Protective Effects of *Scutellaria barbata* Against Rat Liver Tumorigenesis

Zhi-Jun Dai*, Wen-Ying Wu*, Hua-Feng Kang*, Xiao-Bin Ma*, Shu-Qun Zhang, Wei-Li Min, Wang-Feng Lu, Shuai Lin, Xi-Jing Wang*

Abstract

*Scutellaria barbata* D. Don (*S. barbata*), a traditional Chinese medicine, is used to treat cancers, inflammation, and urinary diseases. This study aimed to determine any protective effects of *S. barbata* crude extract (CE-SB) against rat liver tumorigenesis induced by diethylnitrosamine (DENA). Liver malfunction indices in serum were measured by biochemical examination. Hematoxylin and eosin staining was performed to examine liver pathology. Contents of malondialdehyde (MDA) and superoxide dismutase (SOD) were measured in liver homogenates to evaluate oxidative stress. The levels of liver malfunction indices in the CE-SB groups, especially in the CE-SB high dose group, were lower than that of the model group (*P*<0.05). The results from histological examination indicated that the number of liver nodules in the CE-SB groups decreased compared with the model group (*P*<0.05). Content of MDA determined in liver was significantly decreased, and level of SOD elevated by CE-SB. CE-SB can inhibit experimental liver tumorigenesis and relieve hepatic injury in rats.

Keywords: Anti-tumor - *Scutellaria barbata* - hepatoma - tumorigenesis - diethylnitrosamine - rat

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Introduction

Liver cancer is one of the most common cancers with high incidence and mortality. Curative surgery or liver transplantation is rarely possible and often leads to tumor recurrence (Jemal et al., 2011). Although many therapy strategies for liver cancer exist, the therapeutic outcome remains very poor (Farazi et al., 2006). So, the preventive control of liver cancer has been emphasized for the limited liver cancer treatment and grave prognosis. Many Chinese herbs that could be potential antitumor drug sources have been discovered (Vickers et al., 2002). The herb has been used in clinics to treat lung cancer, digestive system cancers, hepatoma, breast cancer, and chorioepithelioma. *Scutellaria barbata* D. Don (*S. barbata*) is a perennial herb, which mainly grows throughout southern China. The S. barbata D. Don herb is known in traditional Chinese medicine as Ban-Zhi-Lian, and it has been used as an anti-inflammatory and antitumor agent as well as a diuretic in China and Korea (Lin et al., 1996; Lee et al., 2004; Yin et al., 2004; Goh et al., 2005; Suh et al., 2007; Dai et al., 2008). Furthermore, our previous research found that CE-SB have antitumor activities both in vitro and in vivo (Dai et al., 2011).

Diethylnitrosamine (DENA) is a well-known potent hepatocarcinogenic agent present in tobacco smoke, water, cured and fried meals, cheddar cheese, agricultural chemicals, cosmetics, and pharmaceutical products (Lee et al., 1998). DENA induces damage in many enzymes involved in DNA repair and is normally used to induce liver cancer in experimental animal models (Bhosale et al., 2002). The DENA-induced disease process is similar to human liver cancer. In the present study, the protective effects of CE-SB against DENA-induced rat liver tumorigenesis were investigated.

Materials and Methods

Reagents and animals

Sixty adult male Sprague–Dawley rats weighing 200 g to 220 g were purchased from the Experiment Animal Center, Medical School of Xi’an Jiaotong University, China. The rats were housed (five per cage) in an air-conditioned room at a constant temperature of 23 °C±1 °C and a humidity of 60±10% with a 12 h light/dark...
cycle for 1 week before the experimental period. Food and water were available ad libitum. The experiments were conducted according to Institutional and National Guidelines. DENA, enzymes, and coenzymes were obtained from Sigma Chemical Co. (St. Louis, MO, USA). CE-SB was purchased from Xi’an zhong-xin Biotechnology Development Ltd. (Xi’an, P.R. China). Other chemicals were AnalaR grade. DENA was prepared as a saline solution with a concentration of 8 mg/mL.

**Experimental Protocol**

HCCs were induced as described previously (Futakuchi et al., 1999) with slight modifications. The rats were given a single intraperitoneal injection of DENA at a dose of 100 mg/kg body weight to induce liver carcinogenesis, followed by 10 mg/kg per day in drinking water for 5 d per week for another 8 weeks.

After 24 weeks, the surviving animals were sacrificed using 200 g/L ethyl carbamate as anesthesia. The body, liver, spleen, and lungs were weighed, and an autopsy was performed. The liver nodules or tumors that were visible on the liver surface and the liver nodules which is greater than 3 mm in diameter were counted and measured. Multiple samples were taken from the tumors and from the apparently non-neoplastic liver portions.

**Animal grouping and treatment**

The animals were divided into four groups (n = 15): the normal control group, the model control group, the 6 g/kg CE-SB treatment group, and the 12 g/kg CE-SB treatment group. Cancer was induced using the same protocol as described before. In addition, CE-SB was co-administered at a daily oral dose of 6 g/kg or 12 g/kg per day for 5 d per week for the total experimental period.

**Histopathological examination of liver tissue**

Hepatic tissues were fixed in a 40 g/L formaldehyde solution in 0.1 mol/L phosphate-buffered saline (pH 7.4), and embedded in paraffin. Section slides (5-6 μm thick) were prepared and stained with hematoxylin and eosin (HE).

Each liver was microscopically examined for lesion distributions, and were classified as focal, multifocal, diffused, and no detected abnormalities. The patterns of liver injury and lesion grading, which were classified as minimal, moderate, and marked, were determined in the coded slides. The liver sections were also observed for any necrosis/abscesses, degenerative changes, structural disruption, and any other changes.

**Biochemical estimations**

The separated plasma was analyzed for total protein and albumin content. The separated serum was analyzed for AST and ALT activities using a kit provided by Bicon, Germany. ALP was analyzed using a kit provided by Biolabo, France. Levels of MDA were determined as described by Yagi (1976). Activity of SOD was measured using commercial available kit purchased from Nanjing Jiancheng BioEngineering (Nanjing, China).

A 10% homogenate was obtained in a Tris-sucrose buffer (pH 7.4), and was centrifuged at 105,000 ×g at 4 °C for 30 min using a DuPont Sorvall Ultracentrifuge (USA) to isolate the cytosolic fraction, which was used for the assay of γ-Glutamyl transferase (γ-GT), glutathione-S-transferase (GST) and Alpha-L-fucosidase (AFU) activities.

**Statistical analysis**

All values were expressed as mean ± SEM. Statistical analysis was performed with one-way analysis of variance (ANOVA) test and student t test using the statistical software SPSS 13.0. *P<0.05 was considered statistically significant.

**Results**

**Protective effects of CE-SB on DENA-Induced liver tumorigenesis**

The rats in normal control group were active (eating and drinking). As shown in Table 1, the body weight in the CE-SB groups and the model group were significantly

| Table 1. Protective Effects of CE-SB on DENA-Induced Liver Tumorigenesis ( ±s) |
|---------------------------------|------------------|-----------------|-----------------|-----------------|-----------------|
| Group             | Dosage (g/kg/d) | Liver nodules | Body weight (g) | Liver weight (g) | Liver / body weight ratio (%) | Mortality rate (%) |
| Normal            | -               | 0              | 496.3±36.45     | 11.62±2.34       | 2.34             | 0 (0/15)         |
| Model control     | -               | 40.22±4.83     | 343.6±276.52*   | 18.72±8.63*      | 5.45*            | 40.0(6/15)       |
| CE-SB 12          | 12              | 13.3±12.12*    | 418.27±59.64*   | 14.73±3.26*      | 3.52*            | 6.7(1/15)        |
| CE-SB 6           | 6               | 24.5±4.05*     | 425.46±54.32**  | 16.28±3.57**     | 3.83**           | 20.0(3/15)       |

*P<0.05 versus normal group; *P<0.05 versus model control group
Figure 2. Liver Sections of the Different Groups. (A) Liver tissue of the normal control group showed hepatic lobule having normal architecture. The liver cell cord was arranged in neat rows, and a clear nucleus was observed (HE×200); (B) Liver tissue of the model control group showed nuclear pleomorphism. The lobular architecture of the model control group was normal, and the general concept of the white nodules of hepatocellular carcinoma cells were destroyed with low differentiation degree, significant atypical, and were mostly level III histological grade (HE×200); (C) CE-SB-treated rat liver tissues showed some degenerative changes and a vacuolated cytoplasm with high cell differentiation and were mostly level I-II histological grade (HE×200).

Figure 3. Effects of CE-SB on Non-enzymatic Liver Functions in DENA-induced Liver Tumorigenesis. Values are expressed as means ± standard deviations, and determined using the ANOVA test. *P<0.05 versus normal group, #P<0.05 versus model control group.

lower than the normal group (P<0.05). The liver weight and the liver/body weight ratio in the CE-SB groups were significantly higher than the normal group, whereas liver nodules, liver weight, and liver/body weight ratio were significantly lower than the model group. However, the body weight of rats in the CE-SB groups were significantly higher than the model group, whereas the liver nodules and liver/body weight ratio were significantly lower than the model group (0.05). No significant difference was observed between the two CE-SB groups (P>0.05).

6 rats were died in the model group, 3 in the 6 g/kg CE-SB group, and 1 in the 12 g/kg CE-SB group. The mortality rate (%) in the model group, 6 g/kg CE-SB group and 12 g/kg CE-SB group were 40.0%, 20.0% and 6.7%, respectively.

Histopathological observation

The liver tissues of the normal control group were dark red with smooth surface, sharp edges, and medium texture (Figure 2A). The rats in the model control group and CE-SB groups were all successively induced with liver cancer. Multiple gray-white nodules of varying sizes were scattered in the liver tissues of the model control group (Figure 2B), of which four cases had huge tumor formation with a diameter greater than 3 cm (Figure 2C). The liver tissues of two cases in the 12 g/kg CE-SB group showed smooth surface and normal texture, but only a small focal nodule formation. The liver tissues of the 6 g/kg CE-SB group showed multiple liver surface nodules, but the nodules were significantly reduced compared with the model control group (Figure 2D).

No case with spontaneous hepatocellular carcinoma was found by HE staining in the normal control group. The normal control group showed complete hepatic lobule structure. Moreover, the liver cell cord arranged in neat rows, and a clear nucleus (Figure 3A). The normal lobular architecture of the model control group and the general concept of the white nodules of hepatocellular carcinoma cells were destroyed with a low differentiation degree, significant atypical, and were mostly level III histological grade (Figure 3B). The microscopy results showed high cell differentiation in the liver cancer cells in the ESB groups and mostly level I-II histological grade (Figure 3C).

Effect of CE-SB on Non-enzymatic Liver Functions in DENA-Induced Liver Tumorigenesis

DENA significantly decreased total protein, albumin, and A/G levels, which indicated poor liver function and inability to fight infections. On the contrary, the administration of CE-SB normalized total protein, albumin, and A/G levels. It indicated that CE-SB could ameliorate liver function in DENA-induced liver damage. The co-administration of CE-SB increased total protein, albumin, and A/G ratios. Moreover, DENA significantly increased total bilirubin (TBIL) and total bile acid (TBA) contents compared with the normal control group. CE-SB

Figure 4. Effect of CE-SB on Enzymatic Liver Functions in DENA-induced Liver Tumorigenesis. Values are expressed as means ± standard deviations, and determined using the ANOVA test. *P<0.05 versus normal group, #P<0.05 versus model control group.

Figure 5. Effect of CE-SB on Tumor Markers in DENA-induced Liver Tumorigenesis. Values are expressed as means ± standard deviations, and determined using the ANOVA test. *P<0.05 versus normal group, #P<0.05 versus model control group.
The numbers of hyperplastic nodules are correlate with hepatic histopathological damage induced by DENA. induced liver weight increases and improved the SB treatment also significantly inhibited the DENA-induced levels of tumor markers induced by DENA. CE-SB improve the serum biochemical indices, and inhibit to significantly reduce the numbers of the tumor nodules, which is inhibite by CE-SB. It indicated that CE-SB may ameliorate the liver injury induced of DENE by antioxidant effect. In this study, we found  the rats treated with DENA showed a increased MDA levels and an reduced SOD values are shown in Figure 6. The MDA levels were significantly increased and SOD actives were reduced in rats with DENA-induced liver cancer, which is inhibit by CE-SB. It indicated that CE-SB may be useful to ameliorate liver injury caused by DENA-induced liver tumorigenesis.

**Effect of CE-SB on Enzymatic Liver Functions in DENA Induced Liver Tumorigenesis**

DENA significantly elevated serum ALT, AST, and ALP activities compared with the normal group (Figure 4). The co-administration of CE-SB restored serum AST and GGT activities with significantly decreased ALP activity compared with the model control group (P<0.05).

**Effect of CE-SB on Tumor Markers in DENA-Induced Liver Tumorigenesis**

As shown in Figure 5, serum γ-GT, GST, and AFU activities were significantly elevated in the model control group compared with the normal group. The co-administration of CE-SB decreased the activities of serum tumor markers in rats with DENA-induced liver cancer, which were significantly different from the model control group (P<0.05).

**Effect of CE-SB on Oxidative Stress in DENA-induced Liver Tumorigenesis**

MDA and SOD values are shown in Figure 6. The MDA levels were significantly increased and SOD actives were reduced in rats with DENA-induced liver cancer, which is inhibit by CE-SB. It indicated that CE-SB may be useful to ameliorate liver injury caused by DENA-induced liver tumorigenesis.

**Discussion**

In conclusion, the protective effects of CE-SB crude extract against rat liver tumorigenesis were observed. The levels of liver function indexes in the CE-SB groups were lower than that of the model group. The results of the histological examination demonstrated that the number of liver cancer nodus in the CE-SB groups decreased compared with the model group. CE-SB can inhibit experimental liver tumorigenesis and can relieve hepatic injury in rats. However, further studies are necessary to clarify the detailed mechanism involved in the antitumor effects of CE-SB.

![Figure 6. Effect of CE-SB on Oxidative Stress in DENA-induced Liver Tumorigenesis. Values are expressed as means ± standard deviations, and determined using the student’s t-test test. *P<0.05 versus normal group, #P<0.05 versus model control group](image-url)
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References


