

EFFECTS OF CIJI HUA'AI BAOSHENG GRANULE FORMULA (CHBGF) ON LIFE TIME, PATHOLOGY, PERIPHERAL BLOOD CELLS OF TUMOR CHEMOTHERAPY MODEL MOUSE WITH H<sub>22</sub> HEPATOMA CARCINOMA CELLSShengyan Xi<sup>1</sup>, Rongjian Hong<sup>2</sup>, Jingru Huang<sup>3</sup>, Dawei Lu<sup>1</sup>, Linchao Qian<sup>1</sup>, Pengcheng Li<sup>1</sup>, Lei Wen<sup>1\*</sup>, Yanhui Wang<sup>1\*</sup>

<sup>1</sup>Department of Traditional Chinese Medicine, Medical College of Xiamen University; Cancer Research Center of Xiamen University, Xiamen 361102, China. <sup>2</sup>Shanghai Medical College, Fudan University, Shanghai 200032, China. <sup>3</sup>Laboratory Center, Medical College of Xiamen University, Xiamen 361102, China.

\*Co-correspondence Tel: (+86)-592-2188689; Fax: (+86)-592-2183069  
E-mail: 2076110@126.com; xishengyan@xmu.edu.cn

## Abstract

**Background:** Ciji Hua'ai Baosheng Granule Formula (CHBGF) is a traditional Chinese empirical formula that can help the tumor patients who have received chemotherapy antagonize the toxin and side-effects so as to improve and prolong the life. This study is to evaluate the effects of CHBGF on improving life quality in terms of survival time, pathology of tumor tissue and ameliorating peripheral blood cells in mouse chemotherapy model with subcutaneous transplanted tumor or ascitic tumor of H<sub>22</sub> hepatoma carcinoma cells at an overall level.

**Materials and Methods:** 71 mice among the 92 Kunming mice were injected subcutaneously into the right anterior armpit with H<sub>22</sub> hepatoma carcinoma cells, after 7 days, which had formed tumors and were used peritoneal injection of Cytosan (CTX) (200mg/kg) to establish the mouse chemotherapy model with transplanted tumor, and then which were commensurately divided into 8 groups by random digits table. 21 mice were injected into peritoneal cavity to use CTX and the same method to establish the model. The groups for evaluating the effects on the survival time were the model, CHBGF and positive control group respectively with 7 mice in each group. The groups for evaluating the effects on anti-cancer were the model group, three treatment groups and positive control group with 10 mice in each group. The survival-time-observing groups were given intragastric administration of normal saline, CHBGF (64g/kg) once a day, and peritoneal injection of 5-Fluorouracil (25mg/kg) once every other day respectively. The survival time of each group was observed. The five anti-cancer-observing groups were given intragastric administration of normal saline, CHBGF (64g/kg, 32g/kg and 16g/kg) once a day, and peritoneal injection of 5-Fluorouracil (25mg/kg) once every other day respectively. After treatment for 21 days, the transplanted tumors were peeled off. Blood was collected through pricking eyeball and analyzed by hematology analyzer. And postchemotherapy transplanted tumor inhibition ratios were calculated. Pathological changes of tumor tissues and blood smears were observed with light microscope.

**Results:** The life prolonging rate of CHBGF (64g/kg) group with transplanted tumor is 20.14%, and their survival time was longer than that of the 5-Fluorouracil group ( $P<0.05$ ). Life prolonging rate of CHBGF (64g/kg) group with ascitic tumor is 64.15%, the survival time was longer than that of the model group ( $P<0.01$ ) and the 5-Fluorouracil group ( $P<0.05$ ). The growth of the transplanted tumor in model group was faster than that in CHBGF (64g/kg) group and 5-Fluorouracil group ( $P<0.05$ ). The tumor average weight of the positive drug and the CHBGF (64g/kg, 32g/kg) groups was lighter than that of the model group ( $P<0.05$  or  $P<0.01$ ). The inhibition ratios of CHBGF (64g/kg, 32g/kg and 16g/kg) groups are 31.15%, 21.31%, and 13.11% respectively. Under light microscope, in the positive drug and three CHBGF groups the pathological deteriorated severity of tumor tissue observed was milder than that in the model group, the distribution of WBC in CHBGF groups was more obvious than that of the model and 5-Fluorouracil groups. The WBC and PLT decrease in CHBGF (64g/kg, 32g/kg and 16g/kg) groups is less than the model and the 5-Fluorouracil group ( $P<0.05$  or  $P<0.01$ ), the number of RBC and HGB just in the CHBGF (64g/kg) group was more than that of the model group or the 5-Fluorouracil group ( $P<0.05$ ).

**Conclusion:** Ciji Hua'ai Baosheng Granule Formula can prolong the survival time of the mice chemotherapy model of both subcutaneous transplanted tumor and ascitic tumor of H<sub>22</sub> hepatoma carcinoma cells, has some determinate inhibitory effects on the growth of subcutaneous transplanted tumor chemo-treated, and has the therapeutic effect on antagonizing decrease of WBC and PLT caused by chemotherapy.

**Key words:** Ciji Hua'ai Baosheng Granule Formula (CHBGF); tumor chemotherapy model; transplanted tumor; ascitic tumor; survival time; pathology; peripheral blood cell; H<sub>22</sub> hepatoma carcinoma cell.

## Introduction

Hepatic cancer is one of the most common human malignancies worldwide and its incidence rate is increasing year by year, with over 0.748 million new cases and 0.696 million deaths in 2010 (Jemal et al., 2011). To date, chemotherapy is still the major remedy for patients with the advanced hepatic cancer. The application of many anti-cancer chemotherapeutics used currently like 5-Fluorouracil, Cyclophosphamide and others is very extensive, but these chemotherapeutics will generate unacceptable level of toxicity to normal cells and tissue. Drug toxicity especially the myelosuppression reaction limits the effectiveness of current hepatic cancer chemotherapy, increasing the necessity for the development of new therapeutic methods. Natural products, including traditional Chinese medicine (TCM), recently have received strong interest as the therapeutic drugs for hepatic cancer as they have relatively few side effects and have been long used as alternative remedies for a variety of diseases including cancer (Shen et al., 2012; Lin et al., 2012). TCM formula is a complex combination of many natural products, each of which contains numerous chemical compounds. Therefore, TCM formulas are considered to be the multi-component and multi-target agents exerting their therapeutic function in a more holistic way and discovering naturally-occurring agents is a promising approach for

<http://dx.doi.org/10.4314/ajtcam.v11i4.16>

anti-cancer treatment (Zhuang et al., 2012). Ciji Hua'ai Baosheng Granule Formula (CHBGF) is the traditional Chinese empirical formula set up by Yanhui Wang, a famous traditional Chinese physician and Prof. of Xiamen University Medical College. It mainly includes Dangshen (*Radix Codonopsis*), Huangqi (*Radix Astragali Mongolici*), Tiannanxing (*Rhizoma Arisaematis Erubescens*), Muli (*Concha Ostreae*), Lingzhi (*Ganoderma Lucidum*), Sanleng (*Rhizoma Sparganii*), Zaojiaoci (*Spina Gleditsiae*), Buguzhi (*Fructus Psoraleae*), Chenpi (*Pericarpium Citri Reticulatae*) and Fuling (*Poria*) etc. (Wang, 2004; Wang et al., 2004). According to the traditional Chinese medical theory, CHBGF can strengthen vital *qi* to eliminate pathogenic factor, drastically remove blood stasis, dissipate phlegm and resolve hard masses, and nourish blood. During the clinical practice we used CHBGF to treat many kinds of tumors postchemotherapy and find that CHBGF can alleviate the tumor patients' symptoms, but the correlated study and research have no reports (Wang, 2004; Wang et al., 2004). This study investigated the effect of CHBGF on the general state of health, tumor inhibition, pathology, survival time, and peripheral blood cells in mouse chemotherapy model with subcutaneous transplanted tumor or ascitic tumor of H<sub>22</sub> hepatoma carcinoma cells to aim at providing modern pharmacology basis for its clinical application.

## Materials and Methods

### Animal and Tumor Cell

A total of 92 Kunming mice, with specific pathogen-free (SPF) degree, weighing 18±2g, aged 4-6 weeks, male and female in equal, were obtained from the Experimental Animal Center of Xiamen University in Xiamen, China [License No. SCXK (Min) 2013-0001]. H<sub>22</sub> hepatoma carcinoma cell suspension was provided by Cancer Research Center of Xiamen University (Xiamen, China), and transferred by the mice hydroperitoneum once per week.

### Experimental Drugs

CHBGF [Dangshen (*Radix Codonopsis*) 12g, Huangqi (*Radix Astragali Mongolici*) 10g, Zhebeimu (*Bulbus Fritillariae Thunbergii*) 10g, Tiannanxing (*Rhizoma Arisaematis Erubescens*) 10g, Chenpi (*Pericarpium Citri Reticulatae*) 10g, Fuling (*Poria*) 20g, Houpo (*Cortex Magnoliae Officinalis*) 5g, Zhiqiao (*Fructus Aurantii Submaturus*) 5g, Baizhu (*Rhizoma Atractylodis Macrocephalae*) 10g, Sharen (*Fructus Amomi*) 5g, Yizhiren (*Fructus Alpiniae Oxyphyllae*) 5g, Baibiandou (*Semen Lablab Album*) 10g, Maiya (*Fructus Hordei Germinatus*) 10g, Sanleng (*Rhizoma Sparganii*) 10g, Zaojiaoci (*Spina Gleditsiae*) 10g, Hehuanpi (*Cortex Albiziae*) 15g, Muli (*Concha Ostreae*) 15g, Lingzhi (*Ganoderma Lucidum*) 10g and Buguzhi (*Fructus Psoraleae*) 10g], each Chinese herb's formula granule was provided by Jiangyin Tianjiang Pharmaceutical Co. Ltd. (Jiangyin, China), altogether were equivalent to 192g of crude drug, was stored at -20°C. 5-Fluorouracil for Injection was supplied as a colorless fluid, 250mg, 10mL each ampoule, product lot No.120112, was produced by Shanghai Xudong Haipu Pharmaceutical Co. Ltd. (Shanghai, China). 0.9% Sodium Chloride Injection, 100mL each ampoule, product lot No.120320103, was produced by Fuzhou Neptunus Fuyao Pharmaceutical Co. Ltd. (Fuzhou, China). CTX (Cyclophosphamide for Injection), 200mg/ampoule, product lot No. 111104, was produced by Jilin Tonghua Mao Xiang Medicine Co. Ltd. (Tonghua, China).

### Main Reagents

Dehydrated alcohol (Sanlin Pharma Fujian Co. Ltd.); Haematoxylin staining solution, 0.7% acidized eosin staining solution, methanol, were all made by Zhongshan Golderbridge Biotechnology Co. Ltd, Beijing, China; 10% neutral Formaldehyde Solution, Distilled Water was prepared by the laboratory center of Medical College of Xiamen University, Xiamen, China.

### Instruments

IDEXX VetAutoread Hematology Analyzer (PT. Sambada healthy TBK, JL. Pertinggalan, Indonesia), TGL-16M high-speed refrigerated centrifuge (Xiang Yi Centrifuge Instrument Co. Ltd, Changsha, China), LeicaRM2035 Histotome (LEICA Co., Solms, Germany), Olympus B202 Microscope (Olympus Optical Co. Ltd, Tokyo, Japan), SF2000 Electronic Digital Display Calipers (Guilin Guanglu Measuring Instrument Co., Ltd, Guilin, China), TP-200D and TP-1000A Electronic Balance (Xiang Yi Balance Instrument Co. Ltd, Changsha, China), Chamber for Counting Blood Cells (Shanghai Qiujing Biochemical Reagent & Instrument Co., Shanghai, China).

### Modeling

Hydroperitoneum mice with H<sub>22</sub> hepatoma carcinoma cells were transferred until ivory white hydroperitoneum could be sucked. Take suction of hydroperitoneum at the asepsis condition, drop on the counting slide to count under an inverted microscope (×100). The concentration was regulated by the normal saline, and the single cell suspension with 3×10<sup>7</sup> cells/mL was counted by Chamber for Counting Blood Cells. Select 71 mice randomly. 0.2mL suspension (about 6×10<sup>6</sup> cells) was inoculated subcutaneously to the right armpit or peritoneal cavity under the asepsis condition. 7 days later after inoculation, 71 mice that all formed the transplanted tumors, were injected by Cytoxan (CTX) at the dosage of 200mg/kg to peritoneal cavity to establish the cancer mouse chemotherapy model with subcutaneous transplanted tumor of H<sub>22</sub> hepatoma carcinoma cells. The other 21 mice were injected the same amount of H<sub>22</sub> hepatoma carcinoma cells into peritoneal cavity, and 24 hours later, the same method was used to establish the ascitic tumor chemotherapy model.

### Mice Breeding

The mice were bred in animal house (SPF degree) with barrier system assisted with apinoid laminar flow chamber in the Experimental Animal Center of Xiamen University, and used for experiment after 1 week with diet and water access freely.

## Drugs Dispensing

Mix every CHBGF's drug Granule and completely dissolve with appropriate amount of distilled water to prepare the CHBGF solution with concentration of 3.2g/mL, 1.6g/mL and 0.8g/mL respectively. Dilute 5-Fluorouracil for Injection by 0.9% Sodium Chloride Injection to the concentration 1.25mg/mL. And dilute CTX by 0.9% Sodium Chloride Injection.

## Animal Grouping and Administering

The 71 mice that had formed tumors were injected by Cytosin (CTX) (200mg/kg) to establish the mouse chemotherapy model with the transplanted tumor, and then they were commensurately divided into 8 groups by random digits table. The other 21 mice were divided into 3 groups by the same mean. Two kinds of models, each three groups for evaluating the effects on survival time were model group, CHBGF group and the positive control group respectively with 7 mice in each group. Another five groups for evaluating the effects on anti-cancer were model group, the three treatment groups and the positive control group with 10 mice in each group. And then, the survival-time-observing groups of solid tumor were respectively given intragastric administration of normal saline (0.9%, 0.2mL/10g), CHBGF (64g/kg, 0.2mL/10g) (equivalence to 20 times of human dosage of medicine material crude slices) once a day, and peritoneal injection of 5-Fluorouracil (25mg/kg, 0.2mL/10g) once every other day until death emerged in the control group. The survival-time-observing groups of ascitic tumor were the same administering as the above, but the observing days were the successive 10 days. And then record the survival time of each mouse to calculate the life prolonging rate. The five anti-cancer-observing groups were respectively given intragastric administration of normal saline (0.9%, 0.2mL/10g), CHBGF (64g/kg, 32g/kg, 16g/kg, 0.2mL/10g) once a day, and peritoneal injection of 5-Fluorouracil (25mg/kg, 0.2mL/10g) once every other day for successive 21 days.

## Life Prolonging Rate and Tumor Inhibition Ratio Calculating

The life prolonging rate (LFR) was calculated.  $LFR = (\text{average survival days of experimental group} - \text{average survival days of control group}) / \text{average survival days of control group} \times 100\%$ . To the five anti-cancer-observing groups, on the next day after administering at the last time, the mice were weighed, mice were put to death by cervical dislocation according to regulation, and the tumors were peeled off completely to weigh. The postchemotherapy tumor inhibition rate (IR) was calculated.  $IR = (\text{average tumor weight of model group} - \text{average tumor weight of medication administration group}) / \text{average tumor weight of model group} \times 100\%$ .

## Blood Cell Calculating and WBC Blood Smear Observing

Pick the eyeballs to collect peripheral blood on the next day when administration was over, and use VetAutoread Hematology Analyzer to analyze automatically, and calculate the number of red blood cell (RBC), white blood cell (WBC), hemoglobin (HGB) and blood platelet (PLT). Meanwhile, drop the blood on one terminal of microscopic slide, and use cover glass with smoothed edge to push blood drop to spread out evenly, and dry, then drop Wright's stain to fix the slide for 1 minute, rinse clean and dry naturally. Then the slides were observed under microscope.

## Preparation of Pathological Section and Observation

Fresh tumor tissues were put into formalin neutral solution to fix, and then the tissues were dehydrated routinely and embed with paraffin to be made serial section with 4 $\mu$ m thickness. The sections were dried at 50 $^{\circ}$ C, stained by haematoxylin, and rinsed with tap water. And then they were processed with 70% saline ethanol to separate color, and rinsed following retained with acidizing Feosin, rinsed again, dehydrated, and cleared. Finally the sections were mounted with neutral balsam, and then put under the inverted microscope. The pathology in tumors was watched in 400 times fields-of-view to observe oncopathological changes.

## Statistics

The data were expressed as ( $\bar{x} \pm s$ ) and statistical software, Statistical Product and Service Solutions (SPSS) 19.0 (IBM, Armonk, NY, USA), was used for one-way analysis of variance [One-Way ANOVA (analysis of variance)]; and the least significant difference (LSD) method was chosen.  $P < 0.05$  was regarded as statistically significant difference.

## Results

### Effects of CHBGF on Life Prolonging Time of Tumor Mice Chemo-treated

The survival time of subcutaneous transplanted tumor mice and ascitic tumor mice chemo-treated in CHBGF group was obviously longer than that in the negative control group and 5-Fluorouracil group ( $P < 0.05$ ,  $P < 0.01$ ) (Table 1&2). The life prolonging rates (LFR) of two CHBGF groups were 20.14% and 64.15% respectively. It hinted that the formula 3.2g/mL CHBGF solution had a certain action to

prolong the life of both H<sub>22</sub> subcutaneous transplanted tumor and ascitic tumor mice chemo-treated.

**Table 1:** Effect of CHBGF on life prolonging rates (LFR) in mouse chemotherapy model with subcutaneous transplanted tumor of H<sub>22</sub> hepatoma carcinoma cells ( $\bar{X} \pm s$ )

Group	Dose(g/kg)	n	Body weight (g)		Survival time (d)	LFR (%)
			Before administration	After administration		
Model	--	6	22.66±0.83	35.90±3.34	22.00±2.28	--
5-Fluorouracil	0.025	7	22.41±0.70	28.96±2.44 <sup>a</sup>	18.28±2.75 <sup>a</sup>	-16.91
CHBGF	64.0	7	22.75±0.86	31.20±4.99	26.43±3.82 <sup>ac</sup>	20.14

Notes: <sup>a</sup> P<0.05 vs. the model group; <sup>c</sup> P<0.05 vs. the 5-Fluorouracil group

**Table 2:** Effect of CHBGF on life prolonging rates (LFR) in mouse chemotherapy model with ascitic tumor of H<sub>22</sub> hepatoma carcinoma cells ( $\bar{X} \pm s$ )

Group	Dose(g/kg)	n	Number of survival mouse	Survival rate (%)	Survival time (d)	LFR (%)
Model	--	6	0	0	5.83±0.75	--
5-Fluorouracil	0.025	7	3	42.86	8.00±1.53 <sup>b</sup>	37.22
CHBGF	64.0	7	5	71.43	9.57±0.79 <sup>bc</sup>	64.15

Notes: <sup>a</sup> P<0.05 vs. the model group; <sup>b</sup> P<0.01 vs. the model group; <sup>c</sup> P<0.05 vs. the 5-Fluorouracil group

**Table 3:** Effect of CHBGF on tumor weight in mouse chemotherapy model with subcutaneous transplanted tumor of H<sub>22</sub> hepatoma carcinoma cells ( $\bar{X} \pm s$ )

Group	Dose (g/kg)	n	Body weight		Tumor weight (g)	IR (%)
			Before administration	After administration		
Model	--	9	20.66±1.18	33.47±3.33	1.22±0.30	--
5-Fluorouracil	0.025	7	20.85±1.16	28.85±2.54 <sup>a</sup>	0.40±0.09 <sup>b</sup>	67.21
CHBGF(high)	64.0	9	20.92±0.83	31.91±3.28 <sup>c</sup>	0.84±0.17 <sup>b</sup>	31.15
CHBGF(middle)	32.0	8	20.55±1.00	30.90±3.26	0.96±0.16 <sup>a</sup>	21.31
CHBGF(low)	16.0	9	20.75±0.79	31.56±1.96	1.06±0.34	13.11

Notes: <sup>a</sup> P<0.05 vs. the model group; <sup>b</sup> P<0.01 vs. the model group; <sup>c</sup> P<0.05 vs. the 5-Fluorouracil group

**Table 4:** Effect of CHBGF on peripheral blood cells in mouse chemotherapy model with subcutaneous transplanted tumor of H<sub>22</sub> hepatoma carcinoma cells ( $\bar{X} \pm s$ )

Group	Dose (g/kg)	n	PLT ( $\times 10^9/L$ )	RBC ( $\times 10^{12}/L$ )	WBC ( $\times 10^9/L$ )	HGB (g/L)
Model	--	9	176.67±17.97	10.28±0.76	6.72±1.14	156.89±6.49
5-Fluorouracil	0.025	7	171.71±11.64	10.87±0.75	5.50±0.81 <sup>ac</sup>	150.14±9.86
CHBGF(high)	64.0	9	246.22±35.42 <sup>bd</sup>	11.10±0.80 <sup>a</sup>	9.79±1.01 <sup>bd</sup>	160.56±7.35 <sup>c</sup>
CHBGF(middle)	32.0	8	225.38±30.54 <sup>bd</sup>	10.27±0.52	7.84±0.63 <sup>ad</sup>	157.50±8.32
CHBGF(low)	16.0	9	219.56±32.86 <sup>bd</sup>	10.25±0.90	7.40±1.42 <sup>ad</sup>	151.22±9.73

Notes: <sup>a</sup> P<0.05, <sup>b</sup> P<0.01 vs. the model group; <sup>c</sup> P<0.05, <sup>d</sup> P<0.01 vs. the 5-Fluorouracil group

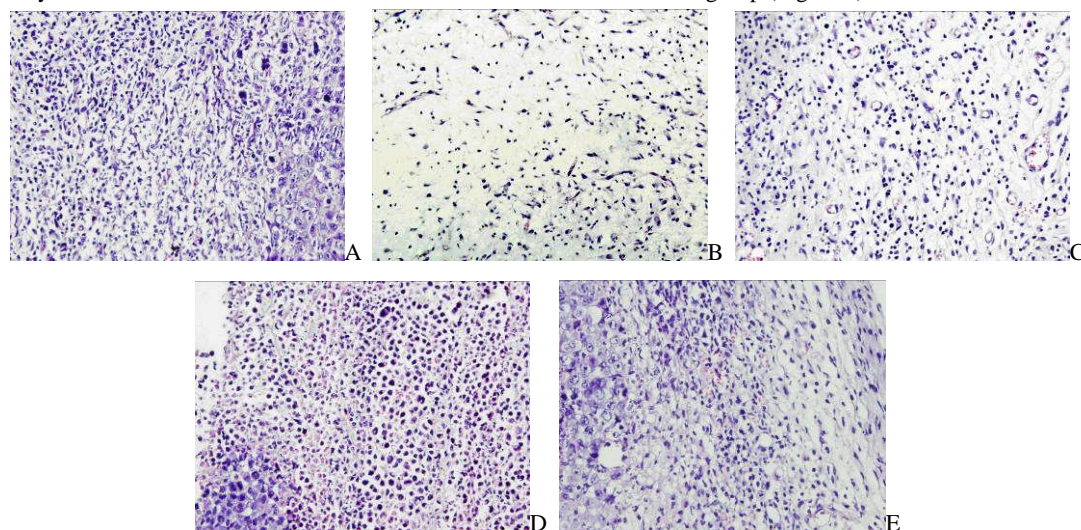
### Inhibitory Effects of CHBGF on Growth of Transplanted Tumor Chemo-treated

The average tumor weight of the CHBGF (64g/kg), CHBGF (32g/kg) groups and 5-Fluorouracil groups was lower than that of model group (P<0.05; P<0.01), and the postchemotherapy inhibition rates (IR) of four treated groups were 67.21%, 31.15%, 21.31%, and 13.11% respectively. It manifested that CHBGF with a certain concentration had an inhibitory action to the growth of H<sub>22</sub> transplanted tumor by chemo-treated (Table 3).

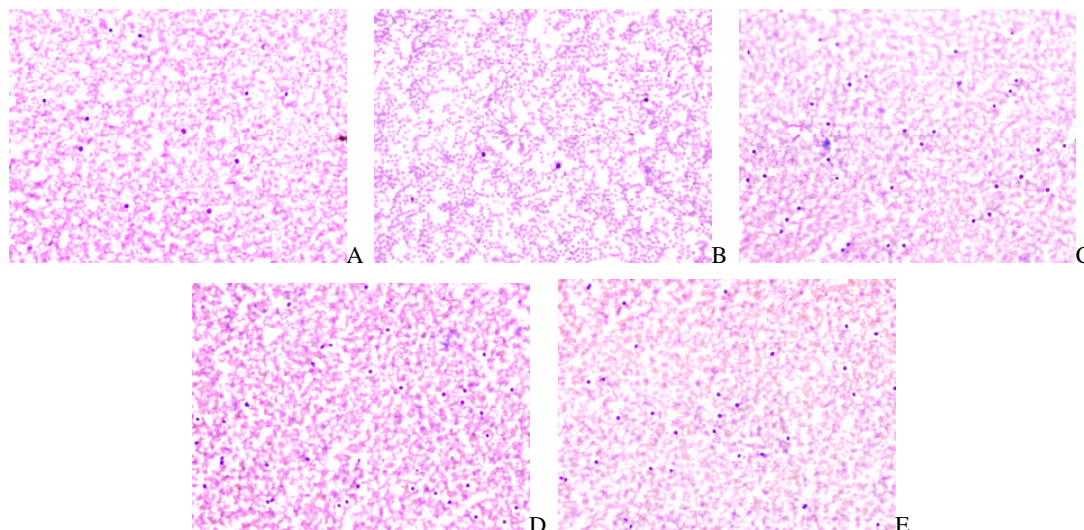


**Effects of CHBGF on Pathological Change of Transplanted Tumor Chemo-treated**

Most tumor masses when peeled off had clear dividing lines in tissue; some of them had invaded adhesions. The tumors were light red and show as ellipsoids, their texture was hard, and had pallid tissue section. Most of the tumor tissue in model group had sublobe, and unsmooth, irregular surface. Under the inverted microscope, a great quantity of H<sub>22</sub> hepatoma carcinoma cells could be seen in tumor tissue of each group; they show as round or oval shape, different sizes and disordered arrangement; their nuclei were stained to deep blue and some were large karyoplasm. The tumor tissue in model group had very conferted and scrambled cancer cells arrangement, and more megakaryocytes with deep staining, and obvious karyokinesis; proliferation of H<sub>22</sub> hepatoma carcinoma cells was high. After treatment, the tumor tissue in the 5-Fluorouracil group shows the sparse distribution of H<sub>22</sub> hepatoma carcinoma cells, more interstitial substances, and few megakaryocytes and appearance of karyokinesis. The tumor tissue in the CHBGF (64g/kg), CHBGF (32g/kg) groups were the slight concentration of H<sub>22</sub> hepatoma carcinoma cells; the disordered arrangement of cancer cell was not more than that of model group, the appearance of karyokinesis and cell distribution were also fewer than that in the model group (Figure 1).



**Figure 1:** Pathological diagram of the chemo-treated transplanted tumor of H<sub>22</sub> hepatoma carcinoma cells in each group (400×, HE staining). A: Model group; B: 5-Fluorouracil group; C: CHBGF (high) group; D: CHBGF (middle) group; E: CHBGF (low) group.



**Figure 2:** Blood smear diagram of the chemo-treated transplanted tumor of H<sub>22</sub> hepatoma carcinoma cells in each group (400×, Wright staining). A: Model group; B: 5-Fluorouracil group; C: CHBGF (high) group; D: CHBGF (middle) group; E: CHBGF (low) group.

**Effects of CHBGF on Peripheral Blood Cells of Transplanted Tumor Mice Chemo-treated**

Compared with the model and the 5-Fluorouracil groups, the number of WBC and PLT in three CHBGF groups was obviously more than that of the other two groups ( $P < 0.05$ ;  $P < 0.01$ ). But the number of RBC and HGB just in the CHBGF (64g/kg) group was more than that

of model group or the 5-Fluorouracil group ( $P < 0.05$ ) (Table 4). Under the microscope, the red blood cells manifested slightly purple red color; the white blood cells were stained to deep blue color. The number of WBC of the continuous chemotherapy group was quite few. WBC number of the three CHBGF group was big which was obviously more than that of the model and 5-Fluorouracil group (Figure 2). The blood smear pictures were in line with the results of peripheral blood analyzed automatically by Hematology Analyzer. These results manifested that CHBGF with a certain concentration had a promotive effect on the upswing of WBC reduced by chemotherapy and antagonizing the reduction of PLT.

## Discussion

Malignant tumor is one of the clinical refractory diseases, especially the hepatic carcinoma, which has a swift progression, a short natural life span, and the death rate at the middle and advanced stage of which is high, so now there is still no commendable therapeutic method to treat it. The classical chemotherapy often brings about many more serious toxic and side-effects to the patients and makes them hard to bear. Peripheral blood cells count is one of the routine examination targets for chemotherapy, which can reflect the immune state. Recent research data manifested that about 90% of the tumor patients who had been received chemotherapy would show the bone marrow depression symptom of WBC reduction; the severe patient may suffer serious infection, even life was threatened (Zhang et al., 2013; Li et al., 2013). Traditional Chinese medicine thinks that liver cancer has the syndrome of intermingled deficiency and excess. "Deficiency" mainly lies in *qi* deficiency of spleen, *yin* deficiency of liver and kidney, *yang* deficiency of spleen and kidney, and "excess" mainly lies in *qi* stagnation and blood stasis, dampness heat and stagnated toxin (Wang et al., 2009). To the treatment, TCM physicians should strengthen spleen and fortify the center, regulate the *qi* movement, remove toxic substance, and activate blood circulation to dissipate blood stasis. To the patient in the advanced stage, they should often fortify the spleen and nourish the kidney, consolidate body resistance and bank up the original *qi*. Chemotherapy, TCM thought it was one kind of pathogenic toxin compared with healthy *qi* (Wang, 2004). TCM treatment of tumor after chemotherapy, should be given more emphasis on fortifying the spleen and boosting *qi*, and supplementing kidney and essence that combined removing phlegm, stasis and stagnation by Prof. Yanhui Wang (Wang et al., 2004). In formula "CHBGF", Dangshen (*Radix Codonopsis*) matching Huangqi (*Radix Astragali Mongolici*), Chenpi (*Pericarpium Citri Reticulatae*), Fuling (*Poria*), etc., can boost, regulate *qi* and fortify the spleen; Sanleng (*Rhizoma Sparganii*) and Zaojiaoci (*Spina Gleditsiae*) can invigorate blood and dissolve stasis; Tiannanxing (*Rhizoma Arisaematis Erubescens*), Zhebeimu (*Bulbus Fritillariae Thunbergii*) and Muli (*Concha Ostreae*) can dissolve phlegm and dissipate masses; Lingzhi (*Ganoderma Lucidum*) can supplement the deficiency; Hehuanpi (*Cortex Albiziae*) and Muli (*Concha Ostreae*) can calm the mind, Buguzhi (*Fructus Psoraleae*) can supplement the kidney, which are quite in line with the therapeutic principles for liver cancer after chemotherapy.

This experimental results show that CHBGF could not only prolong the survival time of mouse chemotherapy model with solid and ascitic tumor of H<sub>22</sub> hepatoma carcinoma cells, but also inhibit synergistically the growth of transplanted tumor chemo-treated. The inhibition ratio was inferior to that of 5-Fluorouracil, but in the aspect of the body weight change in anterior-posterior experiments, the toxic, side-effects and the follow-up survival time of tumor retention, 5-Fluorouracil could improve the inhibition ratio but not yet prolong the survival time. Though the direct tumoricidal efficacy of CHBGF was not as good as chemotherapy drug, the inhibitory action existed in all three dosage groups of CHBGF, and the increasing of body weight of the three groups of CHBGF was more than the continuous chemotherapy mice. The upswing of WBC reduced by chemotherapy and the antagonizing effect on PLT reduction of the three CHBGF groups, and the number of RBC and HGB just in the CHBGF (64g/kg) group were all more obvious than the model and continuous chemotherapy mice, which manifested that CHBGF had a certain action on relieving the toxic and side-effects caused by chemotherapy, and this formula was worth further studying and researching.

The chemotherapy for tumor and the benefits of patients obtained from the treatment were often ignored by modern medicine doctors to some degree, but the application of Chinese materia medica was more and more thought highly of. The antagonizing tumor of Chinese medicinals has their unique advantages. At present, the therapeutic effects of Chinese medicinal or its chief components mainly concentrate upon improving immunity (Zhang et al., 2010), inhibiting tumor capillary angiogenesis (Xi et al., 2012) and metastasis (Li et al., 2012), killing the neoplastic cells directly (Xu et al., 2005), promoting them apoptosis (He et al., 2012) and so on. So formula may play a multi-target, multi-angle and multi-path anticancer effectiveness, such as to increase the tolerance of body to chemotherapy, relieve the adverse reactions, and then reduce the complications and improve the curative effect. In the formula "CHBGF", some recent researches show that coarse polysaccharides of Dangshen (*Radix Codonopsis*) could inhibit the S<sub>180</sub> ascites tumor (Li et al., 2011), parenteral solution of Huangqi (*Radix Astragali Mongolici*) could restrain tumor growth of tumor-bearing mice whose mechanism was likely related to improve mice's cellular immune function (Fan et al., 2013), and Huangqi polyoses (astragalins) also can prevent leukopenia of carcinoma of large intestine caused by chemotherapy (Lv et al., 2009), combined with Lingzhi (*Ganoderma Lucidum*) which may be more effective on WBC (Zhang et al., 2012), Sanleng (*Rhizoma Sparganii*) and some components of Chenpi (*Pericarpium Citri Reticulatae*) which can induce apoptosis of hepatoma carcinoma cells (Li et al., 2006; Li et al., 2009), and Psoralen, component of Buguzhi (*Fructus Psoraleae*), some components of Fuling (*Poria*) which both had the antineoplastic activity (Wu et al., 1998; Cheng et al., 2008), Buguzhi (*Fructus Psoraleae*) which can also increase the WBC number reduced by CTX (Lin et al., 2007), Tiannanxing (*Rhizoma Arisaematis Erubescens*), Muli (*Concha Ostreae*), Lingzhi (*Ganoderma Lucidum*) and Zaojiaoci (*Spina Gleditsiae*) which may all antagonize transplanted tumor through strengthening immunity (Wang et al., 1997; Jin et al., 2011; Liu et al., 2009). So it is thus clear that the anti-cancer or anti-side-effect action of every Chinese medicine of CHBGF chosen by Prof. Yanhui Wang has been confirmed by animal or clinical experiments.

This study first confirmed that "CHBGF", the empirical formula, can prolong the life span of mouse chemotherapy model of H<sub>22</sub> hepatoma carcinoma cells, also has a certain action on inhibiting their growth of tumor, meanwhile, it can antagonize the reduction of WBC and PLT caused by chemotherapy, which provides some experimental data for the TCM clinical treatment to postchemotherapy of tumor at the angle of integrated pharmacodynamics. And its concrete mechanisms will be the next investigative focal points.

## Acknowledgments

This work was supported by the Xiamen Science and Technology Key Program Plan Grant (No.3502Z20100006), the National Natural Science Foundation of China (No.81202659) and the Scientific Research Start Foundation for New Teacher of Xiamen University (ZK1014). We gratefully thank Fu Chen, the experimentalist of the Medicine Laboratory Animals Center of Xiamen University for his help in laboratory procedure, and Jinghua Qiu, the laboratory assistant of the pathology department of Medical College of Xiamen University for his help in making tumor pathological section, and Dandan Feng and Anning Zhu, the undergraduates of TCM department of Medical College of Xiamen University for help in preparing detection.

## References

- Cheng JS, Zhao J, Huang H. (2008). Facile preparation of Carboxymethyl Pachymaran and the observation on its antitumor effects. *Youjiang Medicine*, **36(4)**: 386-388.
- Fan SL, Wang ZJ, Ren YL. (2013). The cellular immunity mechanism of tumor inhibitory action of Huangqi Zhusheyeye on H<sub>22</sub> tumor-bearing mice. *Guide of Chinese Medicine*, **11(13)**: 76-77.
- He GZ, Deng SX, He QS, Li SJ, Wu ML, Wei LG, Wang WJ, Wang P, Cao F, Cao G, An CW, An CX. (2012). Effects of flavone components from *Spina Gleditsiae* on proliferation, apoptosis and metastasis of HepG<sub>2</sub> cell line. *Journal of Natural Science of Hunan Normal University*, **35(1)**: 77-81.
- Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. (2011). Global cancer statistics. *CA: A Cancer Journal for Clinicians*, **61(2)**: 69-90.
- Jin L, Liu JY, Sun SY, Jiang ZJ. (2011). Antitumor and immunoregulation effects of Ganoderma spore oil soft capsule on mice of hepatocarcinoma H<sub>22</sub> cells-derived tumor. *China Journal of Traditional Chinese Medicine and Pharmacy*, **26(4)**: 715-718.
- Li J, Niu GX. (2012). Experiment research on anti-tumor effect of *Scutellaria Barbata D.Don* on H<sub>22</sub> lymphatic metastasis. *Chinese Archives of Traditional Chinese Medicine*, **30(11)**: 2472-2473.
- Li L, Zhang P, Kang HZ, Pan W. (2013). Risk factors for nosocomial infections during myelosuppression period of tumour chemotherapy. *Chinese Journal of Nosocomiology*, **23(18)**: 4394-4395, 4398.
- Li LY, Peng WN, Qian SH. (2009). Study of methoxyflavone components of *Pericarpium Citri Reticulatae* on inducing tumor apoptosis of H<sub>22</sub> tumor-bearing mice. *Journal of Chinese Medicinal Materials*, **32(10)**: 1596-1598.
- Lin GM, Guo YH. (2007). Experimental study on pharmacodynamics of *Psoralea Corylifolia* L. *Chinese Archives of Traditional Chinese Medicine*, **25(11)**: 2347-2348.
- Lin MH, Lin JM, Wei LH, Xu W, Hong Z, Cai Q, Peng J, Zhu D. (2012). *Hedyotis diffusa* Willd extract inhibits HT-29 cell proliferation via cell cycle arrest. *Experimental and Therapeutic Medicine*, **4(2)**: 307-310.
- Li RY, Gao JP. (2011). The primary study of pharmacological action in vivo with abdominal cavity S<sub>180</sub> tumor bearing mice of the *Radix Codonopsis* coarse polysaccharides. *Journal of Changzhi Medical College*, **25(2)**: 94-96.
- Li SY, Zhou YD. (2006). The inhibitory action research of *Rhizoma Curcumae Phaeocaulis*, *Rhizoma Sparganii* and *Herba Hedyotidis* on tumor cells. *Journal of Practical Traditional Chinese Internal Medicine*, **20(3)**: 246-247.
- Liu MH, Huang XW, Xiao SH, Zhong L, Ren MP, Tian J. (2009). Effects of extractive from *Spina Gleditsiae* (ESG) on tumor growth and cytokines in tumor-bearing mice. *Cancer Research on Prevention and Treatment*, **36(5)**: 365-367.
- Lv JL, Sun JY, Yu NR, Yuan Y, Lu CJ, Liu HY. (2009). Study of astragaloside injection preventing leukopenia before carcinoma of large intestine treated by chemicals. *Journal of Chinese Medicinal Materials*, **32(1)**: 166-168.
- Shen AL, Hong F, Liu LY, Lin JM, Zhuang QC, Hong Zf, Peng J. (2012). Effects of Pien Tze Huang on angiogenesis in vivo and in vitro. *Chinese Journal of Integrative Medicine*, **18(6)**: 431-436.
- Wang YH. (2004). The application of TCM method 'He' in preventing and curing the toxic and side-effect from chemotherapy to malignant tumor. *Journal of Gansu College of Traditional Chinese Medicine*, **21(3)**: 9-11.
- Wang YH, Shen XY. (2004). Emphasis of TCM recuperative medical care on post-chemotherapy to malignant tumor is 'treatment should focus on the principal cause of a disease'. *Journal of Gansu College of Traditional Chinese Medicine*, **21(4)**: 6-7.
- Wang Y, Ma AL, Zhang HZ, Xue BH, Zhao ZJ, Fu FH, Zhou GY. (1997). Experimental studies on the antitumor effect of Oyster extract. *Chinese Journal of Marine Drugs*, **16(1)**: 18-22.
- Wang YY, Yan SY. (2009). *Practical Chinese Internal Medicine* (2nd edition). Shanghai: Shanghai Scientific and Technical Publishers, 693-694.
- Wu SH, Zhang ZH, Zhao JB. (1998). An experiment study on antitumor activity of Psoralen on mammary cancer cell line EMT<sub>6</sub> in vitro and in vivo. *China Journal of Chinese Materia Medica*, **23(5)**: 303-305.
- Xi SY, Zhang Q, Liu CY, Xie H, Yue LF, Gao XM. (2012). Effects of hydroxy safflower Yellow-A on tumor capillary angiogenesis in transplanted human gastric adenocarcinoma BGC-823 tumors in nude mice. *Journal of Traditional Chinese Medicine*, **33(2)**: 243-248.
- Xu LC, Bian KJ, Liu ZM, Zhou J, Wang G. (2005). The inhibitory effect of the curcuminol on women cancer cells and synthesis of RNA. *Tumor*, **25(6)**: 570-572.
- Zhang JJ, Liao XY, Weng XC. (2012). Efficacy of compound of Ganoderma Lucidum and Astragalus extracts on tumor-bearing mice treated by cyclophosphamide. *Journal of Shanghai University (Natural Science)*, **18(4)**: 425-429.
- Zhang P, Lv MY, Liu Q, Bai RZ, Zhang ZJ, Xu FG. (2013). Advances in research on the effects of traditional Chinese medicines on platinum antitumor drugs. *Progress in Pharmaceutical Sciences*, **37(5)**: 207-214.
- Zhuang QC, Hong F, Shen AL, Zheng L, Zeng J, Lin W, Chen Y, Sferra TJ, Hong Z, Peng J. (2012). Pien Tze Huang inhibits tumor cell proliferation and promotes apoptosis via suppressing the STAT3 pathway in a colorectal cancer mouse model. *International Journal of Oncology*, **40(5)**: 1569-1574.
- Zhang ZL, Tang JH, Chen Y, Wang XY, Wang SZ, Wang WJ. (2010). Study of ethanol extract from *Rhizoma Arisaematis Erubescens* on anti-cancer activity. *Shaanxi Journal of Traditional Chinese Medicine*, **31(2)**: 242-243.