

Study on the antithrombotic activity of *Umbilicaria esculenta* polysaccharide



Yufeng Wang^{a,*}, Jiangjuan Shao^b, Shulin Yao^a, Shengsheng Zhang^a, Jingfan Yan^a, Huiqin Wang^a, Yangyang Chen^a

^a College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, China

^b College of Pharmacy, Nanjing University of Traditional Chinese Medicine, Nanjing 210046, China

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ABSTRACT

Umbilicaria esculenta as a traditional food is known to have many pharmacological activities, such as cholesterol synthesis inhibition, anti-inflammation and anti-tumor. The antithrombotic activities of UEP isolated from the lichen were examined in vitro and in vivo for the first time. The in vitro anticoagulant activity of UEP was tested by its PT, APTT and TT. The more prolongation of APTT suggested a more obvious inhibition of the intrinsic coagulation systems than the extrinsic. Its antithrombotic properties were evaluated using an arteriovenous shunt thrombosis model in rats, and its inhibition of thrombus formation increased in a dose-dependent manner. It also caused a dose-dependent increase in tail transection bleeding time. Oral administration of UEP also showed a significant dose dependent preventive effect against thrombotic death or paralysis. UEP has a potent antithrombotic effect in vitro and in vivo, which may be used as a novel, effective and promising antithrombotic agent.

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1. Introduction

Thrombosis diseases are primary causes of death, especially cardiovascular disease and stroke, and their incidences have been increasing annually around the world (Juliana, Elaine, Philip, & Marcello, 2005; Li et al., 2010). Thrombosis, associated with blood coagulation and endothelial lesions, is the main source of thromboembolic complications (Fan et al., 2010; Jin et al., 2004; Juliana et al., 2005; Martinichen, Carbonero, Sasaki, Gorin, & Iacomini, 2005). Thrombus formation is the key factor, which is widely accepted that it determines the pathogenesis of cardiovascular or cerebrovascular disorders (Kang, Lim, & Yuk, 1999; Li et al., 2010). The search for antithrombotic agents is urgent.

Thrombus is mainly composed of platelet aggregate, which is a primary pathogenic mechanism of thrombosis. Platelets play a key role at the site of damaged blood vessels. Adhesive ligands will generated at the injury site, and promote platelets activation and coagulation cascades (Han et al., 2012; Li, Ji, Cheng, Li, & Ng, 2002). This thrombus is the source of thromboembolic material, so regulation of it may be an approach for the disease, such as antithrombotic therapy.

A number of drugs with antithrombotic effects, have been developed for preventing thrombosis. Some platelet inhibitors, such as

aspirin, heparin and ticlopidine, have been proven to be effective. Heparin has been widely used in the therapy (Li et al., 2011; Rana, Zeynep, Nurcan, Ali, & Yusuf, 2011; Sa, Kim, & Choi, 2011). In fact, these inhibitors such as aspirin are only partially effective in the prevention of thrombus formation. They also have side effects including internal bleeding, prolonged bleeding time, and palpitation gastrointestinal symptoms and hemorrhage, so alternative drugs for these are in high demand (Li et al., 2011; Rana et al., 2011; Sa et al., 2011).

As an alternative source, antithrombotic agent isolated from traditional Chinese medicine or food with much safety has attracted attentions. Lichens are symbiotic microorganisms of algae and fungi, with 13,500 distinct species. Among them, only about 100 lichens have been researched (Bargagli, Sanchez, & Monaci, 1999; Branislav, Marijana, & Slobodan, 2008; Carbonero et al., 2006a, 2006b; Moosung & Kyungae, 2006), and *Umbilicaria esculenta* has been used as a traditional food in China. *U. esculenta* has been used to treat inflammation and bleeding, and it proved to have inhibitory activities of cholesterol synthesis, antitumor and glycosidase (Lee & Kim, 2000; Muller, 2001; Tomas, Jitka, & Valery, 2003). Polysaccharides isolated from *U. esculenta* (UEP, *U. esculenta* polysaccharide) have several components. Among them, three main polysaccharides have been characterized. Two of them were (1/3)- and (1/6)-linked β -glucans, namely laminaran and pustulan, respectively. The former consisted of a main chain of (1/6)-linked α -mannopyranosyl residues, part of them being substituted at O-2, O-4, and O-2,4 by complex, branched side chains

* Corresponding author. Tel.: +86 25 84396583; fax: +86 25 84396583.

E-mail addresses: joywangyu@sina.com, yufengwang@sohu.com (Y. Wang).

containing α -mannopyranosyl and β -galactofuranosyl units. The other was a galactofuranomannan, and mainly a D-galacto-D-glucod-mannoglycan ($-4\text{Man}\beta 1-4\text{Glc}\beta 1-4(\text{Gal}\alpha 1-6)\text{Man}\beta 1-4\text{Man}\beta 1-$), which was analyzed as well as a galactose-free polysaccharide formed on partial hydrolysis (Carbonero et al., 2006b; Karunaratne, 2012). UEP has antimicrobial activities against many microorganisms. Moreover, UEP can boost human immunity, eliminate oxygen free radicals and inhibit lipid peroxidation. Furthermore, it can also reduce the occurrence of cardiovascular event, which was closely related with the incidence of thrombosis (Lee & Kim, 2000; Muller, 2001; Tomas et al., 2003).

Up to now, its active constituents and antithrombotic activity have not been investigated in depth. In our previous study, a new method for separating its UEP was developed with AB-8 macroporous resins. Based on our preliminary study of UEP, this objective was to investigate its antithrombotic activity and provide evidences for clinical applications. Therefore, we tested its effects on rat thrombus formation *in vivo* and *in vitro*. In addition, its *in vivo* antithrombotic effects on arteriovenous shunt thrombosis model in rats were also examined. This work would contribute to evaluate whether *U. esculenta* could be used as a functional food or medicine ingredient.

2. Materials and methods

2.1. Chemicals and reagents

The AB-8 resin was supplied by Anhui Sanxin Resin Technology Co., Ltd. (Anhui Province, China). Deionized water was used throughout and purified by a Mill-Q water-purification system from Milipore (Bedford, MA, USA). All other chemicals and reagents were of analytical grade and obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), unless otherwise stated.

2.2. Collection of *umbilicaria esculenta*

U. esculenta were obtained at July 2010 from Huangshan Mountain (30°N , 1864 m altitude) in Anhui Province, China. They were identified according to their morphological characteristics (Lee & Kim, 2000; Muller, 2001; Tomas et al., 2003).

2.3. Preparation of UEP

U. esculenta (100 g) were cleaned and lyophilized (a Model 2K-XL Lyophilizer, Virtis corporation, American), and ground with a Model ZN-100 Grinder (Zhongnan Pharmaceutical Machinery Factory, Shanghai, China) to obtain their powders. The powders were successively extracted with deionized water (800 ml) at 100°C for 3 h, and the residue was isolated. Each aqueous extract was added to excess absolute ethyl alcohol (ratio of 3:1, v/v) to form a precipitate. The precipitate was isolated by a Model 5804R centrifugation ($4680 \times g$ for 20 min, at 25°C , Eppendorf corporation, Germany) and dried or lyophilized.

Then it was further purified at room temperature in a glass column (Φ 26 mm $\times$ 300 mm) wet-packed with the AB-8 resin. For adsorption, the concentration, processing volume and flow rate of the sample were 2.0 mg/ml, 3.2 BV and 1.5 BV/h, respectively; the pH of 7 and temperature of 25°C were also suitable. For desorption, the AB-8 resin column was firstly washed by deionized water with a flow rate of 1 BV/h and then ethanol solution (ethanol: water of 50:50, v/v) of 4.4 BV with a flow rate of 2.0 BV/h; the pH and the temperature was the same as that of the adsorption process. A peristaltic pump (HL-2, Nanpu Huxi Equipment Factory, Shanghai, China) was used to pump the crude UEP solution through the packed column, and the effluent or eluate was collected in an auto-partial collector (DBS-100, Nanpu Huxi Equipment

Factory, Shanghai, China) with 6 min per test tube. After treatment with the AB-8 resin, the pure UEP (99.8%) was obtained and lyophilized.

2.4. Animals

Experiments were carried out with male Sprague–Dawley rats weighing 250–300 g (Nanjing University of Traditional Chinese Medicine, China). The rats were maintained in a temperature-controlled room ($24 \pm 1^\circ \text{C}$). The rats had free access to diets before experiments and were acclimatized for at least one week. All experiments were performed in accordance to internationally accepted guidelines on laboratory animal use.

2.5. *In vitro* anticoagulant activity assay

The rats were anesthetized intramuscularly with ketamine (100 mg/kg) and xylazine (16 mg/kg). Blood was collected from the abdominal aorta, then anticoagulated with citrate (3.8%; 1:9, v/v). The blood was centrifuged (1000 rpm, 10 min) to obtain its PRP (platelet-rich plasma). The residual blood was centrifuged (3000 rpm, 15 min) to obtain its PPP (platelet-poor plasma). Its platelets were centrifuged (2500 rpm, 10 min) to obtain its pellets and washed thrice. They were then suspended in a Tyrode-Hepes solution (1 mM Ca^{2+} , 0.35% BSA; heparin 50 units/ml; pH 7.4). In the solution, heparin at the concentration was used to prevent plasma from coagulating and has no effect on aggregation induced by ADP in PRP (Antonio & Marchien, 2001; Gulzar, Nurbiya, Kerimjan, & Abdulla, 2011; Lee, Park, Jung, Lee, & Oak, 2010). The platelet concentration was adjusted to 4.5×10^8 platelets/ml. They were stored at 4°C .

Experiments were carried out using UEP or heparin (as a positive control) dissolved in saline at various concentrations, respectively. As controls, saline was added to plasma in a ratio of 1:10. The plasma (90 μL) was mixed with a 10 μL solution of UEP (0–50 μg), or heparin (0–10 μg) and incubated at 37°C for 7 min. Prothrombin time (PT), activated partial thromboplastin (APTT) and thrombin time (TT) were measured using kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, China) and an automated coagulometer (Medtronic Inc, USA). In brief, 1 μL of the incubated plasma was mixed with 50 μL of cephalin in the process plate, and the coagulation was started by addition of CaCl_2 (1 mM), 100 μL of thromboplastin and 100 μL of bovine thrombin into the 100 μL of incubated plasma for APTT, PT and TT assay, respectively.

The decreasing percentage of platelet aggregation rate was also calculated and compared, as well as IC_{50} was calculated, according to the Born method (Lu et al., 2011; Wu et al., 2009; Xie et al., 2007).

2.6. *Ex vivo* determination of APTT

The rats were anesthetized with ketamine (100 mg/kg intramuscularly) and xylazine (16 mg/kg intramuscularly). The carotid artery was exposed and dissected free from surrounding tissue. Heparin (0–500 $\mu\text{g/kg}$), or UEP (0–1500 $\mu\text{g/kg}$) was administered into it. After 5, 15, 30 and 60 min samples of blood was collected (0.5 ml in 3.8% trisodium citrate, 9:1, v/v). Each was centrifuged ($2000 \times g$, 10 min), and plasma was stored at 4°C . APTT was evaluated using the kit and the automated coagulometer.

2.7. *In vivo* arteriovenous shunt thrombosis

Thrombus formation was promoted with a combination of stasis and hypercoagulability. The rats were anesthetized by intraperitoneal injection of chloral hydrate (350 mg/kg) and an arteriovenous shunt tube was placed between the right carotid

artery and left jugular vein. The left jugular vein and the right carotid artery were cannulated for injection of UEP, heparin, and thromboplastin. The 14 cm polyethylene tube (containing 5-cm long of suture silk thread) was filled with saline. UEP (0–5 mg/kg) or heparin (0–100 µg/kg) was carried with a single bolus injection, respectively. Ends of the tubing were pinched, and the silk thread with thrombus was gently removed from the shunt tube, after blood circulated through the shunt tube for 15 min. Its wet weight was measured, and the formed thrombus was calculated.

2.8. Tail transection bleeding time

Bleeding time determined the ability to form hemostatic plug. Rats were also anesthetized. The right carotid artery of rats was cannulated for administrations of UEP (0–100 mg/kg) or heparin (0–50 mg/kg). The compounds were administered for 5 min before the tail transection. The bleeding was carried out by the section of the tail extremity 5 mm from the tip. The tails were blotted with tissue papers every 30 s, and the bleeding time was calculated as the time up to the moment that the bleed flow stopped for more than 60 s. Bleeding time beyond 600 s was considered as the cut off time.

2.9. In vivo antithrombosis assay

The antithrombotic test was carried by the pulmonary thrombosis experiment. UEP (20, 40, 80 and 100 mg/kg), heparin (20, 40, 80 and 100 mg/kg), or saline were administered orally. After 1 h, the mixture of collagen (12 mg/mouse) and epinephrine (2 g/mouse) was injected into the tail vein to induce the pulmonary thrombosis. As an index, paralysis is the loss of hind limbs' function for more than 15 min. The number of dead or paralyzed rat was calculated, and the percentage of protection was recorded.

2.10. Analysis of UEP

The content of the polysaccharide was determined by the modification method of phenol-sulfuric acid, using glucose as standard and deionized water as blank (Bargagli et al., 1999; Branislav et al., 2008; Carbonero et al., 2006a, 2006b; Moosung & Kyungae, 2006). The sugar solution (0.6 ml) and the phenol solution (0.3 ml) were added to screw cap tubes (13 × 100 mm), which were capped and vortex-stirred. Then 1.5 ml of concentrated sulfuric acid was added slowly down the side of the tubes. The tubes were closed, vortex-stirred for 5 s and incubated for 15 min at 110 °C (DF-1 collector-type thermostat oil bath, Jintan Ronghua Instrument Manufacture Co, Ltd., Jintan, China). They were then allowed to cool down to room temperature before measuring the absorbance at 490 nm in a Model 752 UV spectrophotometer (Shanghai Jinghua Instrument Co, Ltd., Shanghai, China).

2.11. Statistical analysis

All experiments were repeated three times, each with three replicates (three fingers for one replicate). The data were expressed as means ± SD (standard deviations) of triplicate determinations (n = 3 × 3). One-way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) were carried out to assess the significance of the differences between means using Statistical Analysis System software. The significance of the level was set at p < 0.05.

Table 1

Effects of UEP and heparin (as a positive control) on in vitro plasma coagulation time measured by APTT, TT and PT.

Sample	Dose for plasma (µg/mg)	APTT (s)	PT (s)	TT (s)
UEP	0	27.1 ± 0.9	14.8 ± 0.6	19.2 ± 0.7
	5	58.3 ± 1.2*	15.1 ± 0.9	44.6 ± 0.5*
	10	86.5 ± 1.3*	17.1 ± 1.0*	69.5 ± 0.7*
	20	147.4 ± 1.0*	19.8 ± 0.5*	119.3 ± 0.9*
	30	208.3 ± 2.1*	21.5 ± 1.2*	187.4 ± 1.8*
	40	266.7 ± 1.6*	26.0 ± 0.8*	260.4 ± 1.5*
Heparin	50	324.9 ± 1.7*	28.7 ± 1.0*	327.5 ± 2.1*
	0	27.1 ± 0.9	14.8 ± 0.6	19.2 ± 0.7
	2	89.3 ± 1.8*	17.8 ± 0.5*	60.2 ± 1.3*
	4	165.2 ± 1.1*	18.5 ± 0.5*	117.8 ± 1.7*
	6	223.7 ± 1.6*	19.4 ± 0.3*	208.5 ± 2.2*
	10	308.6 ± 2.3*	22.5 ± 0.6*	303.4 ± 1.6*

Data are means ± SD (n = 3). Statistical significance compared to control values is denoted by asterisks where *p < 0.05 compared with controls, DMRT's tests.

3. Results and discussion

3.1. In vitro anticoagulant activity and ex vivo determination of APTT

To investigate the interactions of UEP with coagulation factors, the effects of UEP on coagulation time were investigated by the classical coagulation assays APTT, PT and TT (shown in Table 1). APTT is related to the intrinsic coagulation phase in plasma, PT with the extrinsic phase, and TT with the third coagulation phase in plasma (Branislav et al., 2008; Carbonero et al., 2006a; Muller, 2001; Nishikawa & Tanaka, 1970; Tomas et al., 2003). These tests are often referred to as functional tests because they monitor clot formation. UEP was able to prolong APTT and TT in a concentration-dependent manner. UEP prolonged APTT and TT beyond 300 s at concentrations greater than 50 µg/ml, 5-fold more UEP than heparin being required to prolong APTT and TT to 300 s. At 10, 20, 30 and 40 µg/ml of plasma, the anticoagulant activity was 3.2 (86.7 ± 1.3 s), 5.4 (147.4 ± 1.0 s), 7.7 (208.3 ± 2.1 s), and 9.8 (266.7 ± 1.6 s) times greater than that of the control (27.1 ± 0.9 s), in respect to APTT. The anticoagulant effect of UEP was also evaluated by the TT test, the anticoagulant activity being about 3.6 (69.5 ± 0.7 s), 6.2 (119.3 ± 0.9 s), 9.8 (187.4 ± 1.8 s), and 13.6 (260.4 ± 1.5 s) times greater than that of the blank control (19.2 ± 0.7 s) at the same concentrations.

In order to determine whether UEP could exert its anticoagulant action in vivo, APTT ex vivo was tested (shown in Table 2). APTT determined for UEP doses of 100, 200, 400, 600, 800, 1000 and 1200 µg/kg, after 5 min of drug administration, was increased in a dose-dependent manner by 2.4, 3.3, 6.2, 9.3, 10.9 and 13.8-fold, respectively. A 2.4-fold more UEP than heparin was required to prolong APTT to 300 s.

UEP affected intrinsic and extrinsic coagulation systems simultaneously, and the more prolongation of APTT suggested a more obvious inhibition of the intrinsic coagulation system than the extrinsic, whereas the prolongation of TT indicated a little inhibition of thrombin-mediated fibrin formation. Since the anticoagulant effect of heparin is not mainly mediated by a modulation of the extrinsic system, it appears that UEP is a fine inhibitor of the intrinsic pathway. UEP showed an importance in vitro anticoagulation action, evidenced by an increased dose-dependence of APTT and TT. UEP has a potential anticoagulant action in vivo.

UEP and heparin significantly inhibited ADP activating-induced platelet aggregation in a concentration dependent manner with the IC₅₀ value of 82.7 and 71.4 µmol L⁻¹. Although the IC₅₀ of UEP is higher than that of heparin, UEP still has the therapeutic potential

Table 2
Effects of UEP and heparin on ex vivo plasma coagulation time measured by APTT.

Sample	Dose for rats (μg/kg)	APTT (s)
UEP	0	20.3 ± 1.3
	100	49.6 ± 1.5*
	200	67.2 ± 1.0*
	400	125.9 ± 1.8*
	600	188.1 ± 1.5*
	800	221.4 ± 1.7*
	1000	255.4 ± 1.3*
	1100	281.8 ± 2.0*
	1200	306.2 ± 2.1*
Heparin	0	20.3 ± 1.3
	25	21.9 ± 1.6
	50	22.4 ± 0.9
	75	33.6 ± 1.1*
	100	52.8 ± 1.5*
	200	73.9 ± 2.4*
	300	122.5 ± 1.3*
	400	176.0 ± 1.7*
	500	305.1 ± 2.0*

Data are means ± SD ($n=3$). Statistical significance compared to control values is denoted by asterisks where * $p < 0.05$ compared with controls, DMRT's tests.

if its single functional factor will be separated in the future, and the dose may be decreased.

3.2. In vivo arteriovenous shunt thrombosis

The antithrombotic activity of UEP was further investigated in rats by the arteriovenous shunt thrombosis model, being observed on thrombus formation (shown in Fig. 1). In saline-treated rats (as a blank control) the average thrombus weight was 35.2 ± 1.8 mg. For UEP, its inhibition of thrombus formation increased in a dose-dependent manner. The percentage of thrombosis inhibition was about 40.9, 53.7, 71.6, 80.0 and 91.5% at 100, 200, 300, 400 and 500 μg/kg body weights, respectively. Sections of the vena cava and the jugular vein were isolated and ligated so that the stasis in this region promotes thrombus generation (Bargagli et al., 1999; Kunio, Tadaihiro, & Shoji, 1974; Li et al., 2011; Muller, 2001; Rana et al., 2011; Sa et al., 2011; Tomas et al., 2003; Watana & Shibata, 1986).

UEP was an effective antithrombotic agent, and prevented thrombosis in a dose-dependent manner and a dose of 700 μg/kg body weight completely prevented thrombosis after 15 min shunt. Although heparin was more effective than UEP and completely prevented thrombosis in the same experimental model, at a dose of

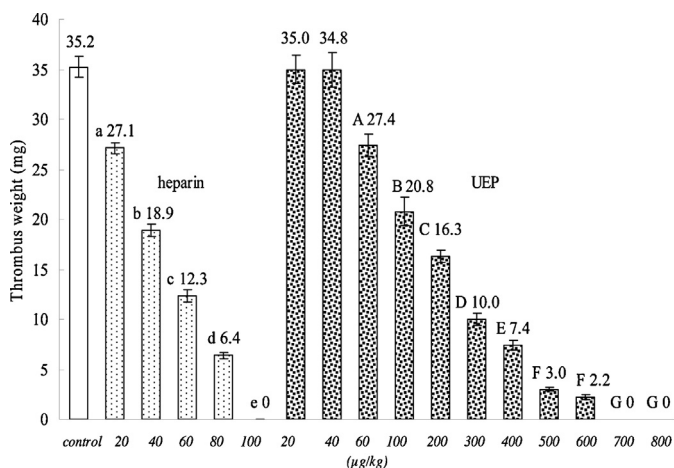


Fig. 1. Antithrombotic effects of UEP and heparin on the arteriovenous shunt thrombosis model in rats. Data are means ± SD ($n=3$). The error bars represent the standard deviation. Values marked by the same letter are not significantly different ($p < 0.05$), compared with controls, DMRT's tests.

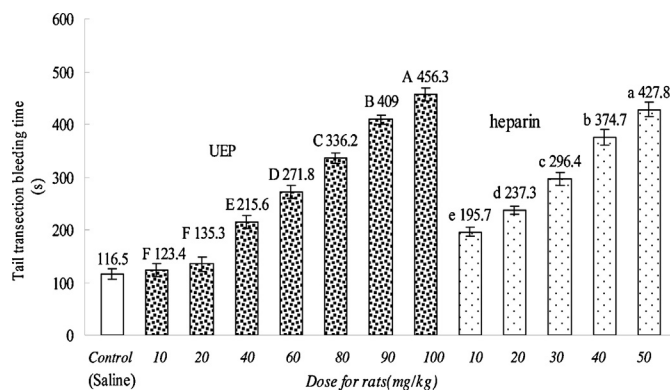


Fig. 2. Effects of UEP and heparin on the tail transection bleeding time in rats. Data are means ± SD ($n=3$). The error bars represent the standard deviation. Values marked by the same letter are not significantly different ($p < 0.05$), compared with controls, DMRT's tests.

100 μg/kg body weight, only a concentration 7 times greater of UEP was necessary to obtain the same effect as heparin.

3.3. Tail transection bleeding time

The tail transection bleeding time of rats was measured to determine the antihemostatic effects of UEP, based on blood loss from a cut rat tail, after intravascular administration of UEP (shown in Fig. 2). The tail bleeding time of saline treated rat was measured to be 116.5 ± 8.2 s. The bleeding time was significantly prolonged by UEP as well as by heparin.

Both UEP and heparin caused dose-dependent increases in the tail transection bleeding time. In the saline-treated blank control group, the bleeding time averaged at 116.5 ± 8.2 s. UEP caused an increase in bleeding time of 85.1, 133.3, 188.6, 251.1 and 291.7% at 40, 60, 80, 90 and 100 mg/kg body weight, respectively. Heparin produced a strong hemorrhagic effect at 50 mg/kg: the increase in bleeding times was 267.2%. The ideal clinical anticoagulant would reliably and predictably inhibit thrombin without substantially increasing the risk of bleeding (Carbonero et al., 2006a; Kim & Lee, 2006; Lee & Kim, 2000; Li et al., 2010; Tomas et al., 2003). UEP produced a short prolongation of the rat tail transection bleeding time, when compared with equivalent heparin.

3.4. In vivo antithrombosis activity

Intravenous injection of a mixture of collagen and epinephrine into the tail vein of rats caused pulmonary thrombosis, resulting in death and giving a protection rate of zero in the blank control group (shown in Table 3). Oral administration of UEP exhibited a significant dose dependent preventive effect against thrombotic death or paralysis, giving a protection rate of 100, 77.8, 44.4, 22.2 and 11.1% in dose of 250, 200, 150, 100 and 50 mg/kg, respectively. Heparin, as a positive control, showed a protective effect by 77.8, 88.9, 100% at dose of 10, 20, 30 mg/kg, respectively. UEP could prevent platelet aggregation. Though its antithrombotic potency is lower than that of heparin, UEP may be a potential source of antithrombotic agent.

Pathological platelets aggregation is critical in arterial thrombosis which may cause serious disorders, such as heart attacks, unstable angina and stroke (Antonio & Marchien, 2001; Lee & Kim, 2000; Moosung & Kyungae, 2006; Nishikawa & Tanaka, 1970; Watana & Shibata, 1986). For treatments and preventions of these diseases, the inhibition of platelet aggregation is important. Although current drugs can treat or prevent these disorders to a certain extent, these drugs, such as aspirin, can produce hemorrhagic events, upper gastrointestinal bleeding and long bleeding time. It is also necessary to overcome some of the problems associated

Table 3

Effects of UEP and heparin on in vivo pulmonary thrombosis in rats.

Sample ^a	Dose for rats (mg/kg)	Number of rats (dead and paralyzed)/number of tested rats	Protection rate (%)
Saline (control)	–	9 (7 and 2)/9	0
UEP	20	9 (6 and 3)/9	0
	50	8 (5 and 3)/9	11.1*
	100	7 (3 and 4)/9	22.2*
	150	5 (3 and 2)/9	44.4*
	200	2 (0 and 2)/9	77.8*
	250	0/9	100*
	300	0/9	100*
Heparin (positive control)	10	2 (1 and 1)/9	77.8*
	20	1 (0 and 1)/9	88.9*
	30	0/9	100*

Data are means $\pm$ SD ($n = 3$). Statistical significance compared to control values is denoted by asterisks where * $p < 0.05$ compared with controls, DMRT's tests.

with heparin, such as bleeding, thrombocytopenia, problems with animal pathogen contamination due to its animal origin, and poor bioavailability (Branislav et al., 2008; Lee & Kim, 2000; Lee et al., 2010; Li et al., 2011; Moosung & Kyungae, 2006; Tomas et al., 2003). A number of alternative anticoagulants need to be developed. The search for novel sources to suppress the platelet aggregation is urgent. Besides heparin, other polysaccharides are increasingly used for medical applications, taking advantage of some intrinsic functional properties elicited by biological interactions. Polysaccharides are important because of their biological and pharmacological properties, and some groups (sulfation as an example) are related to anticoagulant and antithrombotic effects and other biological activities (Antonio & Marchien, 2001; Branislav et al., 2008; Lee & Kim, 2000; Muller, 2001; Tomas et al., 2003). Anticoagulant and antithrombotic activities are among the most widely studied properties of polysaccharides (Branislav et al., 2008; Kunio et al., 1974; Moosung & Kyungae, 2006; Rana et al., 2011; Sa et al., 2011). Studies on a number of other polysaccharides have been carried out related to in vivo and in vitro anticoagulant and antithrombotic properties with the aim of finding substitutes for heparin.

U. esculenta has been used as a traditional food and medicine, but the biological potency of the lichen was not investigated sufficiently. Although its phenolic compounds were extracted, and could prevent platelet aggregation and protect against cardiovascular diseases, the amount of total phenolic compounds in the methanol extract of *U. esculenta* was only 1.96% (Kim & Lee, 2006). Our experimental results suggested that *U. esculenta* polysaccharide could be a potential source of antithrombotic agents, and the antithrombotic activity of UEP might be due to anticoagulant activity. Considering the traditional uses of the lichen as a medicine, most of them have been employed to treat many kinds of inflammation, bleeding and poisoning. The antithrombotic effect of UEP may be used for treatments and preventions of relative disorders. Further studies about UEP action mechanism are necessary and under investigation.

4. Conclusions

U. esculenta polysaccharide is provided with significant preventive effect on thrombosis. The more prolongation of APTT suggested a more obvious inhibition of the intrinsic coagulation systems than the extrinsic. Its antithrombotic properties were evaluated using an arteriovenous shunt thrombosis model in rats, and its inhibition of thrombus formation increased in a dose-dependent manner. Oral administration of UEP also showed a significant dose dependent preventive effect against thrombotic death or paralysis. UEP, as a novel, effective and promising antithrombotic agent for the treatment of various thrombotic diseases, may be beneficial for

the individuals with high risks of thrombotic and cardiovascular diseases.

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