


Research Article

Study on the Hemostatic Effect of Semen Vaccariae Before and After Charcoal Preparation

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Abstract

Aims: This study is aimed to verify the records in ancient Chinese classics that semen vaccariae was mainly used for traumatic hemorrhage caused by metal sharp weapons but need to be burned into ash first. **Methods:** The bleeding time and clotting time of tail-amputated bleeding experiment and capillary coagulation experiment of mice were measured to evaluate the hemostatic effect of semen vaccariae before and after charcoal preparation. **Results:** While semen vaccariae was used topically to mice, the bleeding time of charcoal group was significantly shortened ($P < 0.05$), but there was no significant difference in raw group. While semen vaccariae was used orally to mice, the bleeding time and clotting time in the low and high dose groups of raw groups decreased significantly ($P < 0.05$ or 0.01). In addition, the bleeding time in the high dose of raw group also decreased significantly ($P < 0.05$). However, animal death and animal weight loss were found in the raw group during the experiment. The clotting time was also decreased significantly in low, middle and high dose charcoal groups ($P < 0.05$ or 0.01). **Conclusions:** Semen vaccariae used topically after charcoal preparation has hemostatic effect, while semen vaccariae used orally both before and after charcoal preparation shows hemostatic effect, but raw semen vaccariae used orally has potential risk. This study to some extent verifies the records in ancient Chinese classics.

Keywords

Semen Vaccariae, Hemostatic Effect, Charcoal Preparation, Tail-Amputated Bleeding Experiment

1. Introduction

Semen vaccariae is a traditional Chinese herb, first recorded in the *Classic of Shennong Materia Medica*. It is the dried mature seed of *Vaccaria hispanica*, a plant of Caryophyllaceae [1]. *Classic of Shennong Materia Medica* records semen vaccariae is mainly used to stop bleeding caused by metal sharp weapons [2]. In addition, *Treatise on typhoid and Miscellaneous Diseases* written by Chinese medical

saint Zhang Zhongjing also records semen vaccariae powder as the primary therapeutic drug for bleeding caused by metal sharp weapons and clearly points out it should be burned to ashes at first [3]. These kinds of burned ashes are called charcoal medicine commonly in China [4]. The following ancient books of Chinese materia medica have a lot of similar records suggesting the hemostatic effect of semen vac-

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cariae. For example, the *Compendium of Materia Medica* in the Ming Dynasty records that it is mainly used to treat blood loss caused by swords [5]. Obviously, many Chinese medicine experts have paid attention to the hemostatic effect of semen vaccariae. However, after systematic search, we can hardly find the modern research reports on the hemostatic effect of semen vaccariae. There are only a few discussions and clinical reports [6-9]. Further, the hemostatic effect of semen vaccariae has not been mentioned in many modern textbooks of traditional Chinese medicine [10-13], as well as all versions of Chinese Pharmacopoeia [14]. Thus, it seems that there is a need to testify the hemostatic effect of semen vaccariae recorded in the classics through the modern research. In this light, this study is going to investigate the hemostatic effect of semen vaccariae used by topically and orally through the model of amputated tail bleeding mice, as well as investigate the necessity of burning to ashes by comparing the difference before and after charcoal preparation of semen vaccariae. Based on these investigations, we hope to verify the records in ancient Chinese classics and then hope it beneficial to the modernization of traditional Chinese medicine.

2. Material and Methods

2.1. Plant Material

The semen vaccariae were provided by Anhui Xiehe Cheng Pharmaceutical Co., Ltd (No.220818), Anhui Province, China. The material complied with the specification of the Pharmacopoeia of China (2020) [14].

2.2. Animal Material

2.2.1. Animal of Topical Experiment

Male Kunming mice weighing 19.3-24.0 g were obtained from Guangdong Medical Laboratory Animal Center and were housed in an environment with a temperature of 22 to 26 °C and 12/12 h light-dark cycle in SPF grade laboratory. Water and food was freely available to mice during external experiments. The animal experimental design and protocols used in this study were approved by the Animal Ethics Committee of this institution.

2.2.2. Animal of Oral Experiment

Both sexes of Kunming mice (15.1-19.4 g, half male and half female) were obtained from Guangdong Medical Laboratory Animal Center. The feeding environment of mice during the oral administration experiment is the same as that of the topical experiment. The animal experimental design and protocols used in this study were approved by the Animal Ethics Committee of this institution.

2.3. Experimental Material

Yunnan Baiyao Powder was provided by Yunnan Baiyao Group Co., Ltd. (No.ZHA2001, No.ZGA2102). Starches were provided by Guangdong Food and Drug Vocational College (No.cc21001), CMC-Na was provided by Fuchen (Tianjin) Chemical Reagent Co., Ltd. (No.20170220).

2.4. Preparation of Sample

2.4.1. Raw Sample

500 g of semen vaccariae were powdered by multifunctional crusher (2500C multifunctional crusher, Yongkang Aizela Electric Appliance Co., Ltd) and filtrated through sieves with screen mesh 250. The powders stored in airtight package and at low temperature.

2.4.2. Charcoal Sample

According to the 2020 edition of the Chinese Pharmacopoeia [14], burnt black surface and burnt brown internal are the standards of charcoal preparation. Base on this standard, 500 g of semen vaccariae were carbonized in 20 min under 250°C by stir-frying machine (CY-10Y electric fryer, Hangzhou Fuyang Kanghua Machinery Equipment Factory). The products were powdered and filtrated through sieves with screen mesh 250. The powders stored in airtight package and at low temperature.

2.4.3. Sample of Oral Experiment

2.05 g raw powders and 2.05 g charcoal powders were put in a mortar, respectively. Add a small amount of 0.8% CMC-Na and grind it for suspension. Repeat several times until the final volume reaches 10 mL, resulting in a high-dose suspension of 205 mg/mL. Take 4 mL of high-dose suspension, dilute with 0.8% CMC Na to 8 mL, and obtain a medium dose suspension of 102.5 mg/mL. Take 3 mL of medium dose suspension and dilute to 6 mL with 0.8% CMC-Na to obtain a low-dose suspension of 51.25 mg/mL.

2.4.4. Positive Control of Oral Experiment

0.2 g Yunnan Baiyao powders were put in a mortar. Add an appropriate amount of 0.8% CMC-Na and grind it for suspension. Repeat several times until the final volume reaches 10 mL, resulting in a 20 mg/mL suspension of Yunnan Baiyao.

2.5. Group and Dose

2.5.1. Group of Topical Experiment

40 healthy KM SPF mice were randomly divided into 4 groups based on tail length: negative control group (starch), positive control group (Yunnan Baiyao powder), raw powder group, and charcoal powder group, with 10 animals in each

group.

2.5.2. Group of Oral Experiment

80 healthy KM SPF mice were randomly divided into 8 groups based on body weight: negative control group (0.8% CMC-Na), positive control group (Yunnan Baiyao), low, medium, high-dose groups of raw powders, and low, medium, high-dose groups of charcoal powders, with 10 mice in each group.

2.5.3. Dose of Oral Experiment

The dosage of semen vaccariae for adults (60 kg) is 10 g/d, so the adult dose is 0.167 g/kg. The conversion coefficient from adult dose to mouse dose is 12.33 [15], so the medium dose for mice is 2.05 g/kg. Based on the ratio of low, medium and high dose of animals (1:2:4), the low dose and high dose of animals are 1.025 g/kg and 4.1 g/kg, respectively. The adult dose of Yunnan Baiyao as the positive control is 2 g/d, so the adult dose was 0.033 g/kg. According to the conversion coefficient, the mice dose was 0.4 g/kg, as shown in Table 1.

Table 1. Group and dose for oral experiment.

group	<i>n</i>	Concentration (mg/mL)	oral volume (mL/kg)	Dose (g/kg)
negative control	10	—	20	—
positive control	10	20	20	0.4
raw group (low dose)	10	51.25	20	1.025
raw group (medium dose)	10	102.5	20	2.05
raw group (high dose)	10	205	20	4.1
charcoal group (low dose)	10	51.25	20	1.025
charcoal group (medium dose)	10	102.5	20	2.05
charcoal group (high dose)	10	205	20	4.1

2.6. Method

2.6.1. Topical Experiment

The mice were placed in a fixed cylinder and cut off at 1 cm from the tip of the tail. The timing began when the tail was cut off, and the broken end of the tail was inserted into 0.2g of powder. After 90 s, the tail was removed from powder and blood drops were adsorbed with filter paper to observe bleeding once every 20 s. The timing is stopped when no blood seepage is observed at the broken end of the tail and no obvious blood stains are detected when adsorbed with filter paper. The time from artificial wound formation to bleeding cessation was taken as the hemostatic time (HT). The general clinical conditions of the animals were observed once a day until the end of the experiment, and the hemostatic time was measured and recorded.

2.6.2. Oral Experiment

The experimental animals in all groups were given the corresponding concentration of test article or 0.8% CMC-Na by intragastric administration of 20 mL/kg once a day for 4 consecutive days (D1~D4). D3, 1 h after administration, the mice were fixed and cut off at 1 cm from the tip of the tail.

When the blood flowed out spontaneously, the timing began, and the blood was adsorbed with filter paper strip every 30 s. The bleeding time is recorded until the bleeding stops naturally (no blood when the filter paper is adsorbed). D4, 1 h after administration, a capillary glass tube was inserted approximately 4–5 mm deep into the static plexus which is after the mice angular ball, gently turned back, and blood was allowed to flow into the glass tube, for a period of time the timing began when blood flow into the glass, until the capillary glass tube was full. After removal of the blood, the capillary tube was laid flat on the table. A length of 0.5 mm was broken every 20 s and slowly opened it to the left and right to observe whether the broken section had blood clotting filaments or not. Until the blood clotting filaments appeared, and the timing stopped. The time experienced was blood clotting time.

During the experiment, the survival, body shape, skin, feces, muscle tension, gait, spirit and breathing were observed. The animals were weighed at the beginning and end of the experiment, and the bleeding time and clotting time were recorded.

2.7. Statistical Analysis

The data are expressed by ($\bar{x} \pm s$), and the SPSS 21.0 software is used for statistical analysis. One-way ANOVA was used for statistics. LSD method was used to compare the

variance uniformity between groups, and Dunnett's T3 method was used to compare the variance between groups if the variance was uneven. The test level was $\alpha = 0.05$.

3. Result

3.1. Topical Experiment

In the course of the experiment, the general clinical condition of the animals was normal and there was no animal death. Compared with the negative control groups, the bleeding time in the positive control group and charcoal powder group were significantly shorter than that in the negative control group ($P < 0.05$ or 0.01). There was no significant difference in bleeding time between the raw powder group and the negative control group ($P > 0.05$), as shown in Table 2.

The results showed that charcoal powder group had a certain hemostatic effect.

Table 2. Bleeding time in topical experiment ($\bar{x} \pm s$),

group	n	bleeding time (s)
negative group	9	229±38.0
positive group	10	150±56.2**

group	n	bleeding time (s)
raw group	10	229±74.3
charcoal group	10	175±30.2*

Note: There was one abnormal value in the negative control group (SPSS 21.0 showed that the value was greater than 3 standard deviations), so it was excluded. Compared with the negative control group: *, $P < 0.05$, **, $P < 0.01$

3.2. Oral Experiment

In general observation, in D3, two animals of raw group (high dose) died before the bleeding test, and one animal in low dose group as well as one in middle dose group died in the course of bleeding test. D4, one animal in positive control died before the blood clotting test, and one animal in raw group (high dose) died after the blood clotting test. Before the beginning of the experiment (D1), there was no significant difference in animal weight among all groups ($P > 0.05$). At the end of the experiment (D4), compared with the negative control group, the weight of the low, middle and high dose groups of raw powder significantly decreased ($P < 0.05$ or 0.01), but there was no significant difference among the other groups ($P > 0.05$), see Table 3. These results of general observation show that the semen vaccariae has potentially risk for oral use of raw products.

Table 3. Change of mice weight in oral experiment ($\bar{x} \pm s$).

group	Dose (g/kg)	Weight (g)			
		n	D1	n	D4
negative control	—	10	23.0±1.0	10	26.1±2.5
positive control	0.4	10	22.8±0.9	9	25.3±1.2
raw group (low dose)	1.025	10	22.8±0.9	9	23.1±2.5*
raw group (medium dose)	2.05	10	22.7±1.2	9	21.8±3.3**
raw group (high dose)	4.1	10	22.8±0.9	7	21.3±4.3**
charcoal group (low dose)	1.025	10	22.7±1.0	10	25.7±2.1
charcoal group (medium dose)	2.05	10	22.7±0.9	10	24.8±2.0
charcoal group (high dose)	4.1	10	22.8±0.9	10	25.8±1.1

Note: Compared with the negative control group: *, $P < 0.05$, **, $P < 0.01$

Compared with the negative control group, the bleeding time and clotting time in positive control group decreased significantly ($P < 0.05$). The bleeding time and clotting time in low dose of raw powder decreased significantly ($P < 0.05$ or 0.01). The bleeding time in high dose of raw powder de-

creased significantly ($P < 0.05$), as well. In addition, the clotting time decreased in the low, middle and high dose groups of charcoal powder ($P < 0.05$ or 0.01), as shown in Table 4. These results suggest that under the condition of oral experiment, the raw product of semen vaccariae can shorten the

clotting time and bleeding time of animals, and the charcoal product can also shorten the clotting time of animals.

Table 4. Bleeding time and clotting time in oral experiment ($\bar{x} \pm s$).

group	Dose (g/kg)	n	bleeding time (s)	n	clotting time (s)
negative control	—	10	793 ±432	10	179 ±98
positive control	0.4	10	453 ±220*	9	99 ±59*
raw group (low dose)	1.025	9	342 ±215**	9	94 ±54*
raw group (medium dose)	2.05	9	591 ±297	9	168 ±67
raw group (high dose)	4.1	8	400 ±445*	7	149 ±61
charcoal group (low dose)	1.025	10	940 ±549	10	87 ±55**
charcoal group (medium dose)	2.05	10	517 ±191	10	90 ±59**
charcoal group (high dose)	4.1	10	585 ±352	10	98 ±59*

4. Discussion and Conclusion

In the light of the records of many Chinese materia medica classics that semen vaccariae was mainly used for traumatic hemostasis caused by metal sharp weapons, we to some extent verified the hemostatic effect of semen vaccariae through tail-severing bleeding test and capillary coagulation test in mice in this study. Moreover, the results of comparing the difference of hemostatic effect before and after charcoal preparation indicate that charcoal preparation is necessary for semen vaccariae to show hemostatic effect in topical application. This indication shows that traditional Chinese medicine processing plays an important role in the efficacy of traditional Chinese medicine.

However, in animal experiments, oral administration of raw products caused death and significant weight loss, suggesting that oral administration of raw products has potential risks, which requires vigilance. On the contrary, both the ancient works of materia medica and Chinese Pharmacopoeia have not recorded any toxicity of semen vaccariae [10-14]. The reason of this contrary may be that the traditional usage of semen vaccariae is decocting with water, while in this experiment, semen vaccariae is directly taken orally with the raw powder. In other words, these two usages are not the same. Therefore, the toxicity of semen vaccariae needs further more research.

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Conflicts of Interest

All the authors do not have any possible conflicts of interest.

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