

Antithrombotic Action of New Earthworm Powder (HLP)
with Rat AV-Shunt Model

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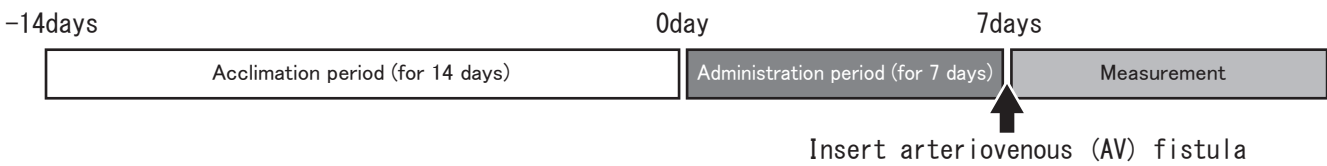
Purpose of the Study

Due to the fact that earthworm powder has fibrinolytic function, it has been used as dietary supplement ingredient not only in Japan, but also in many countries around the world. In order to confirm the reliability of oral administration of earthworm powder (Waki Pharmaceutical Co., Ltd.) in animals, this study examines its antithrombotic action on thrombus formed in rats. (The rats used in the test tend to form thrombus easily)

Test method

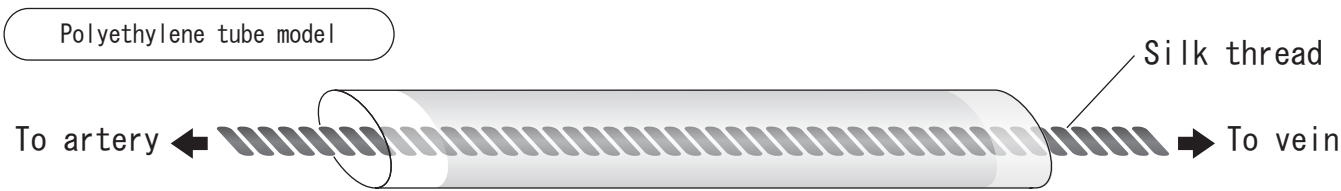
Test substances: Control (water), new earthworm powder (HLP provided by Waki Pharmaceutical Co., Ltd)
Test animal : Rats (Slc:SD, male)
Administrated amount : 5mL/kg
Number of rats : 10 in each group (total of 20 rats)

Test schedule



AV-shunt testing

Polyethylene tube containing silk thread is inserted between arteries and veins of anesthetized rats. After forming AV-shunt, blood is circulated for 20 minutes. Increase or decrease of the thrombus is judged by measuring the wet weight of thrombus attached to the silk thread.



Supervisor for animal testing



Professor Yasuhiko Tabata of Department of Biomaterials. Field of Tissue Engineering
Institute for Frontier Medical Sciences, Kyoto University

Age of the test animal

At the time of arrival: 7 week old
At the time of grouping: 8 week old
At the time of administration: 8-10 week old

Group structure and administration method

Plasmin activity test that previously conducted indicates that HLP performs about 2-3 times higher plasmin activity than the second generation earthworm powder. In this test, 50mg/kg (A-2) that is equivalent to human dose of the second generation earthworm powder 240mg is administered to rats.

group	test substance	given dose	volume	number of model
A-1	water	–	5ml/kg	10
A-2	HLP powder	50mg/kg	50ml/kg	10

Administration of test substance

- ①Number of dose and period
Administer water or HLP once a day between 9:00 and 12:00 for 7 days. However, the last administration takes place one hour before forming AV-shunt.
- ②Administration method
Administer orally by using disposable syringe and feeding needle.
- ③Reason for choosing the administration method
As the accurate amount can be administered.
- ④Calculation of the volume
Based on their latest weight.

Measurement

- ①Weight
Weight (g) is measured at the beginning of the administration, 4 days after the administration, and before the AV-shunt testing.
- ②Wet weight of thrombus
1 hour after the administration of test substance, the blood is circulated through AV-shunt tube for 20 minutes (Fig. 1, 2). Then the blood flow is blocked and the thread in polyethylene tube (PE 240) is withdrawn immediately to measure the wet weight of the thrombus. Wet weight of the thrombus (mg) is determined by subtracting the thread weight (mg) measured beforehand from the total wet weight. As for each administrated group, the inhibition rate of the wet weight of the thrombus in each rat calculated at the following formula.

The inhibition rate of the
wet weight of thrombus (%)

=

※1

the test group average wet weight of the thrombus

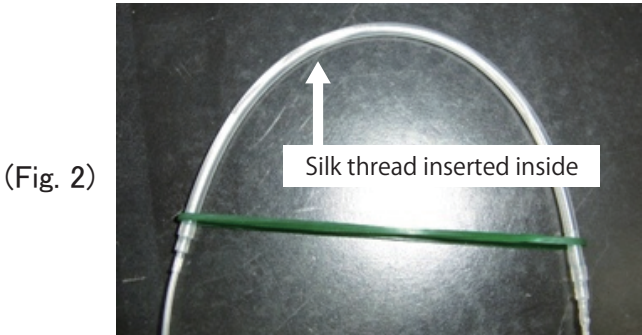
× 100

※1 : (the test group average wet weight of the thrombus – the wet weight of the thrombus)



(Fig. 1)

AV-shunt testing



(Fig. 2)

AV-shunt used in the test

- ③ Visual check of the thread
Take photograph of the thread with thrombus.

Testing procedure

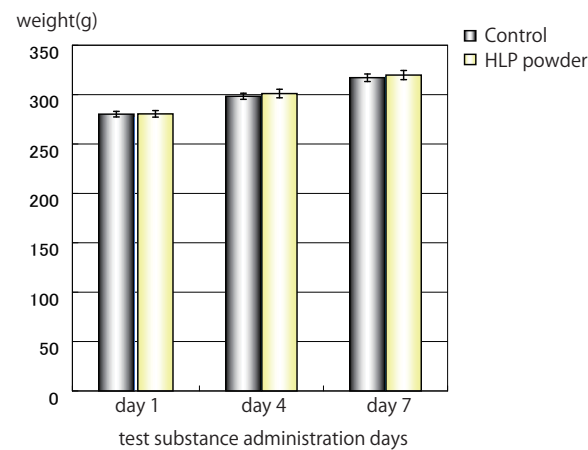
- 1) Divide the rats into groups at the end of quarantine and acclimation periods.
- 2) Administer test substance for 7 days.
- 3) At the end of the administration, form AV-shunt and allow blood to flow for 20 minutes. Measure the wet weight of the thrombus and take photograph of the thread.

Statistical approach

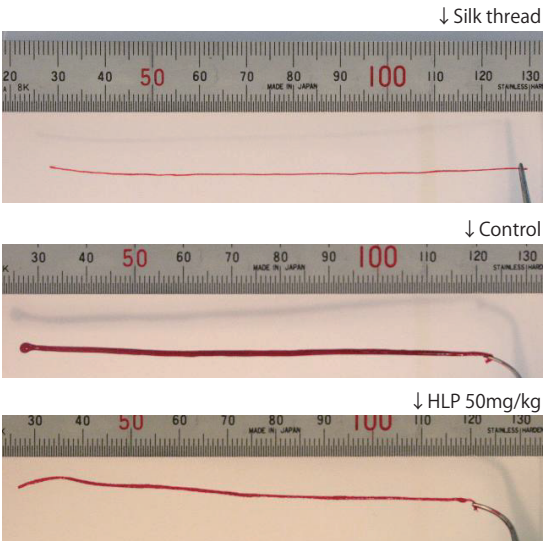
The wet weight of the thrombus is shown as the average \pm standard deviation. A comparison test is performed between A-2 group and A-1 group. Perform Student t-test when uniformity of dispersion is confirmed in F-test, and perform Aspin-Welch t-test when it is not confirmed. StatLight® (Yukms Co., Ltd.) is used for statistical analysis at a significance level of less than 5%.

Results

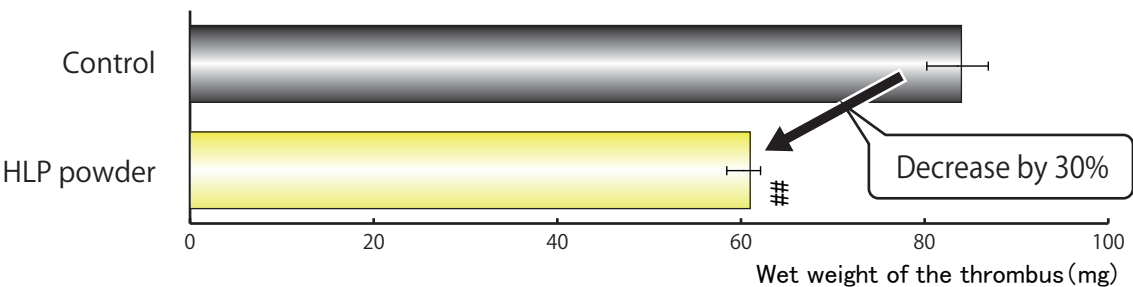
- ① Weight
The following chart shows the test results. The administration of test substances did not lead to weight change in rats.



- ② Below are the photograph of the threads of test substances.



- ③ Wet weight of the thrombus and thrombus suppression rate
The following chart shows the test results. It indicates that HLP has an effect of decreasing the wet weight of the thrombus compare to the control group. The thrombus suppression rate was about 30 %.



Summary

By administering HLP for 7 days, thrombus formation was significantly suppressed in a density- dependent manner and antithrombotic effect was confirmed. It is one of the subjects for future analysis to further elucidate the mechanism of action of HLP in terms of the antithrombotic effect.

Cooperation

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Director of Laboratories Maru, Yamashita of Pharma Foods International Co., Ltd.
Medical department of West China School of Public Health, Sichuan University
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