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EVALUATION OF THE ACUTE AND SUBCHRONIC TOXICITY OF ZIWAN BAIBU TANG

Rui-hua Xin[1], Wen-jing Peng[1], Xiao-lei Liu[1], Yong-jiang Luo[1], Gui-bo Wang[1], Chao-ying Luo[1], Jia-sheng Xie[1], Jin-yu Li[1], Ge Liang[2], Ji-fang Zheng[1]*

[1] Lanzhou Institute of Husbandry and Pharmaceutical Sciences of CAAS, Key Laboratory of New Animal Drug Project of Gansu Province, Engineering & Technology Research Center of Traditional Chinese Veterinary Medicine of Gansu Province, Key Laboratory of Veterinary Pharmaceutics Development, Ministry of Agriculture, Lanzhou 730050, PR China
[2] Sichuan Animal Science Academy, Chengdu 610066, PR China.
***Corresponding author E-mail:** xinruihuamys@126.com and zhengjifang100@126.com

Abstract

Background: Ziwan Baibu Tang (ZBT) is used as a traditional Chinese prescription used in the clinic to cure a variety of respiratory diseases. The objective of this study was to evaluate the acute and subacute toxicity of ZBT.

Materials and Methods: Fifty Kunming mice were assigned to 5 groups, and ZBT was administered at different concentrations. Symptoms, mortality and behavioural changes were recorded. Eighty Wistar rats were randomly allocated to 4 treatment groups or a control group for 4 weeks of treatment (half male and half female); general observations and weekly body weight (BW) gain were recorded. At the end of the 4-week experiment, the rats were sacrificed, and blood was collected for haematological and serum biochemical analyses. Samples from the primary organs were fixed in 10% buffered formalin for histopathological studies.

Results: The calculated LD_{50} of acute oral toxicity was 319.16 g/kg BW. After the 4-week treatment, BW did not significantly differ in any of the dose groups, compared with the control group ($P>0.05$), and the rat blood red blood cell (RBC) and haemoglobin (HGB) levels in each dose group did not significantly differ ($P>0.05$). In addition, serum urea and creatine levels in each dose group did not significantly differ from those in the control group ($P>0.05$).

Conclusion: These results indicate that ZBT does not possess toxic potential and that both the acute and subchronic toxicity in animals are very low.

Key words: Ziwan Baibu Tang, acute, subchronic, toxicity, herbal

Introduction

Herbs and herbal preparations have been used to treat disease throughout human history. According to World Health Organization (WHO) statistics, 70–80% of the world's population employs plant-derived traditional treatment methods for health problems (Ahmad et al., 2006; Shirwaikar et al., 2009). Traditional Chinese medicine (TCM) has been used in China for thousands of years to diagnose and treat diseases (Chou et al., 2008). TCM theories are based on syndrome differentiation and holistic medicine. Chinese herbal medicine (CHM), the pharmaceutical and most important component of the TCM system, combines compounds in the form of processed natural products (Li et al., 2013) and is supported by extensive literature and clinical applications covering thousands of years (Xu et al., 2009). A compound formulation is prescribed according to the principle, "Monarch, Minister, Assistant and Guide." A formulation that contains more than two Chinese herbs conforms more closely to TCM theories and better reflects the characteristics of TCM than the administration of a single herb (Jiao et al., 2004). Studies of the effects of CHM formulas are attracting increasing attention globally (Shi et al., 2011).

Ziwan Baibu Tang (ZBT) is a traditional Chinese prescription that is now used in the clinic to cure a variety of respiratory diseases, such as pneumonia, respiratory tract infections, cough and phlegm (Cheng et al., 2014). ZBT consists of 7 crude drugs: *Aster Tataricus, Stemona Japonica, Platycodon Grandiflorus, Cortex Mori, Herba Houttuyniae, Folium Eriobotryae* and *Glycyrrhiza.*

Aster Tataricus is the chief component in this prescription and is clinically used to eliminate phlegm and relieve cough in China. *Aster Tataricus* also possesses diuretic, antibacterial, antitumour, antiviral and anti-ulcer activities (Hou et al., 2006). *Herba Houttuyniae* is commonly used in TCM and provides antiviral, anti-inflammatory, antibacterial, and heat-clearing properties for the treatment of pneumonia and throat swelling; it has also been shown to increase the immune response *in vivo* and *in vitro* (Chen et al., 2014). *Stemona Japonica* (Zhu et al., 2010), *Platycodon Grandiflorus* (Song et al., 2006), *Cortex Mori* (Feng et al., 2004), *Folium Eriobotryae* (Ju et al., 2003) and *Glycyrrhiza* (Tian et al., 2006) are used traditionally as medicinal herbs for the treatment of respiratory tract infections in China, and several research studies have revealed that these herbs provide respiratory therapeutic effects (Chinese Pharmacopoeia Commission, 2010).

The safety of ZBT is generally assumed from its very long history of consumption in China. However, the consumption of plants and plant products whose content, toxicity profile and safe dose have not been determined may cause severe toxicity problems in humans and animals. Despite knowledge of the biological activities of ZBT, toxicological and side effect profiles have not been adequately documented. To provide scientific data for clinical drug safety, the present study investigated the acute and subchronic oral toxicity of ZBT by applying the recommended guidelines for safety or dose-dependent toxicity in mice and rats.

Materials and Methods

Instruments and Reagents

A haematology analyser (SYSMEX, pocH-100iV, Japan), thin semiautomatic microtome (Leica RM 2265), organization stand/dryer (Leica HI 1210), and automatic biochemistry analyser (Mindray Biomedical) were used. An aspartate aminotransferase kit (AST, 150211009), alanine aminotransferase kit (ALT, 150111013), albumin kits (ALB, 150909014), creatinine reagent kit (Creatinine, 151011012), urea kits (Urea, 151311013) and a creatine kinase kit (CK, 152511010) were provided by Mindray Biomedical Co., Ltd.

Preparation of ZBT Extract

ZBT consists of 7 crude drugs; the plant components and their origins are listed in Table 1. Briefly, a mixture of 150 g of *Aster Tataricus*, 150 g of *Stemona Japonica*, 120 g of *Platycodon Grandiflorus*, 120 g of *Cortex Mori*, 180 g of *Herba Houttuyniae*, 150 g of *Folium Eriobotryae* and 130 g of *Glycyrrhiza* was macerated with 12,000 mL of distilled water for 2 h and decocted at 100 ℃ for 1.5 h. The filtrate was collected, and the residue was again decocted for 1.5 h in 10,000 mL of distilled water. The extracts were combined and further condensed at 65 ℃ to obtain the aqueous extract of ZBT (1000 mL).

Table 1: The composition of ZBT

Species	Chinese name	Plant components	Origin	Grams(g)	%
Aster Tataricus	Zi wan	Root, stem	Hebei, China	150	15
Stemona Japonica	Bai bu	Root	Jiangxi, China	150	15
Platycodon Grandiflorus	Jie geng	Root	Liaoning, China	120	12
Cortex Mori	Sang bai pi	Rhizodermis	Henan, China	120	12
Herba Houttuyniae	Yu xing cao	Whole herb	Sichuan, China	180	18
Folium Eriobotryae	Pi pa ye	Leaves	Zhejiang, China	150	15
Glycyrrhiza	Gan cao	Root, stem	Xinjiang, China	130	13

Animals

Young mice weighing 18-22 g were purchased from the Animal Physiology Laboratory of Lanzhou University. Adult female and male Wistar rats (aged two months, weighing 181-199 and 185-205 g, resp.) were supplied by the production facility of the

Animal Physiology Laboratory, Gansu University of Traditional Chinese Medicine (Gansu, China). The accession number was GTCM-150823. The animals were fed in a separate room with a barrier system under a controlled light-dark cycle (12-12 h, lights on 7:00-19:00), ventilation (air exchange rate of 18 cycles/h), temperature (23±2 °C) and relative humidity (55±15%) during the study. The cages and chip bedding were changed twice each week (Wang et al., 2011). The experiments were initiated after acclimatizing the rats for 1 week. The study was performed in accordance with Veterinary Laboratory Biosafety Guidelines (The Chinese Ministry of Agriculture Guide, 2003).

Acute Oral Toxicity Study in Mice

Fifty healthy Kunming mice (equal numbers of males and females, 18-22 g) were randomly assigned to 5 groups. ZBT was dissolved at different concentrations in distilled water and administered to the Kunming mice via oral gavage at doses of 250.00, 300.00, 360.00, 432.00, and 518.40 g/kg body weight (BW) per day. All mice were fasted overnight to eliminate food from the gastrointestinal tract before dosing. After gavage, the mice were observed for 14 days to observe their symptoms, mortality, behavioural changes, skin, eyes, fur and somatic motor activity. Finally, the LD_{50} was calculated using Bliss software.

Subchronic Oral Toxicity Test in Rats

Eighty Wistar rats were assigned to 4 groups (high-dose group, medium-dose group, low-dose group, and control group; 20 rats in each group, half male and female). The treated groups received ZBT at a dose of approximately $10/LD_{50}$, $50/LD_{50}$ and $100/LD_{50}$ every day, and the control group received an equal dose of physiological saline between 9:00 a.m. and midnight every day for 4 weeks. The general observations for clinical signs included behavioural changes, signs of gross toxicity, fur condition and somatic motor activity. The animals were weighed on the first day of administration, and the individual BWs of the rats were recorded every subsequent week. The mean weekly BW gain was calculated for each sex and measured weekly. The rats were allowed unlimited access to food throughout the study.

At the end of the 4-week experiment, the animals were fasted for 12 h and then sacrificed by decapitation under anaesthesia with sodium pentobarbital (30 $mg.kg^{-1}$) intraperitoneally administered. Blood was collected in two tubes: tube 1, containing EDTA, was processed immediately for haematological parameters; tube 2, without additive, was centrifuged at 3000×g at 4 °C for 10 min to obtain serum (stored at -20 °C until analysis). The organs (heart, liver, spleen, lungs and kidneys) were weighed, and the absolute organ weights were converted to relative organ weights based on the organ-to-BW ratio. Organ samples were fixed in 10% formalin for histopathological examination.

Haematological and Serum Biochemical Analysis

Haematology indexes were detected by pocH-100iV (haematology analyser and reagent) and included the white blood cell (WBC), red blood cell (RBC) and platelet (PLT) counts, as well as the haemoglobin (HGB) measurement. The following measurements were obtained: alanine aminotransferase (ALT), aspartate aminotransferase (AST), AST/ALT, albumin (ALB), creatine kinase (CK) and urea.

Histopathological Studies

After weighing the collected organs, all samples were fixed in 10% buffered formalin. In the histological analysis, we assessed the tissue integrity of the organs and observed the presence of degeneration, necrosis, leukocyte infiltration, congestion, blood extravasation or fibrosis.

Statistical Analysis

The LD_{50} was calculated using the Bliss method. The data for weekly BW, relative organ weights, haematology and serum

biochemistry were evaluated by one-way ANOVA using SPSS 17.0 statistical software. All values are expressed as the mean±SD. *P* values less than 0.05 were considered significant.

Results

Acute Oral Toxicity

After administration of ZBT, the mice in the high-dose group lay quietly and rarely moved. In addition, the mice exhibited reduced spontaneous activity and slow movement. Few mice developed movement disorders, shortness of breath or obvious neurological symptoms. As the dose of ZBT increased, the symptoms intensified. Prior to death, the mice exhibited symptoms such as hind limb twitching, disordered hair coat and difficulty breathing. After 7 d, the surviving mice exhibited good appetite, improved vitality and normal movement. The hair coat of the surviving mice became smooth and shiny. Death mainly occurred within 1-2 h following oral gavage of ZBT. The mice were dissected and examined. The following major organ lesions were observed: duodenal haemorrhage, liver darkening and inconspicuous bleeding and bruising. No significant gross lesions were detected in other organs. As shown in Table 2, 0, 4, 9, 9 and 10 deaths were respectively observed in the 5 groups of mice within 7 d after administration of ZBT. The LD_{50} of ZBT in mice and the 95% confidence limits were calculated by the Bliss method using SPSS 13.0 software. The LD_{50} was 319.16 g/kg BW, and the 95% confidence interval was 270.31–363.68 g/kg BW.

Table 2: Mortality of the mice in the acute toxicity experiment

Group	Dose (g/kg BW)	Number of mice	Mortality
1	250.00	10	0
2	300.00	10	4
3	360.00	10	9
4	432.00	10	9
5	518.40	10	10

Observation of Gross Toxicity and Mortality

Experimental observations of the rats indicated that there were no abnormal changes in the experimental groups or control group in terms of food intake, water intake, mental state, respiratory condition, and coat gloss or body temperature. No rats died during the experimental period.

Body Weight Changes and Organ Weights

As shown in Tables 3 and 4, during the experimental period, there were no significant differences in BW between the control group and the groups of rats that received different doses of ZBT ($P>0.05$). Compared with the control group, female rats in the low-dose group exhibited significantly decreased heart weight/BW coefficient ($P<0.05$). By contrast, no significant differences in the heart weight/BW coefficient were observed between the rest of the groups and the control group ($P>0.05$). In addition, there were no significant differences between the control group and the various dose groups in the organ weight/BW coefficients of the liver, spleen, lung and kidney ($P>0.05$).

Haematology

The haematological profiles of the treated and control groups are presented in Table 5. After continuous dosing for 4 weeks, WBC levels decreased significantly ($P<0.05$) in females in the high-dose group compared with the control group, and PLT levels significantly decreased ($P<0.05$) in both sexes in the high-dose group. No significant differences in RBC and HGB levels were observed in the dose groups ($P>0.05$).

Table 3: Weekly body weight gain in subchronic oral administration test

Groups	1 week	2 weeks	3 weeks	4 weeks
Female				
Vehicle	188.05±7.52	203.55±5.12	217.78±6.77	232.07±6.49
3 g/kg BW	188.10±6.91	201.15±5.35	215.50±6.96	230.00±7.22
15 g/kg BW	185.50±8.63	200.75±8.18	214.82±9.50	230.20±8.82
30 g/kg BW	182.65±5.43	194.47±5.79	205.44±6.99	217.06±7.11
Male				
Vehicle	202.87±9.34	218.10±8.43	235.11±7.98	251.96±8.08
3 g/kg BW	200.02±5.63	216.23±6.03	232.42±6.45	247.10±7.51
15 g/kg BW	199.84±10.09	214.58±9.79	229.33±10.09	243.09±9.89
30 g/kg BW	197.55±6.67	209.41±7.00	221.74±7.16	232.90±8.01

Values are means±SEM for 20 rats in each group, half male and half female.

Table 4: Effects of subchronic oral administration on relative organ weights

Group	Heart	Liver	Spleen	Lung	Kidney
Female					
Vehicle	0.68±0.02	6.39±0.66	0.37±0.01	1.01±0.03	0.51±0.01
3 g/kg BW	0.62±0.02	6.47±0.83	0.35±0.01	0.96±0.03	0.48±0.01
15 g/kg BW	0.62±0.02	6.71±0.91	0.34±0.01	0.95±0.03	0.48±0.01
30 g/kg BW	0.58±0.02*	6.25±0.66	0.36±0.01	0.96±0.02	0.48±0.01
Male					
Vehicle	0.69±0.02	6.56±0.83	0..41±0.01	1.12±0.04	0.55±0.01
3 g/kg BW	0.68±0.02	6.78±0.75	0.40±0.01	1.06±0.03	0.54±0.01
15 g/kg BW	0.69±0.03	6.65±0.88	0.39±0.01	1.15±0.04	0.53±0.01
30 g/kg BW	0.69±0.02	6.43±0.56	0.40±0.01	1.10±0.03	0.53±0.01

Values are means±SEM for 20 rats in each group, half male and half female. Statistically significant compared to controls (*P<0.05).

Serum Chemistry

After 4 weeks of continuous administration of ZBT, no significant differences in urea levels were observed in the control group and the various groups of rats that received different doses of ZBT (P>0.05). In addition, there were no significant differences in serum creatinine levels between the control group and the low-dose group or between the control group and the moderate-dose group (P>0.05). Compared with the control group, female rats in the high-dose group exhibited significantly decreased serum ALB levels (P<0.05). Serum ALB levels were also markedly reduced in male rats in the moderate-dose group and high-dose group compared to the control group (P<0.05). No significant differences in serum AST activity were detected between the control group and various dose groups (P>0.05). Compared with the control group, male rats in the moderate-dose group exhibited significantly elevated CK activity (P<0.05). By contrast, there were no significant differences in CK activity between the control group and the low-dose or high-dose group (P>0.05). No dose-response relationship between serum CK activity and ZBT dose was observed (Table 6).

Table 5: Haematological parameters in Wistar rats treated orally with ZBT for 4 weeks

Group	WBC/($\times10^9 \cdot L^{-1}$)	RBC/($\times10^{12} \cdot L^{-1}$)	HGB/g $\cdot L^{-1}$)	PLT/($\times10^9 \cdot L^{-1}$)
Female				
Vehicle	13.59±2.89	8.41±2.14	171.38±23.04	420.88±60.63
3 g/kg BW	12.53±2.48	8.56±2.13	172.69±21.19	441.47±65.21
15 g/kg BW	11.98±3.99	8.24±1.27	168.53±43.90	524.56±56.78
30 g/kg BW	10.43±2.89*	8.60±1.09	175.71±19.73	596.50±74.15*
Male				
Vehicle	10.73±0.68	9.78±1.12	177.64±13.33	232.08±56.28
3 g/kg BW	8.84±0.43	8.34±2.14	170.87±24.02	257.87±88.71
15 g/kg BW	10.69±0.64	8.18±1.16	166.00±25.29	279.13±46.42
30 g/kg BW	9.29±0.64	7.86±2.13	158.44±21.32	384.27±62.07*

Values are means±SEM for 20 rats in each group, half male and half female. Statistically significant compared to controls *P<0.05, **P<0.01.

Table 6: Serum biochemistry in Wistar rats treated orally with ZBT for 4 weeks

Group	AST (U/L)	AST/ALT	ALB (g/L)	Urea (mmol/L)	Creatinine (μmol/L)	CK (μmol/L)
Female						
Vehicle	318.50±19.07	2.61±0.15	34.04±0.25	7.13±0.28	73.83±2.52	5373.67±198.71
3 g/kg BW	358.63±16.52	3.06±0.11	34.21±0.29	6.99±0.22	70.71±1.21	4570.75±379.30
15 g/kg BW	241.88±19.28	2.37±0.15	33.59±0.37	7.14±0.14	70.88±1.67	4811.88±394.28
30 g/kg BW	246.10±11.29	2.69±0.15	30.94±0.26*	6.04±0.29	62.20±1.07	4777.80±296.45
Male						
Vehicle	239.25±7.17	2.22±0.11	35.10±0.14	6.67±0.26	80.33±1.65	3704.44±504.07
3 g/kg BW	268.14±26.44	2.13±0.17	34.34±0.28	7.69±0.27	72.88±1.44	3539.88±497.87
15 g/kg BW	284.71±23.26	2.25±0.12	33.14±0.33*	6.26±0.26	69.29±1.80	5297.57±306.58*
30 g/kg BW	199.50±3.77	1.82±0.20	33.93±0.61*	6.54±0.34	68.40±2.40	3260.50±700.20

Values are means±SEM for 20 rats in each group, half male and half female. Statistically significant compared to controls *P<0.05, **P<0.01.

Histopathology

In the histological investigation, organs including the heart, liver, spleen, lung and kidney exhibited no sign of pathological changes compared with the corresponding organs in the control group (Figure 1).

Discussion

Safety studies of plants and plant products and establishing the effectiveness and safety of plants and plant products via scientific research are increasingly emphasized. The complexity of herbal preparations and their natural biological variations requires their safety, effectiveness and quality to be established (Shin et al., 2011; Lee et al., 2012). ZBT is a traditional Chinese prescription that is used in clinical practice. ZBT was developed by modifying Zi Wan San, an ancient medical prescription collected in "Tai Ping Sheng Hui Fang" (The Peaceful Holy Benevolent Prescriptions). ZBT consists of 7 Chinese herbs, including *Aster Tataricus*, *Stemona Japonica*, *Platycodon Grandiflorus* and *Cortex Mori*. *Aster Tataricus* relieves cough and asthma, clears away lung-heat, moistens intestines and relaxes bowels (Hou et al., 2006). *Cortex Mori* purges the lung of pathogenic fire, relieves the symptoms of asthma and induces diuresis to alleviate oedema (Feng et al., 2004). *Herba Houttuyniae* opens inhibited lung-energy,

stops coughing and clears away heat and toxic substances (Chen et al., 2014). *Glycyrrhiza uralensis* expels phlegm, stops coughing, relieves acute symptoms, removes toxic substances and coordinates the effects of various drugs in the prescription (Tian et al., 2006). Therefore, ZBT produces effects such as relief of cough and asthma, heat clearing and detoxification. In addition, as natural medicines, TCMs possess unique advantages, including anti-stress activity, improvement of nonspecific immunity and regulation of metabolism. Combined with these unique advantages, ZBT exhibits good clinical effectiveness in the treatment of bronchial asthma, phlegm-heat panting and cough.

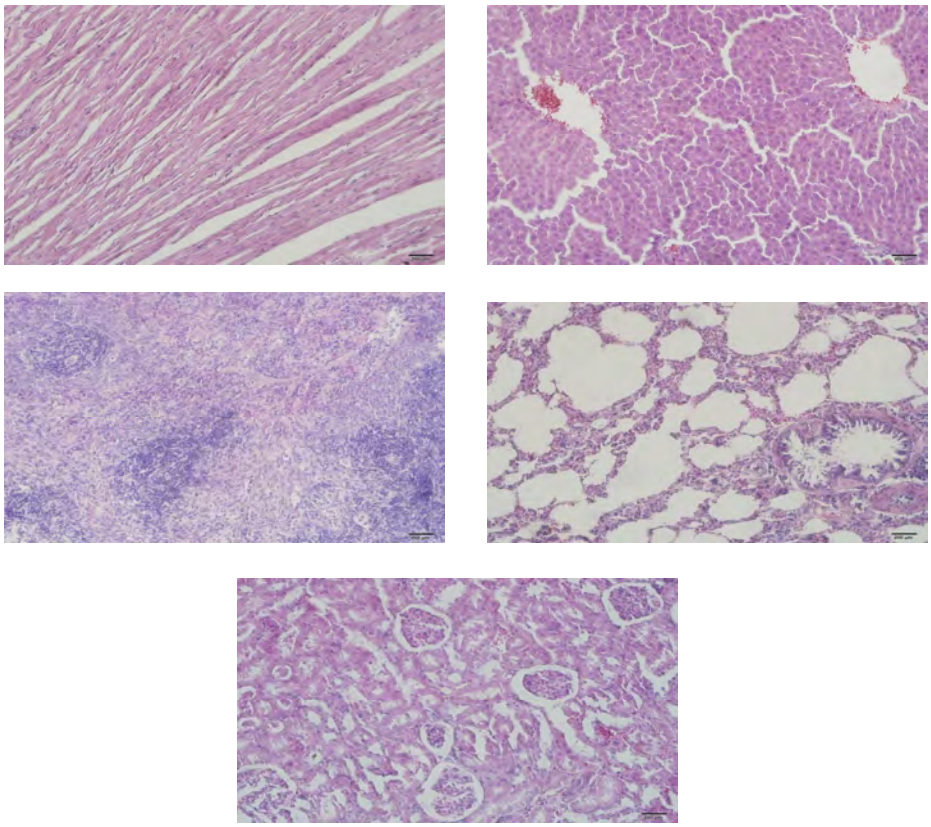

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Figure 1: Histopathological photomicrographs obtained at 4 weeks
Liver 200×, Heart 400×, Spleen 200××, Lung 200×, Kidney 200×

There were no significant differences in BW between rats in the control group and the groups exposed to different doses of ZBT during the experimental period ($P>0.05$). However, the BW of the rats in the various dose groups decreased in a dose-dependent manner, indicating that high doses of ZBT impact the appetite of rats. Increasing the dose of ZBT may have reduced food intake by the rats. Consequently, the control group exhibited greater weight gain, but the effect of ZBT on BW was not significant. Compared with the control group, female rats in the high-dose group exhibited a markedly reduced heart weight/BW coefficient ($P<0.05$). By contrast, no significant differences in the heart weight/BW coefficient were detected between the control group and the low- or moderate-dose group ($P>0.05$). The coefficient of organ weight/BW is a crucial detection index in the evaluation of drug safety and provides a quantitative reference for drug evaluation. However, it is impossible to identify changes in organs based on the coefficients alone (Yuan et al., 2003). Combining these coefficients with the results of a series of blood biochemical indices in the rats revealed no significant differences in AST and CK between the control group and various dose groups ($P>0.05$). Histopathological examination of heart tissue sections from the rats in the various dose groups revealed no abnormalities, indicating that ZBT had little effect on the hearts of female rats at doses of less than 30 g/kg BW.

The haematopoietic system can serve as an indicator of toxic effects because of its sensitivity to toxic compounds (Adeneve et

al., 2006). Compared to the control group, the groups that received different doses of ZBT exhibited varying decreases in WBC levels in the blood. Among the ingredients of ZBT, the medicinal herb *Aster tataricus* contains shionone, a type of flavonoid. Shionone promotes S-phase (the phase of DNA synthesis) entry in mouse Kupffer cells and significantly increases the number of cells in the mitotic phase, indicating that shionone is capable of promoting the proliferation of liver Kupffer cells and activating the function of Kupffer cells. In addition, shionone significantly promotes the phagocytic activity of lung macrophages and peritoneal macrophages (Li et al., 1991). In the present study, no gross abnormalities were detected in various organs and tissues by histopathological examination. Therefore, ZBT does not appear to affect WBCs in the blood of rats. During the experimental period, blood PLT counts were significantly increased in both female rats and male rats exposed to high doses of ZBT compared with the control group. This result is consistent with findings by Chen et al. that adrenocortical hormones stimulate the haematopoietic activity of bone marrow and may increase the number of platelets (Chen et al., 2003). Compared to the control group, the male rats in the various dose groups exhibited significantly decreased blood RBC and HGB levels (P>0.05). One of the components of ZBT, *Glycyrrhiza uralensis*, contains enoxolone. The adrenocortical hormone-like activity of enoxolone likely initiates negative feedback regulation *in vivo*, thereby inhibiting the production of RBC and HGB. The specific mechanism remains to be further investigated. The results of the present study indicate that ZBT had little effect on the WBC, RBC, HGB and PLT levels in the blood of rats.

Most drugs cause liver damage during transformation in the liver. The extent of damage is related to the dosage and concentration of the drugs. Liver damage often leads to increased ALT and AST activity and elevated ALB content (Wang et al., 2010). However, because the increases in AST and ALT were not necessarily parallel and transaminase activity only reflects liver disease activity, the AST/ALT ratio is used as an index to evaluate liver health. The AST/ALT ratio is a highly sensitive index and accurately reflects the degree of liver damage and prognosis (Wang et al., 2001). Urea and creatinine are two indices commonly used to evaluate renal function. Under normal conditions, the production and excretion of urea and creatinine are in a state of dynamic equilibrium. However, when the kidney undergoes pathological changes, serum urea and creatinine levels significantly change. AST and CK are abundantly expressed in the myocardium. Under normal conditions, the activities of AST and CK are very low in the serum. When the myocardial tissue is injured, cell permeability increases. Large amounts of AST and CK enter the blood, resulting in significantly increased AST and CK activities. Serum AST and CK measurements exhibit high sensitivity and specificity and are recognized as the "gold standard" for the detection of cardiomyocyte damage (Meng et al., 2005).

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After administration of ZBT for 4 weeks, serum ALB levels did not significantly differ between the control group and female rats in the low-dose group or moderate-dose group (P>0.05). By contrast, serum ALB levels were significantly reduced in the high-dose group compared with the control group (P<0.05). However, there were no significant differences in the AST/ALT ratio between the control group and the various dose groups (P>0.05). These results indicate that ZBT did not cause liver damage in the female rats. Urea levels in the female and male rats that received various doses of ZBT did not significantly differ from those in the control group (P>0.05). In addition, creatinine levels did not significantly differ between the control group and the various dose groups (P>0.05). These results indicate that 4 weeks of continuous administration of ZBT had little effect on the kidney in rats. Compared with the control group, the high-dose group exhibited markedly reduced serum AST levels (P<0.05). However, CK content did not significantly differ among the groups (P>0.05). The combined experimental results indicate that ZBT did not induce myocardial injury in the female rats. Serum ALB levels indicated that compared to the control group, 4 weeks of continuous administration of ZBT did not cause liver damage in rats. CK levels were drastically increased in the moderate-dose group compared

to the control group (P<0.05). However, there were no significant differences in serum AST activity between the control group and the low-, moderate- or high-dose groups (P>0.05). The above results suggest that ZBT had little effect on the rat myocardium.

Conclusion

The results of the present study demonstrate that the oral administration of ZBT has no significant impact on rat organs. After 4 weeks of continuous administration, the high-dose group (30 g/kg BW) was exposed to ZBT at a level far exceeding the recommended dosage in clinical applications. Therefore, ZBT should be considered an effective and highly safe Chinese herb medicine suitable for long-term clinical application.

Conflict of Interests: The authors of this paper declare no conflict of interest.

Authors' Contributions: Rui-Hua Xin and Wen-Jing Peng equally contributed to this work.

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References

1. Adeneye, A. A., Ajagbonna, O. P., Adeleke, T. I., Bello, S. O., (2006). Preliminary toxicity and phytochemical studies of the stem bark aqueous extract of *Musanga cecropioides* in rats. Journal Of Ethnopharmacology. 105, 374-379.
2. Ahmad, I., Aqil, F., Ahmad, F., Owais, M., (2006). Herbal medicines: prospects and constraints in Modern Phytomedicine: Turning Medicinal Plants Into Drugs. Wiley-Vch.
3. Shirwaikar., R. Verma., R. Lobo., and A. Shirwaikar., (2009). Phytotherapy-safety aspects. Natural Product Radiance 8, 55–63.
4. Chen, Jing., Jianguo, Fang., Chunyang, Shi., (2014). The research of anti-inflammatory pharmacological action mechanism of *Herba houttuyniae*. Chinese Traditional and Herbal Drugs. 2, 321-328.
5. Chen, Xueqian., Jin Youyu. (2003). New materia medica [M]. People's medical publishing house. 565.
6. Cheng, Long., Ruihua, Xin., (2014). The study of expectorant, cough and asthma action of Ziwan Baibu Tang. China Animal Husbandry & Veterinary Medicine. 5, 46-49.
7. Chou, P.B., Morse, C.A. and Xu, H. (2008). A controlled trial of Chinese herbal medicine for premenstrual syndrome. J. Psychosom. Obst. Gyn., 29, 185–192.
8. Committee for the Pharmacopoeia of P.R. China, (2010). Pharmacopoeia of P.R. China, Part I. China Medical Science and Technology Press, P.R. China.
9. Feng, Binghong., Yuhong, Zhao., Jianhua, Huang., (2004). The active components of *Cortex mori* and pharmacological research. ZHONGYAOCAI. 27, 140-150.
10. Hou, Haiyan., Li, Chen., and Junxing, Dong., (2006). Phytochemistry and pharmacology of an important Chinese medicine: *Aster tataricus*. Chinese Pharmaceutical Journal. 3, 163-172.
11. Jiao, Z.L., (2004). Exploitation and research of traditional Chinese medicine compound prescriptions. Shaanxi J. Tradit. Chin. Med. 25, 357-359.
12. Ju, Jianhua., Liang, Zhou., Geng, Lin., Dong, Liu., Liwei, Wang., Junshan Wang., (2003). Anti-inflammatory and antitussive activity research of Terpene acids composition in *Folium Eriobotryae*. Chinese Pharmaceutical Journal. 10, 752-753.
13. Lee, M. Y., Shin, I. S., Seo, C. S., Kim, J. H., Han, S. R., Shin, H. K., (2012). Subchronic oral toxicity studies of the traditional herbal formula Bangpungtongseong-san in Crl: CD, (SD) rats. Journal of Ethnopharmacology, 144, 720-725.
14. Li, Chunhua., Yan, Xiuying., (1991). The influence of the Asters ketone on liver and kidney cell hyperplasia in mice. Journal of

Shanxi medical university. 22, 88-90.

15. Li, T., and Peng, T. (2013). Traditional Chinese herbal medicine as a source of molecules with antiviral activity. Antivir. Res. 97, 1-9.
16. Meng, Fufen., Zhang, Jianlong., Ma, Qi., (2005). Dynamic changes of heart function and cardiac enzymes in septic rats. Journal of Xinjiang Medical University. 28, 128-130.
17. Shi, X., Lu, X.G., Zhan, L.B., Qi, X., Liang, L.N., Hu, S.Y., Yan, Y., Zhao, S.Y., Sui, H. and Zhang, F.L., (2011). The effects of the Chinese medicine ZiBu PiYin recipe on the hippocampus in a rat model of diabetes-associated cognitive decline: a proteomic analysis. Diabetologia, 54, 1888-1899.
18. Shin, I. S., Yu, Y. B., Seo, C. S., 2011. Subchronic toxicity of Sipjeondaebo-tang (SDT) in Sprague-Dawley rats. Regulatory Toxicology and Pharmacology. 59, 375-384.
19. Song, Yang., Yun, Qi., (2006). Pharmacological research progress of *Platycodon grandiflorus*. Chinese pharmacy. 2, 140-141.
20. Tian, Qinglai., Yueping, Guan., Bo, Zhang., Huizhou, Liu., (2006). Research progress of pharmacological effects of active ingredients in *glycyrrhiza*. Natural Product Research and Development. 2, 285-289.
21. Wang, Guibo., Xie, Jiasheng., Zheng, Jifang., (2010). The study of impact of Shegan Dilong Mahuang San on chicken liver and kidney function and pathological anatomy. Chinese animal husbandry and veterinary medicine. 37, 172-175.
22. Wang, Ying., Jia, Jie., (2001). Change and clinical significance of AST/ALT ratio in various liver diseases. China Tropical Medicine. 3, 222-223.
23. Xu, J., and Yang, Y., (2009). Traditional Chinese medicine in the Chinese health care system. Health Policy, 90, 133-139.
24. Yuan, Benli., (2003). The significance and shortcoming of organ/body weight ratio used in drug safety evaluation. Chinese Journal of New Drugs. 12, 960-963.
25. Zhu, Jianyu., Huifen, Yan., (2010). The research progress of *Stemona japonica* alkaloids and its pharmacological effects. Journal of Shanghai Institute of Technology (Natural Science). 1, 12-18.