Anticoagulant Polysaccharide from *Percottus Glenii*

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\textbf{Keywords:} *Percottus Glenii*, Anticoagulant, Polysaccharide.

\textbf{Abstract.} This paper studied the anticoagulant effects of polysaccharides extracted from *Percottus glenii*. The \textit{in vitro} clotting time was measured using semi-automatic coagulometer. The \textit{in vivo} bleeding time and capillaries clotting time were determined by docking method of mice tail. Results showed that the extracted polysaccharides of *P. glenii* could significantly prolong bleeding time, \textit{in vitro} and \textit{in vivo}, indicating the anticoagulant effect of *P. glenii* extracts.

\textbf{Introduction}

Thrombotic disease refers to a semi-purified blood clot in the blood vessel or heart ingredient intimal surface, the accumulation of this substance in blood vessels, can slow down the blood flowing, and affect our normal life activities [1]. Venous thrombosis occurs at an annual incidence of 1 per 1000 adults worldwide [2, 3]. The traditional methods of treating thrombotic diseases are usually thrombolysis [4], anticoagulation and antiplatelet [5]. Heparin [6] is a mucopolysaccharide, composed of D-glucosamine, L-iduronic acid, N-acetyl-glucosamine and glucuronic acid and it is the most largely polysaccharide clinically employed as anticoagulant and antithrombotic drug. However, heparin has important disadvantages, including marked structural diversity depending on the tissue of origin [7]. Therefore, to find a new anticoagulant drug is particularly urgent.

*P. glenii* is round body, nearly fusiform, the rear side of the flat, large head and slightly flat front. *P. glenii* is commonly known as the old man, which is a small freshwater. *P. glenii*, has a nutrient-rich, delicious flavor and other characteristics, thus was liked by the consumers. In this experiment, the *P. glenii* was used as raw materials to extract the active substance and to study the anticoagulant effect, in order to develop an effective anti-clotting drug. We are hoping to make up for some deficiencies of existing drugs to provide a new option for anticoagulant therapy.

\textbf{Materials and Methods}

\textbf{Materials and Reagents}

*P. glenii* purchased in the Jilin Academy of Fishery Sciences

Thrombin time kit: Shanghai Sun Biotechnology Co., Ltd.

95% ethanol, ethanol, NaCl, NaOH, sodium citrate, all reagents were of analytical grade

\textbf{Instrument}

Centrifuge: LDZ4-2, Ltd.Beijing Lei Boer Centrifuge

Mashed tissue homogenizer: JJ-2, type Runhua Electric Co., Ltd. Changzhou

Rotary Evaporator: RE-2000B, Haiya Rong Biochemical Instrument Factory

Freeze dryer: FD-1B, Beijing Boyikang Laboratory Instruments Co., Ltd.

Semi-automatic coagulation instrument: PUN-2048B, Beijing Prolong Technology Co., Ltd.
Electronic balance, thermostat water bath

**Experimental Animals**

ICR mice: body weight 18-22g, Medical Center Ministry of Jilin University.

Rabbit Plasma: Blood was obtained (3.2% sodium citrate) from healthy rabbits weighing 2.0-2.5 kg, Medical Center Ministry of Jilin University

**Polysaccharide Prepared**

Testing Method was performed according to Qin et al. [8, 9] with minor modifications. The minced *P. glenii* plus saline was homogenized and centrifuged at 4000 rpm for 20 min, the precipitate was added with 0. 1M NaOH solution (1: 5) 30 alkaline hydrolysis for eight hours. Alkaline hydrolysis was 4000 rpm centrifugal for 20 min, to obtain a supernatant. Combined twice supernatant was concentrated using a rotary evaporator. Concentrate plus 95% ethanol solution (1: 4) 4 stilled 12h, 4000 rpm centrifuged 20 min, then the precipitate was collected and lyophilized by a freeze-drying machine to give a lyophilized powder sealed and stored for later use.

**Animals**

30 mice (male: female=1:1) were randomly divided into three groups. Blank control group (distilled water), low-dose group (750 mg / kg) , high-dose group (1000 mg / kg) were gavage. For 15 days, once a day. The mice were fasted for 12 hours before they were used.

**Bleeding Time Was Measured in Mice Tails Law.** One hour after the last administration the mice were placed in a holder, maintaining the vertical tail, marking at the 3mm location of the tail, using surgical scissors to cut the mark. After the blood flowing 30 s, using filter paper to absorb the drop of blood, until no bleeding further, then the bleeding time was calculated [10].

**Determination of Capillary Clotting Time.** One hour after the last administration, with an inner diameter of 0. 5 mm glass capillaries inserted into mice bled adjoin[11] , the capillary clotting time was determined. When blood capillary column was about 5 cm, the capillary was broken down every 30 s to observe the coagulation phenomenon, then the clotting time was calculated [12, 13].

**Determination of in Vitro Clotting Time.** According to the kit instructions the clotting time of APTT, PT and TT were measured [14]. For APTT: 80 µL of plasma and 20 µL of crude polysaccharide solution (50 mg/mL) were mixed and incubated at 37 for 1 minute. Then added 100 µL of ellagic acid, at 37 incubated for 5 min. The reaction by the addition of CaCl₂ (100 µL) were measured. For PT: 80 µL of plasma and 20 µL of crude polysaccharide solution (50mg/mL) were mixed and incubated at 37 3 minutes. The reaction by the addition of thromboplastin (200 µL) was measured. For TT: 160 µL of plasma and 40 µL of crude polysaccharide solution (50mg/mL) were mixed and reacted by adding thrombin solution (200 µL) then the TT was measured. Experiments were performed three times or more parallel test.

**Results and Discussion**

**Tail Bleeding Time Method of Analysis of the Effect of Polysaccharide**

In cutting tail test (Table 1), *P. glenii* polysaccharide extract could significantly prolong bleeding time, compared with a significant difference from the control group. *P. glenii* polysaccharide showed anticoagulant activity.

| Table 1. Tail Bleeding Time Method of Analysis of the Effect of Polysaccharide. |
|-------------------------------|-----------------|-----------------|
| Group                         | g·kg⁻¹           | Bleeding/s       |
| NS                            | 0               | 67±10            |
| Polysaccharide                | 0.75            | 113±20**         |
| Polysaccharide                | 1               | 125±16**         |

NS, Normal saline
* Significant difference compared with the control group, the difference was significant (P <0.05), **.Compared with the control group (P <0.01)
Analysis of the Effect of Anticoagulant Polysaccharide

In the capillary method test (Table 2), the experimental \textit{P. glenii} polysaccharide extract could significantly prolong the clotting time, as compared with the control group. \textit{P. glenii} polysaccharide having anticoagulant activity.

<table>
<thead>
<tr>
<th>Group</th>
<th>g·kg$^{-1}$</th>
<th>Clotting/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>0</td>
<td>7.03±1.33</td>
</tr>
<tr>
<td>Polysaccharide</td>
<td>0.75</td>
<td>10.44±1.17*</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>13.4±12*</td>
</tr>
</tbody>
</table>

NS, Normal saline

* Significant difference compared with the control group, the difference was significant (P <0.05), **.Compared with the control group (P <0.01)

Polysaccharide Clotting Effect Analysis

In the APTT tests, the clotting time (48 ± 0.62 s) was significantly increased. In the PT tests, clotting time (11.78 ± 0.47 s) did not change significantly. In the TT tests, the coagulation time (9.81 ± 1.42 s) did not change significantly (Table 3). This indicated that there was no effect of exogenous coagulation pathway. Results showed that the anticoagulant effect of \textit{P. glenii} polysaccharides was mainly through the intrinsic coagulation pathway.

<table>
<thead>
<tr>
<th></th>
<th>Blank Group/s</th>
<th>Test Group(50mg·ml$^{-1}$)/s</th>
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<tbody>
<tr>
<td>APTT</td>
<td>22.53±1.86</td>
<td>48.58±0.62</td>
</tr>
<tr>
<td>PT</td>
<td>10.57±0.72</td>
<td>11.78±0.47</td>
</tr>
<tr>
<td>TT</td>
<td>13.04±1.58</td>
<td>9.81±1.42</td>
</tr>
</tbody>
</table>

Conclusions

\textit{P. glenii} polysaccharide extract on \textit{in vitro} anticoagulant test, mouse bleeding, the clotting time was obviously prolonged, indicating that \textit{P. glenii} polysaccharide extract has anticoagulation effect. Kinds of foods derived from anti-clotting product, were very healthy and had no side effects, it can be directly used into the gastrointestinal tract. And they might be digested and absorbed through the blood circulation into the anticoagulant system. It is expected to develop \textit{P. glenii} into a nutritional health foods, its exploitation has broad market prospects.

References


