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Original Paper

Effect of Zhishenyuanhu Anticoagulant Formula on Hypercoagulability in Rats with Qi Stagnation and Blood Stasis and Arterial and Venous Thrombosis in Rats

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Abstract

Background: *Tumor patients present with hypercoagulable blood and are at risk of venous thromboembolism (VTE). We explored the effect of Zhishenyuanhu anticoagulant formula（ZSYH）on hypercoagulability in rats with qi stagnation and blood stasis and thrombosis in rats.*

Methods: *Part I. Epinephrine (0.6 mg/kg, hypodermic injection, h.i.) was administered every day for 7 days. Six groups were gavaged with saline, saline, rivaroxaban, low-dose, medium-dose, and high-dose ZSYH every day. We collected rats' daily body weight, food intake, clotting time (CT), coagulation and hemorheology results. Part II. Six groups of rats were given the same drugs as in part I for 7 days. Half of rats in each group were used to construct a common carotid artery thrombusis model, while the other half were used to construct an inferior vena cava thrombusis model.*

Results: *ZSYH caused decreases in FIB, shear rate 1, whole blood low shear relative index, and RE. The high dose of ZSYH caused decreases in wet and dry weight of arterial and venous thrombosis.*

Conclusion: *ZSYH is effective in treating hypercoagulability in rats with qi stagnation and blood stasis. The high dose of ZSYH prevent the development of arterial thrombosis and venous thrombosis.*

Keywords

Chinese medicine, hypercoagulability, thrombosis

1. Introduction

In 2020, there were 19.3 million new cancer patients worldwide and about 10 million cancer patients died. Malignant tumor patients often present with hypercoagulable blood and are at risk of venous thromboembolism (VTE). In fact, VTE has become the second leading cause of death in malignant tumor patients, seriously affecting their survival time. Traditional Chinese medicine (TCM) also believes that the blood of cancer patients is in a state of hypercoagulability, and their clinical manifestations are essentially similar to those of blood stasis in TCM. The basis for the formation of hypercoagulability in cancer patients is a deficiency of healthy qi. Deficiency and stasis are intertwined and promote each other.

Clinically, preventing VTE in tumor patients is more important than treating VTE. Currently, guidelines advocate for primary prevention of thrombosis in hospitalized cancer patients using low molecular heparin, which is effective in preventing VTE. However, the shortcomings of low molecular heparin include its high cost, inconvenient use of subcutaneous injections, and susceptibility to subcutaneous bleeding. Rivaroxaban be used in outpatients who start chemotherapy with Khorana score ≥ 2 and without high risk of bleeding or drug interactions. Rivaroxaban, a highly selective direct inhibitor of FXa, is inexpensive, easy to administer orally, and does not require monitoring of coagulation. Nevertheless, the shortcomings are that rivaroxaban decreases drug absorption in gastrointestinal tract, is easily to interactions with other drugs, and has a high risk of gastrointestinal bleeding.

TCM has been a treasure of our country for thousands of years. To address the shortcomings of low molecular heparin and rivaroxaban, we can fully explore the efficacy of Chinese medicine to develop oral drugs that can benefit cancer patients. The medicinal Leech (*Whitmania pigra Whitman*) exhibits various effects, such as anticoagulant and antitumor. Hirudin, its main active ingredient, is the most active and specific thrombin inhibitor now. Codonopsis pilosula is the dried root of *Codonopsis pilosula (Franch.) Nannf.*, *Codonopsis pilosula Nannf. var. modesta (Nannf.) L.T. Shen*, or *Codonopsis tangshenOliv.* in the family of Platycodonaceae. It exhibits various effects, such as improving patients'immune function and adjusting gastrointestinal motor function. Corydalis yanhusuo (*Corydalis Yanhusuo W. T. Wang*) is the dried tuber of corydalis, a plant from the *genus Corydalis* in the poppy family (*Papaveraceae*), with many effects such as antithrombotic, antitumor, and antiulcer. According to TCM principle of monarch, minister, assistant and guide, leech serves as the monarch drug, while Codonopsis pilosula and Yanhuosuo act as assistant and guide drugs, forming Zhishenyuanhu anticoagulant formula (ZSYH). ZSYH has the effects of anticoagulation, benefiting qi, strengthening spleen and stomach, and antitumor. Consequently, we conducted experiments to dassess effect of ZSYH on hypercoagulability in rats with qi stagnation and blood stasis, as well as arterial and venous thrombosis in rats.

2. Method

2.1 Animals and Ethical Approval

Male and female specific pathogen free Sprague-Dawley rats of clean grade (180-220 g) were obtained from Beijing HFK Bioscience Co., Ltd. [certificate SCXK (JING)2019-0008]. Rats were housed in a laboratory animal house of Southwest Medical University at (22±2)°C with free access to water and food for 7 days. All experiments were approved by the Committee on the Ethics of Animal Experiments of the Southwest Medical University [animal ethics approval number: 2070498] and conformed to the National Institutes of Health guidelines (NIH publication No. 86-23, revised 1985) and ARRIVE guidelines.

2.2 Preparation of TCM

The herbs of ZSYH consist of Leech (Scalded Leech, lot: 220628-1), Codonopsis Pilosula (Codonopsis root, lot:230401141), and Corydalis Yanhusuo (Vinegar Roasted Yanhusuo, lot:202306033), which were provided by the Affiliated Hospital of Southwest Medical University. Leech, Codonopsis Pilosula, and Corydalis Yanhusuo were mixed with powder in a weight ratio of 2:1:1 to produce ZSYH, which was then suspended in saline and administered to rats via gavage. Using the adult dose as a medium dose and converting it based on the conversion coefficient of body surface area between human and rats, ZSYH low-dose, ZSYH medium-dose, and ZSYH high-dose group were gavaged 1.3 g/kg, 2.6 g/kg, and 5.2 g/kg of ZSYH, for 7 d, once a day.

2.3 Reagents and Instruments

1% Adrenaline Hydrochloride Injection (Batch No. H42021700) was purchased from Grand Pharma (China) Co., Ltd (Wuhan, China). Rivaroxaban (Batch No.20203738) was purchased from Shanghai Huilun Pharmaceutical Co., Ltd. (Shanghai, China). 10% Chloral hydrate (Batch No. D09HR14324A) was purchased from Shanghai yuanye Bio-Technology Co., Ltd. (Shanghai, China). 30% Fecl3 solution (Batch No. 0321A23) was purchased from Beijing Leagene Bio-Technology Co., Ltd. (Beijing, China). Isoflurane (Batch No. 151987015) was purchased from Shandong Ante Herd Sci-Technology Co., Ltd. (Shandong, China). Prothrombin Time (PT) Assay Kit (Batch No. C202526), Activated Partial Thromboplastin Time (APTT) Assay Kit (Batch No. C2192705), Thrombin Time (TT) Assay Kit (Batch No. C222356), and Fibrinogen (FIB) Assay Kit (Batch No. C235218) were purchased from Shanghai Sun Bio-Technology Co. Ltd. The Sysmex XS-500i Series Hematology Analyzer Supporting Reagents [Diluent (Batch No. G3015), Staining Solution (Batch No. A3003), Hemolysin 4DL (Batch No. R3006), Hemolysin (Batch No. A2006)] were purchased from Sysmex Medical Electronics (Shanghai) Co., Ltd. (Shanghai, China). Sodium Heparin Anticoagulation Tubes (Batch No. 20230305), 3.2% Sodium Citrate Tubes (Batch No. 20230107), EDTA-K2 Tubes (Batch No. 20230302) were purchased from Jiangsu Yuli Medical Instrument Co., Ltd. (Jiangsu, China). Automatic Blood Analyzer

(model: XS-500i) was purchased from Sysmex Medical Electronics (Shanghai) Co., Ltd. (Shanghai, China). Automatic Coagulation Analyzer (Model: CP3000) was purchased from Sekisui Medical Co., Ltd. (Japan). Automatic Blood Rheology Tester (Model: SA-9000) was purchased from Beijing Succeeder Technology Co., Ltd. (Shanghai, China).

2.4 Effect of ZSYH on Hypercoagulability in Rats with Qi Stagnation and Blood Stasis

2.4.1 Establishment of qi stagnation and blood stasis model in rats

The model of qi stagnation and blood stasis was established by administering a subcutaneous injection of 1% epinephrine (0.6 mg/kg) into the back or neck of rats for 7 d.

2.4.2 Group

60 Sprague-Dawley rats, with an equal ratio of males to females,, were classified into control (C), qi stagnation and blood stasis model (QSBS), rivaroxaban (R), ZSYH low-dose (L), ZSYH medium-dose (M), and ZSYH high-dose group (H), each having 10 rats. The rest of the groups were injected subcutaneously with epinephrine 0.6 mg/kg for 7 d to establish the qi stagnation and blood stasis model, except for C. After one hour of daily epinephrine injection, QSBS, R, L, M, H were gavaged with saline, rivaroxaban (1.08 mg/kg), low-dose (1.3 g/kg), medium-dose (2.6 g/kg), and high-dose (5.2 g/kg) ZSYH respectively, and C was gavaged with the same amount of saline.

2.4.3 Body weight and daily food intake

Daily body weight and food intake of rats were recorded. The daily food intake of rats was calculated using the formula: daily food intake (g/100 g) = (input - residual)/ body weight × 100.[16]

2.4.4 Clotting time

One hour after the final administration, rats were placed on the operating table and trimmed 0.5 cm from the end of their tails. The first drop of blood was not discarded, and a 5mm drop was placed on a clean slide. Using clean needles, blood was picked up once every 15 seconds until fibrin filament could be detected, and the time was recorded. This time was noted as the clotting time (CT).

2.4.5 Coagulation and hemorheology

Rats in section 3.4 were anesthetized with 10% chloral hydrate, and blood was collected from the abdominal aorta for coagulation and hemorheology testing.

2.5 Effect of ZSYH on Arterial and Venous Thrombosis in Rats

2.5.1 Group

96 Sprague-Dawley rats, with an equal ratio of males to females,, were classified into sham operation (S), thrombosis model (T), rivaroxaban (RG), ZSYH low-dose (LG), ZSYH medium-dose (MG), and ZSYH high-dose group (HG), each having 16 rats. S, T, RG, LG, MG, and HG were gavaged with saline, saline, saline, rivaroxaban (1.08 mg/kg), low-dose (1.3 g/kg), medium-dose (2.6 g/kg), and high-dose (5.2 g/kg) for 7 d, respectively.

2.5.2 Effect of ZSYH on arterial thrombosis in rats

One hour after the final administration, half of rats in each group were anesthetized with 10% chloral hydrate and placed on the operating table. Left common carotid artery of each rat was bluntly dissected, and a plastic film (1.2 cm × 2.2 cm) was placed under it to isolate it from the surrounding tissues. In S group, a filter paper (0.7 cm x 1.0 cm) filled with saline was wrapped around the left common carotid artery. In the remaining groups, a filter paper (0.7 cm x 1.0 cm) filled with 30% FeCl3 solution was wrapped around the left common carotid artery and sealed with the plastic film. After 15 min, the filter paper was removed, the blood vessel was carefully cut, and thrombus was weighed to obtain its wet weight. Subsequently, it was placed in an oven at 70°C overnight and weighed to obtain dry weight. The thrombosis inhibition rate in each group was calculated using the formula: thrombosis inhibition rate (%) = [(measured value in model group - measured value in experimental group)/measured value in model group] × 100%.

2.5.3 Effect of ZSYH on venous thrombosis in rats

The other half of rats in each group were fasted for 12 hours before a surgery. One hour after the final administration, rats were anesthetized with isoflurane and placed on the operating table. The inferior vena cava and its branches were bluntly dissected through a median incision in the abdomen of each rat. The inferior vena cava ligation point was located at 5 mm below the left renal vein, which itself and its branches were ligated with a 5-0 surgical suture. After its abdominal wound was closed with a 2-0 surgical suture, each rat was supplemented with water and the environment temperature was maintained at 24°C. After 5 hours, each rat was anesthetized again. The inferior vena cava was cut, and thrombus was removed for weighing to obtain its wet weight. Then, it was placed in an oven at 70°C overnight and weighed to obtain dry weight. The thrombosis inhibition rate in each group was calculated using the formula: thrombosis inhibition rate (%) = [(measured value in model group - measured value in experimental group)/measured value in model group] × 100%.

2.6 Statistical Analysis

The data from this experiment were statistically analyzed using SPSS 26.0 (IBM Corp., Armonk, NY, USA) software. If normality and homogeneity of variance were satisfied, data were analyzed using One-way analysis of variance (ANOVA) with least significant difference (LSD) method for two-by-two comparisons between groups, and the results were expressed as mean ± standard deviation ($\bar{x}\pm s$). If the data were skew distribution or heterogeneity of variance, a nonparametric test was used, and the results were expressed as median (interquartile spacing) [M(Q1, Q3)]. $P < 0.05$ indicated statistical significance.

3. Result

3.1 Experimental Results on Effect of ZSYH on Hypercoagulability in Rats with Qi Stagnation and Blood Stasis

3.1.1 Effect of ZSYH on body weight and daily food intake in rats

As shown in Figures 1 and 2, body weight gain of female rats was observed at 7 days: C > M > L > QSBS > H > R. Body weight gain of male rats was observed: C > QSBS, H, and M > L > R. Generally, female and male rats in L, M, and H groups generally had similar daily food intake overall. C had the highest daily food intake, B had the second highest daily food intake, and R group had the lowest daily food intake.

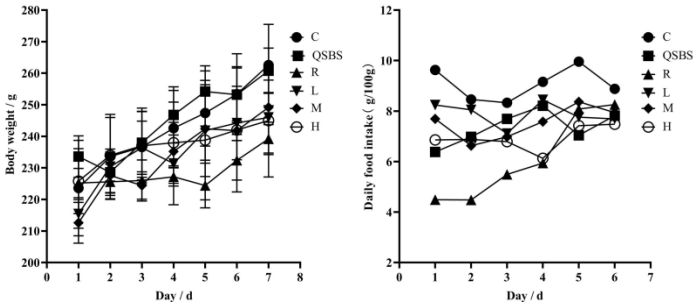

Figure 1. Comparison of Changes in Body Weight and Daily Food Intake of Female Rats of each Group ($\bar{x} \pm s$, n=5)

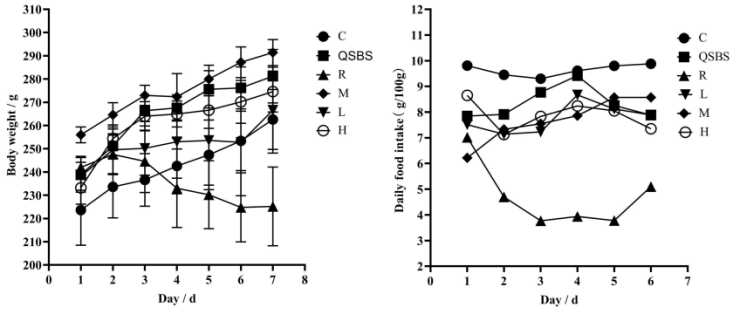

Figure 2. Comparison of Changes in Body Weight and Daily Food Intake of Male Rats of each Group ($\bar{x} \pm s$, n=5)

3.1.2 Effect of ZSYH on CT in rats

As shown d in Figure 3, compared with C, CT of QSBS had a significant increase ($p<0.05$). Compared with QSBS, there were significant increases in CT of R ($p<0.001$), M ($p<0.05$), and H ($p<0.001$). No significant differences were found in the two-by-two comparisons between R, M, and H.

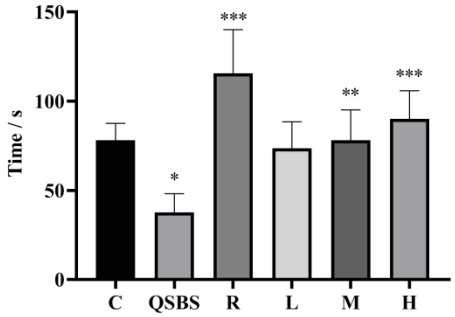

Figure 3. Comparison of Changes in CT of Rats

3.1.3 Effect of ZSYH on coagulation and hemorheology in rats

As shown in Table 1, compared with C, FIB of QSBS had a significant increase ($p<0.001$). Compared with QSBS, there were significant increases in PT ($p<0.05$) and APTT ($p<0.001$) of R, and FIB was significantly decreased ($p<0.001$). Compared with QSBS, there were significant decreases in FIB of L ($p<0.001$), M ($p<0.001$), and H ($p<0.001$), with no statistically significant differences among the three groups.

As shown in Tables 2 and 3, compared with C, there were significant increases in shear rate 1($p<0.001$), whole blood low shear rate (LS, $p<0.001$), plasma viscosity (PV, $p<0.001$), whole blood low shear relative index ($p<0.001$), and erythrocyte aggregation index (RE, $p<0.001$) of QSBS. Compared with QSBS group, there were significant decreases in shear rate 1($p<0.05$), whole blood low shear relative index ($p<0.01$), and RE ($p<0.05$) of R. There were significant decreases in shear rate 1($p<0.001$), whole blood low shear relative index ($p<0.01$), and RE ($p<0.001$) of L. There were significant decreases in shear rate 1($p<0.05$) and RE ($p<0.001$) of M. There were significant decreases in shear rate 1($p<0.05$), whole blood low shear relative index ($p<0.01$), and RE ($p<0.05$) of H.

Table 1. Comparison of Changes in PT, APTT, TT, and FIB in Rats from each Group

Group	N	PT (s) [a]	APTT (s) [a]	TT (s) [a]	FIB ($g \cdot L^{-1}$) [b]
C	10	11.85 (11.28 , 12.13)	22.90 (21.98 , 25.38)	28.55 (27.13 , 29.65)	1.89±0.26
QSBS	10	12.70 (12.15 , 13.75)	23.90 (19.78 , 24.83)	29.75 (28.75 , 30.20)	3.67±0.33*
R	10	17.60 (15.53 , 19.08) **	33.25 (29.08 , 39.48) ***	28.45 (28.00 , 29.03)	2.96±0.39***
L	10	11.90 (11.60 , 12.20)	25.80 (24.33 , 27.95)	29.45 (29.18 , 29.93)	2.97±0.24***
M	10	13.35 (12.13 , 15.95)	23.45 (22.15 , 25.48)	28.95 (28.58 , 29.20)	3.04±0.26***
H	10	15.30 (12.53 , 16.45)	24.50 (22.63 , 30.10)	28.55 (28.20 , 28.80)	2.84±0.26***

Table 2. Comparison of Changes in Shear Rate 1, LS, PV, and Whole Blood Low Shear Relative Index in Rats from each Group ($\bar{x}\pm s$)

Group	N	Shear rate 1 (mpa·s)	LS (mpa·s)	PV(mpa·s)	Whole blood low shear relative index
C	10	22.97±2.96	9.74±1.05	1.14±0.06	20.26±2.81
QSBS	10	33.80±3.42*	12.39±1.51*	1.20±0.05*	27.68±3.71*
R	10	28.91±4.86**	11.49±1.69	1.23±0.05	23.20±3.24***
L	10	28.09±3.48****	11.54±1.23	1.22±0.03	23.08±2.93***
M	10	28.62±2.54**	11.59±0.91	1.21±0.04	25.11±3.29
H	10	29.08±2.95****	11.76±1.06	1.21±0.03	24.06±2.19***

Table 3. Comparison of Changes in MS, HS, Whole Blood High Shear Relative Index, RF, and Casson Viscosity in Rats from each Group [M(Q1, Q3)]

Group	N	MS (mpa·s)	HS (mpa·s)	Whole blood high shear relative index	RF	Casson viscosity (mpa·s)
C	10	4.95(4.45,5.17)	4.04(3.65,4.25)	3.48(3.20,3.58)	5.84(5.57,6.13)	3.19(2.89,3.36)
QSBS	10	5.16(4.33,5.79)	3.90(3.23,4.51)	3.21(2.65,3.71)	8.92(8.36,10.13)*	2.81(2.30,3.32)
R	10	5.38(4.66,5.89)	4.28(3.76,4.66)	3.50(3.17,3.62)	6.82(6.36,7.03)**	3.28(2.89,3.56)
L	10	5.43(5.15,5.76)	4.34(4.15,4.63)	3.56(3.36,3.86)	6.38(5.94,6.65)***	3.34(3.27,3.61)
M	10	5.38(5.16,5.84)	4.29(4.12,4.67)	3.65(3.50,3.88)	6.58(6.41,6.94)***	3.32(3.17,3.61)
H	10	5.27(5.17,5.87)	4.20(4.12,4.66)	3.58(3.46,3.72)	6.70(6.51,6.84)**	3.32(3.18,3.59)

3.2 Experimental Results on Effect of ZSYH on Arterial and Venous Thrombosis in Rats

3.2.1 Effect of ZSYH on arterial thrombosis in rats

As shown in Table 4, compared with S, wet weight and dry weight of T had significant increases ($p<0.001$). Compared with T, there were significant decreases in wet weight and dry weight of HG ($p<0.01$), and thrombosis inhibition rates of wet weight and dry weight was the highest among all groups. There was a significant decrease in wet weight and dry weight of RG ($p<0.05$).

Table 4. Comparison of Changes in Wet and Dry Weight of Common Carotid Artery Thrombus in Rats from each Group [M(Q1,Q3)]

Group	N	Wet weight (mg)	Thrombosis inhibition rates of wet weight (%)	Dry weight (mg)	Thrombosis inhibition rates of dry weight (%)
S	8	0.00 (0.00 , 0.00)	/	0.00 (0.00 , 0.00)	/
T	8	4.20 (4.03 , 5.28) *	/	2.35 (2.23 , 3.23) *	/
RG	8	2.80 (2.30 , 2.98)	45.05	1.25 (1.03 , 1.38) **	54.76
LG	8	3.60 (2.43 , 4.55)	23.08	1.90 (1.28 , 2.25)	30.95
MG	8	2.80 (2.55 , 3.28)	36.81	1.65 (1.33 , 2.05)	36.67
HG	8	2.10 (1.63 , 2.60) ***	53.57	1.00 (0.48 , 1.30) ***	62.38

3.2.2 Effect of ZSYH on venous thrombosis in rats

As shown in Table 5, compared with S, wet weight and dry weight of T had significant increases ($p<0.001$). Compared with T, there were significant decreases in wet weight ($p<0.05$) and dry weight ($p<0.01$) of HG, and thrombosis inhibition rates of wet weight and dry weight was the highest among all groups. There was a significant decrease in wet weight and dry weight of RG ($p<0.05$).

Table 5. Comparison of Changes in Wet and Dry Weight of Inferior Vena Cava Thrombus in Rats from each Ggroup [M(Q1,Q3)]

Group	n	Wet weight (mg)	Thrombosis inhibition rates of wet weight (%)	Dry weight (mg)	Thrombosis inhibition rates of dry weight (%)
S	8	0.00 (0.00 , 0.00)	/	0.00 (0.00 , 0.00)	/
T	8	42.00 (33.98 , 48.65) *	/	17.40 (15.55 , 19.43) *	/
RG	8	5.85 (1.03 , 16.15) **	80.31	3.30 (0.40 , 7.68) **	76.95
LG	8	33.70 (17.88 , 66.70)	2.02	16.05 (9.68 , 21.45)	6.57
MG	8	16.60 (12.88 , 34.13)	46.72	6.00 (4.88 , 10.28)	53.53
HG	8	6.80 (0.00,8.33) **	85.75	2.30 (0.00,2.80) ***	87.87

4. Discussion

Malignant tumor patients often present with hypercoagulable blood and are at risk of VTE. VTE is one of the most common complications, and can lead to bleeding, recurrence of the thrombus, and even death in tumor patients. The incidence of VTE is 4 % to 20 % in cancer patients and 4 times to 7 times higher than in patients without tumors, which seriously affects survival time of cancer patients. Presently, preventive treatment with low molecular heparin and rivaroxaban are widely used. Low molecular heparin has anticoagulant and antithrombotic effects by inhibiting FXa activity, which

prevents FIIa activation. It has the advantages of high bioavailability, a long half-life, and no need for monitoring coagulation. However, it has the disadvantages of high prices, inconvenient use of subcutaneous injections, and the risk of subcutaneous bleeding. Rivaroxaban has an anticoagulant effect by directly and selectively inhibiting the FXa, which inhibits the production of thrombin. It has the advantages of high bioavailability, low price, prompt onset of action, and no need for monitoring coagulation parameters. Nevertheless, it has the disadvantages of being easily interacted with other drugs, affecting absorption in gastrointestinal tract, and bleeding in the gastrointestinal tract.

Hirudin in leeches has an anticoagulant effect by binding to an active site of thrombin and a fibrinogen binding site on thrombin to inhibit thrombin activity and the process of fibrin formation and polymerization. Additionally, hirudin has an antitumor effect by downregulating signaling pathways to inhibit tumor cell proliferation, migration, and invasion while promoting apoptosis. Codonopsis pilosula enhances immunity by promoting lymphocyte proliferation and homing, T-cell activation, and secretion of TNF-a, NO, IL-2, IL-6, etc. Meanwhile, it regulates gastrointestinal motility by decreasing gastrin levels, increasing prostaglandins levels, and regulating intestinal microflora. Corydalis yanhusuo can protect gastric mucosa by increasing blood flow to it and inhibiting the decrease of dopamine transmitters. Anti-tumor effects are achieved by inhibiting cell proliferation, and anti-thrombotic effects are exerted by inhibiting apoptosis of vascular endothelial cells and expression of tissue factor. Therefore, ZSYH consists of leeches, codonopsis pilosulaes, and corydalis yanhusuos, which has the effects of anticoagulation, benefiting qi, strengthening spleen and stomach, and antitumor.

Complex mechanisms of hypercoagulability in cancer patients and various models for blood stasis syndrome modeling have been extensively researched. At present, epinephrine method is widely used to model qi stagnation and blood stasis, assessing the success of the model through general behavior, coagulation function, hemorheology, and other indicators. It was discovered in the experiment that rats in QSBS showed significant decreases in the activity, daily food intake, and CT, while FIB, shear rate 1, LS, PV, whole blood low shear relative index, and RE were significantly increased compared with C. These results indicated that the model of qi stagnation and blood stasis was successful in modeling. Compared to other groups, rats in R had the lowest body weight gain and lowest daily food intake overall, which indicated that rivaroxaban had a negative impact on the gastrointestinal function of rats, whereas ZSYH had a lesser affect on gastrointestinal function than rivaroxaban. Compared with QSBS, rats in L, M, and H showed significant increases in CT and significant decreases in FIB, shear rate 1, whole blood low shear relative index, and RE. These findings indicate that ZSYH can inhibit hypercoagulability and the dose changes have little impact on coagulation and hemorheology. It has been reported that dose threshold for leeches in humans of blood- activating drugs is 1g to 30 g, with a common dose range of 3g to 10 g, indicating a large range of effective doses. The leech doses used in

this experiment were within the dose threshold, which may explain the lack of statistically significant differences in coagulation and hemorheology results between L, M, and H.

Currently, it is believed that formations of arterial thrombosis and venous thrombosis are interconnected, with platelets, inflammation, and other factors playing a significant role in the process of thrombosis.[29] The principle of arterial thrombosis caused by Fecl3 may result from formation of hydroxyl radicals by Fe3+, leading to vascular endothelial damage, accompanied by a decrease in fibrinolytic activity of blood. The process of thrombosis caused by Fecl3 is similar to human thrombophilic disease pathogenesis and is commonly used as a model for studying anticoagulant drugs due to its simplicity and high modeling rate. In the experiment, there was no thrombosis observed in S, while thrombosis was observed in T, indicating the modeling was successful. Compared withT, rivaroxaban had no effect on reducing arterial thrombosis. Its blood concentration varies greatly among individuals and is influenced by factors such as age, liver and kidney function, illness state, and dosage. Additionally, rivaroxaban is often used to prevent systemic venous embolism. These may be why it does not prevent arterial thrombosis. The experiment shows that low and medium dose ZSYH have no effect on arterial thrombosis, while high dose ZSYH has an inhibitory effect on arterial thrombosis. The results of this experiment indicate that high-dose ZSYH can prevent arterial thrombosis. Inferior vena cava ligation is also a common and simple method of modeling venous thrombosis, frequently used in studies of antithrombotic drugs. In the experiment, there was no thrombosis observed in S, while thrombosis was observed in T, indicating that the modeling was successful. The experiment shows that low and medium dose ZSYH have no effect on venous thrombosis, while rivaroxaban and high dose ZSYH have inhibitory effects on venous thrombosis. And rivaroxaban and high dose ZSYH have the same effect in preventing venous thrombosis. The results of this experiment indicate that high-dose ZSYH can prevent venous thrombosis. Meanwhile, the main shortcomings are that we do not purify active ingredients of ZSYH and we have not researched the antithrombotic and anticoagulant mechanisms of ZSYH. We will continue to explore these questions experimentally in the future.

Conclusion

Research results show that ZSYH can be effective in treating hypercoagulability in rats with qi stagnation and blood stasis, and a high dose of ZSYH can prevent the development of arterial thrombosis and venous thrombosis.

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Credit Authorship Contribution Statement

Xingting He: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing—Original Draft. Xuemei Deng: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing—Original Draft. Shuang Chen: Conceptualization, Methodology, Validation, Investigation, Writing - Review &Editing. Kewei Xiang: Validation, Formal analysis, Investigation, Writing—Review &Editing. Qinglian Wen: Methodology, Writing—Review &Editing. Runlan Wan: Methodology, Writing—Review & Editing. Fujiang Xu: Methodology, Writing—Review &Editing. Hongru Yang: Conceptualization, Methodology, Validation, Writing—Review &Editing, Supervision, Project administration.

Conflict of Interests

The authors declare that there are no conflicts of interest.

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Ethical Statement

All experiments were approved by the Committee on the Ethics of Animal Experiments of the Southwest Medical University [animal ethics approval number: 2070498] and conformed to the National Institutes of Health guidelines (NIH publication No. 86–23, revised 1985) and ARRIVE guidelines.

Data Availability Satement

The original data presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

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Note(s)

Figure 1. notes: C: Control group (saline, 7d); QSBS: Qi stagnation and blood stasis model group (saline, 7d); R: Rivaroxaban group (1.08 mg/kg, 7d); L: ZSYH low-dose group (1.3 g/kg, 7d); M: ZSYH medium-dose (2.6 g/kg, 7d); H: ZSYH high-dose group (5.2 g/kg, 7d).

Figure 2. notes: C: Control group (saline, 7d); QSBS: Qi stagnation and blood stasis model group (saline, 7d); R: Rivaroxaban group (1.08 mg/kg, 7d); L: ZSYH low-dose group (1.3 g/kg, 7d); M: ZSYH medium-dose (2.6 g/kg, 7d); H: ZSYH high-dose group (5.2 g/kg, 7d).

Figure 3. notes: CT: Clotting time; C: Control group (saline, 7d); QSBS: Qi stagnation and blood stasis model group (saline, 7d); R: Rivaroxaban group (1.08 mg/kg, 7d); L: ZSYH low-dose group (1.3 g/kg, 7d); M: ZSYH medium-dose (2.6 g/kg, 7d); H: ZSYH high-dose group (5.2 g/kg, 7d). $^{*}p<0.05$vs C; $^{**}p<0.05$, $^{***}p<0.001$vs QSBS.

Table 1. notes: C: Control group (saline, 7d); QSBS: Qi stagnation and blood stasis model group (saline, 7d); R: Rivaroxaban group (1.08 mg/kg, 7d); L: ZSYH low-dose group (1.3 g/kg, 7d); M: ZSYH medium-dose (2.6 g/kg, 7d); H: ZSYH high-dose group (5.2 g/kg, 7d); PT: Prothrombin time; APTT: Activated partial thromboplastin time; TT: Thrombin time; FIB: Fibrinogen. [a]: A nonparametric test was used, and the results were expressed as median (interquartile spacing) [M(Q1, Q3)]; [b]: Data

were analyzed using One-way analysis of variance (ANOVA), and the results were expressed as mean ± standard deviation ($\bar{x}$±s). $^{*}p<0.001$vs C; $^{**}p<0.05$, $^{***}p<0.001$vs QSBS.

Table 2. notes: C: Control group (saline, 7d); QSBS: Qi stagnation and blood stasis model group (saline, 7d); R: Rivaroxaban group (1.08 mg/kg, 7d); L: ZSYH low-dose group (1.3 g/kg, 7d); M: ZSYH medium-dose (2.6 g/kg, 7d); H: ZSYH high-dose group (5.2 g/kg, 7d); LS: Whole blood low shear rate; PV: Plasma viscosity. $^{*}p<0.001$vs C; $^{**}p<0.05$, $^{***}p<0.01$, $^{****}p<0.001$vs QSBS.

Table 3. notes: C: Control group (saline, 7d); QSBS: Qi stagnation and blood stasis model group (saline, 7d); R: Rivaroxaban group (1.08 mg/kg, 7d); L: ZSYH low-dose group (1.3 g/kg, 7d); M: ZSYH medium-dose (2.6 g/kg, 7d); H: ZSYH high-dose group (5.2 g/kg, 7d); MS: Whole blood middle shear rate; HS: Whole blood high shear rate; RF: Erythrocyte aggregation index. $^{*}p<0.001$vs C; $^{**}p<0.05$, $^{***}p<0.001$vs QSBS.

Table 4. notes:S: Sham operation group (saline, 7d); T: Thrombosis model group (saline, 7d); RG: Rivaroxaban group (1.08 mg/kg, 7d); LG: ZSYH low-dose group (1.3 g/kg, 7d); MG: ZSYH medium-dose (2.6 g/kg, 7d); HG: ZSYH high-dose group (5.2 g/kg, 7d); $^{*}p<0.001$vs C; $^{**}p<0.05$, $^{***}p<0.01$vs T.

Table 5. notes:S: Sham operation group (saline, 7d); T: Thrombosis model group (saline, 7d); RG: Rivaroxaban group (1.08 mg/kg, 7d); LG: ZSYH low-dose group (1.3 g/kg, 7d); MG: ZSYH medium-dose (2.6 g/kg, 7d); HG: ZSYH high-dose group (5.2 g/kg, 7d); $^{*}p<0.001$vs C; $^{**}p<0.05$, $^{***}p<0.01$vs T.

Abbreviations

VTE	venous thromboembolism
ZSYH	Zhishenyuanhu anticoagulant formula
CT	clotting time
TCM	Traditional Chinese medicine
APTT	Activated Partial Thromboplastin Time
TT	Thrombin Time
PT	Prothrombin Time
FIB	Fibrinogen
C	Control group
QSBS	Qi stagnation and blood stasis model group
R	Rivaroxaban group
L	ZSYH low-dose group
M	ZSYH medium-dose group
H	ZSYH high-dose group

S	Sham operation group
T	thrombosis model group
RG	Rivaroxaban group
LG	ZSYH low-dose group
MG	ZSYH medium-dose group
HG	ZSYH high-dose group