen (PCNA), vascular endothelial growth factor (VEGF), CD31 and CD31 in human hepatocellular carcinoma (HepG2) cells was assessed by immunocytochemistry using an anti-PCNA monoclonal antibody (1:100), an anti-VEGF monoclonal antibody (1:100) and an anti-CD31 polyclonal antibody (1:200) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) (NanJing JianChen Biotech Company, NanJing, China). The samples were stained by SP staining. All reagents were used in the negative controls except the primary antibodies.

Statistical analysis

Data analysis was expressed as the mean $\pm$ SEM of pooled results obtained from at least ten independent experiments. Statistical analysis was performed by one-way ANOVA test followed by Fisher's protected least significant difference posttest for multiple comparisons using the StatView Program (Abacus Concepts, Berkeley, CA). Significance level was considered as $P < 0.05$.

RESULTS AND DISCUSSION

Liver is the primary organ for glucose metabolism and regulation; therefore it is interesting to elucidate the effects of hyperglycemia in cultured liver cells. The human hepatoma cell line, HepG2 has been used extensively to study hyperglycemia *in vitro* as evidenced by several reports and to understand the mechanism(s) of ethanol-induced hepatic injury (Caro and Cederbaum, 2004; Neuman et al., 1995; Neuman et al., 1998;

Neuman et al., 1999). In particular, HepG2 cells retain the activity of many Phase I, Phase II and antioxidant enzymes ensuring that they constitute a good tool to study cytoprotective, genotoxic and antigenotoxic effects of compounds (Knasmüller et al., 2004; Mersch-Sundermann et al., 2004).

Figure 1 shows the effects of the *S. barbata* ethanol extracts on mice transplanted with HepG2 tumor. There was a significant reduction of the tumor weight in all extracts tested. The differences between experimental groups were compared by ANOVA followed by Student Newman Keuls or Bonferroni tests ($p < 0.05$). On the 16th day, the tumor weight was significantly reduced at doses of 50, 100, 150 and 200 mg/kg, respectively. The effect was dose-dependent.

Proliferating cell nuclear protein (PCNA) is found in the nucleus and is a cofactor of DNA polymerase delta. This protein is associated with DNA synthesis and repair. The encoded protein acts as a homotrimer and helps increase the processivity of leading strand synthesis during DNA replication. It appears during late G1- phase, S-phase of mitosis and persists until the end of the M-phase because of its long biological half-life (Guerini et al., 2005). PCNA which is required for cellular DNA synthesis of a protein is cell G/S on the synthesis of protein whose expression level may reflect the degree of tumor cell proliferation (Stalinska et al., 2009). The higher the degree of tumor cell proliferation, tumor growth is faster, more prone to transfer, the higher the degree of malignancy. Therefore, many scholars have already assessed PCNA expression as a useful indicator of tumor prognosis (Chen et al., 2010).

Figure 2 shows the effect of *S. barbata* ethanol extracts on PCNA protein expression in tumor. The VEGF protein expression level in tumor decreased with increasing concentration of *S. barbata* ethanol extracts. As seen Figure 2, the level of VEGF protein expression in tumor were 47281 ± 4082 , 36913 ± 2639 , 28574 ± 1849 and 18932 ± 1275 , respectively when dose of *S. barbata* ethanol extracts were 50, 100, 150 and 200 mg/kg bw. There was significant ($P < 0.01$) difference in VEGF protein expression level between control group (I) and drug-treated group (II to V). Decreased PCNA protein expression in HepG2 cells may reflect the antitumor activity of *S. barbata* ethanol extracts.

In 1989, Napoleone Ferrara, M.D., and a team of scientists at Genentech first isolated human vascular endothelial growth factor (VEGF), a protein now believed to be one of the most potent sources of angiogenesis (Leung et al., 1989; Ranieri et al., 2006). The need for oxygen and nutrients triggers tumor cells to produce and release the VEGF protein, which leads to the formation of new blood vessels used to feed the tumor. Novel anti-angiogenic cancer therapies based on synthetic and natural molecules target pro-angiogenic growth factors produced by tumors and/or their cell surface receptors in endothelial cells, or even in some cancer cells. Among

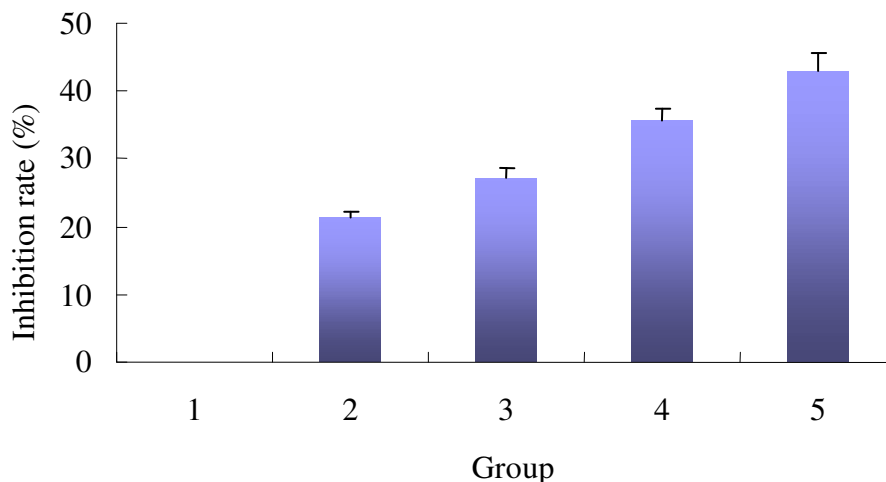


Figure 1. Effect of the *S. barbata* ethanol extracts on tumor weight in mice transplanted with HepG2 cells.

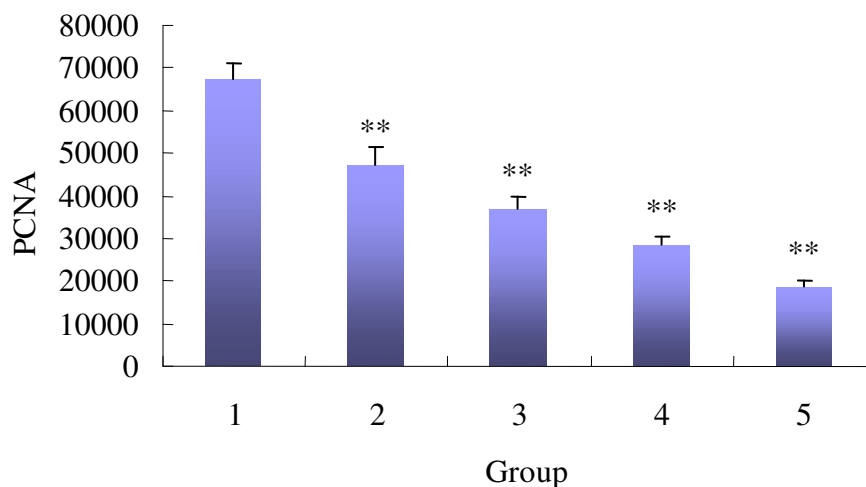


Figure 2. Effect of the *S. barbata* ethanol extracts on PCNA protein expression in tumor. ** $P < 0.01$, compared with control (0 mg/ml body weight); Data analysis were expressed as the mean $\pm$ SEM of pooled results obtained from at least ten independent experiments. Significance level was considered as $P < 0.05$.

these growth factors, vascular endothelial growth factor (VEGF) and its receptors have received special attention, due to: (a) Their central role in endothelial cell physiology and neo-angiogenesis; (b) The detection of VEGF at high concentrations in most of the human tumors and their metastasis; (c) Its frequent association with a bad prognosis in cancer, and (d) The differential nature of tumor angiogenesis, when compared to normal tissues (Ferrara, 2005).

Figure 3 shows the effect of *S. barbata* ethanol extracts on VEGF protein expression in tumor. The *S. barbata* ethanol extracts reduced VEGF protein expression in tumour in a dose-dependent manner. Compared with control group, the results were found statistically significant ($P < 0.01$). We supposed that one of the

mechanisms of the antitumor activity of *S. barbata* ethanol extracts may be due to its effects against tumor angiogenesis by targeting the VEGF protein.

The CD31 protein and factor VIII-related antigen are different markers for endothelial cells and angiogenesis. The CD31 protein, an endothelial cell adhesion molecule (endoCAM-1, PECAM-1), is a membrane glycoprotein belonging to the immunoglobulin superfamily, whereas the Factor VIII antigen-related antigens are hemostasis molecules (Sato et al., 2008). CD31 plays a key role in removing aged neutrophils from the body. CD-31 is also expressed in certain tumors, including epithelioid hemangioendothelioma, epithelioid sarcoma-like hemangioendothelioma, other vascular tumors, histiocytic malignancies, and plasmacytomas. It is rarely found in

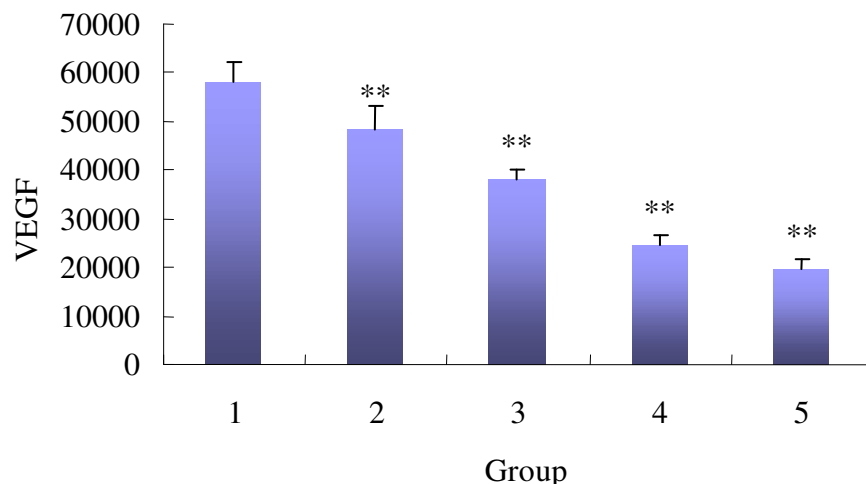


Figure 3. Effect of the *S. barbata* ethanol extracts on VEGF protein expression in tumor. ** $P < 0.01$, compared with control (0 mg/ml body weight); data analysis were expressed as the mean $\pm$ SEM of pooled results obtained from at least ten independent experiments. Significance level was considered as $P < 0.05$.

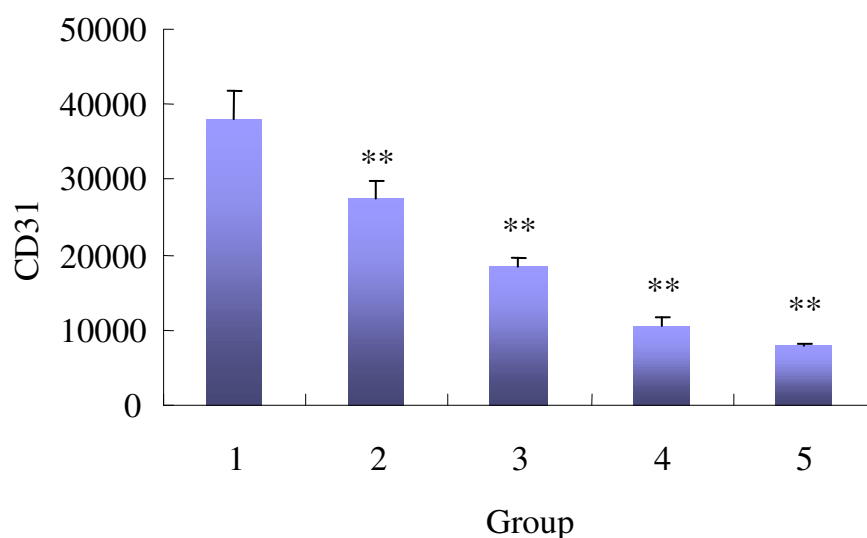


Figure 4. Effect of the *S. barbata* ethanol extracts on CD31 protein expression in tumor. ** $P < 0.01$, compared with control (0 mg/ml body weight); data analysis were expressed as the mean $\pm$ SEM of pooled results obtained from at least ten independent experiments. Significance level was considered as $P < 0.05$.

some sarcomas and carcinomas (Poncelet et al., 2002). Figure 4 depicts a steady increase in the CD31 protein expression in tumor when dose of *S. barbata* ethanol extracts increased from 50 to 200 mg/kg bw. The CD31 protein expression in tumor at the maximum concentration (200 mg/kg bw) of *S. barbata* ethanol extracts was found to be 7832 ± 407 . Compared with control group, the results were found statistically significant ($P < 0.01$). Because of the role of CD31 protein in cancer cells, its expression change is closely related to therapy effect of antitumor medicine. *S. barbata* ethanol extracts reducing CD31 protein expression in HepG2 cell

might partly explain molecular mechanism of antitumor activity of *S. barbata* ethanol extracts.

Conclusion

The present study demonstrates that the *S. barbata* ethanol extracts possess potent hepatoprotective activities. The extract is capable of inhibiting HepG2 cells growth. Further investigations showed that *S. barbata* ethanol extracts can reduce protein expression of PCNA, VEGF, and CD31 in HepG2 cells. The effect is dose-dependent. A possible molecular mechanism is to

S. barbata ethanol extracts playing its effects against tumor angiogenesis by targeting the VEGF protein.

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