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Effect of Milk Processed Arnebiae Radix on Body Temperature of Fever Rats

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Abstract [**Objectives**] To investigate the synergistic effect of Arnebiae Radix after processing. [**Methods**] The effects of raw Arnebiae Radix and milk processed Arnebiae Radix on hypothermia in yeast-induced febrile rats were compared. [**Results**] The processed and unprocessed Arnebiae Radix at high, medium and low doses all had a certain effect on inhibiting the rise of body temperature in rats. The high dose unprocessed group, the medium dose processed group and the high dose processed group had the best inhibitory effect on body temperature, the low dose processed group could delay the fever time, and the low dose unprocessed group had poor inhibitory effect on fever. [**Conclusions**] The prepared Arnebiae Radix has enhanced drug effect, and milk processed Arnebiae Radix can be used to replace common Arnebiae Radix to reduce the dosage of Arnebiae Radix and save Arnebiae Radix resources.

Key words Arnebiae Radix, Fever model, Body temperature

1 Introduction

Arnebiae Radix (Zicao), also named Shanzicao, Zidan, Zifu, is a perennial herb of *Lithospermum* of Boraginaceae. Arnebiae Radix is the root of *Arnebia euchroma* (Royle) Johnst. and *Lithospermum erythrorhizon* of Boraginaceae, which are distributed in Xinjiang, Gansu, western Tibet, northeast China, Hebei, Henan and other places, mainly in Xinjiang and Inner Mongolia[1].

Arnebiae Radix has such effects as cooling blood, promoting blood circulation, clearing away heat and toxic materials, and relaxing bowels; and can be used for treating diseases such as excessive blood heat, purplish black macula, measles, and pyocutaneous disease. Its main active components include acetyl shikonin, β-hydroxyisovaleryl shikonin, shikonin, β, β'-dimethylacryl shikonin, *etc.*[2]. Among them, acetyl shikonin is believed to be the most important active component of Arnebiae Radix.

The processing method of Arnebiae Radix was first recorded in Master Lei's *Discourse on Drug Processing*: "The wax is steamed with water, and the wax is hard and astringent to soften its nature of promoting diarrhea". Although there are many records about the processing methods of Arnebiae Radix in ancient literature, the raw products are often used in clinical application in the past. In Mongolian medicine, Arnebiae Radix is processed by milk. Like Arnebiae Radix in traditional Chinese medicine, it is mostly used as raw medicine[3]. There are lots of processing records of Arnebiae Radix, but it is mostly used as raw medicine in clinical application, but there is no report on the comparative study of the efficacy of Arnebiae Radix before and after processing, so it is necessary to investigate whether Arnebiae Radix after processing has "synergistic" effect through experimental methods.

2 Materials and methods

2.1 Materials SPF grade male SD rats with license number of SCXK (Liao) 2015-0001 were purchased from Liaoning Changsheng Biotechnology Co., Ltd.; normal saline, sodium carboxymethyl cellulose; DXF-20D rotary crusher (Guangzhou Hengtong Machinery Equipment Co., Ltd.) and CX-886 electronic balance (Guangdong Changxie Electronic Products Factory).

2.2 Methods

2.2.1 Processing of Arnebiae Radix. Arnebiae Radix was processed according to the processing method of Arnebiae Radix recorded in *Mongolian Medicine Processing*[4]. 10 kg of Arnebiae Radix was soaked in 15 mL of fresh milk. When Arnebiae Radix was almost absorbed the milk, took out, dried in the sun, crushed and sieved with a 40-mesh sieve for later use.

2.2.2 Building of rat fever model. Ten male SD rats, weighing 180 to 240 g, were divided into normal group and experimental group. On the day of the experiment, the body temperature of the rats was measured three times before the experiment, and the average value was taken as the basal body temperature. The rats were subcutaneously injected with 20% yeast suspension 10 mg/kg and the same volume of saline on the back. After modeling, the

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body temperature was measured every hour for 8 consecutive times, and the rats whose body temperature was greater than 0.8 ℃ higher than the basal body temperature were successful.

The method for preparing the yeast suspension comprises the follow steps of: put a certain amount of dry yeast into a mortar, add a certain amount of water, grind, and take out the yeast when the yeast is pasty to prepare a normal saline suspension containing 20% of yeast for later use.

2.2.3 Experimental grouping. The rectal temperature of rats was measured three times a day in the experimental environment for three consecutive days to make them adapt, and the rats were fasted without water for 12 h before the experiment. On the day of the experiment, the temperature was measured three times, and the average temperature of the three times was taken as the basal body temperature. Rats with body temperature >38 ℃ or rectal temperature fluctuation >0.3 ℃ were eliminated. Appropriate rats were randomly divided into 9 groups: normal group, model group, low dose processed group, medium dose unprocessed group, high dose unprocessed group, Low dose processed group, high dose processed group, medium dose processed group and aspirin group, 5 rats in each group.

2.2.4 Method of administration. Before modeling, except the normal group, all the animals were given the corresponding amount of drugs by gavage, and then the yeast suspension was injected subcutaneously. According to the dose of Arnebiae Radix in the traditional prescription of Mongolian medicine Zicao Siwei Pill (Birimuke-4)[5], three dose levels of high (0.4 g/kg), medium (0.2 g/kg) and low (0.4 g/kg) were set for the experiment in combination with the dose difference between humans and rats. Aspirin group was given 0.05 g/kg by gavage. Rats in the blank group were injected with the same amount of saline according to body weight on the back, while rats in the model group were only injected with yeast suspension on the back. Body temperature was measured at 1, 2, 3, 4, 5, 6, 7 and 8 h after administration, and the change value of body temperature was calculated. The preparation method of the Arnebiae Radix suspension comprises the following steps of: crushing a certain amount of Arnebiae Radix, sieving the crushed Arnebiae Radix with a 40-mesh sieve, and dispersing and suspending the crushed Arnebiae Radix in normal saline of 0.5% sodium carboxymethylcellulose for later use.

3 Results and analysis

3.1 Results of establishment of rat fever model It can be seen from Fig. 1 that the body temperature of the rats in the model group was significantly higher than that in the normal group, and the temperature in the model group was finally increased to (39.10±0.42) ℃, exceeding 0.8 ℃, so it can be judged that the rat fever model was successfully established. As shown in Fig. 2, the body temperature of the rats decreased in the first 2 h after modeling, and began to rise after 4 h.

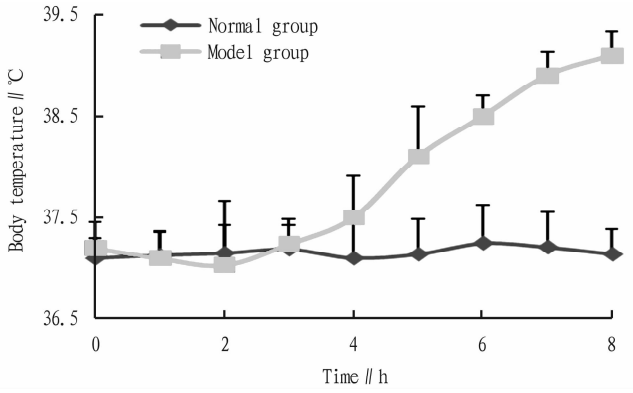


Fig. 1 Body temperature of rats in normal group and model group

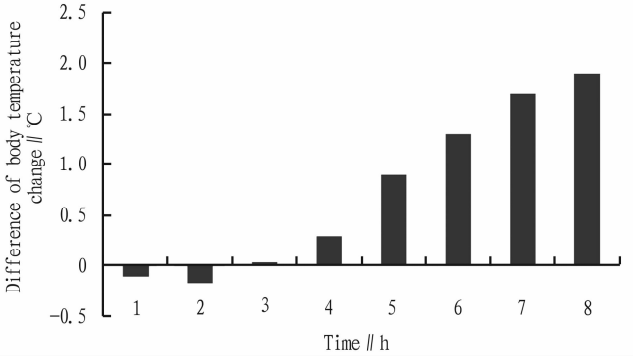


Fig. 2 Fever time after modeling

3.2 Change of body temperature in each group From Fig. 3, it can be seen that during the experiment, the fluctuation of body temperature in the normal group was very small; the body temperature of the rats in the aspirin group did not rise significantly in the first 6 h, but began to rise after 6 h; the body temperature of rats in the model group began to rise after 4 h, and the highest temperature was (39.21±0.39) ℃; the low dose processed group had a weak ability to control the body temperature of rats, and the body temperature of rats increased after 4 h of modeling, and the increase was the largest in all groups; the body temperature of medium dose unprocessed group rats began to increase significantly after 5 h; both the high dose unprocessed group and the medium dose group could inhibit the increase of body temperature in rats; the body temperature of low dose processed group rats increased rapidly. The mean body temperature difference was obtained by subtracting the basal body temperature measured at the beginning of each group from the body temperature of the rats measured at each time point in each group, as shown in Fig. 4.

From the above results, compared with the model group, there was no significant difference between low dose processed group, medium dose unprocessed group, and low dose processed group. Compared with the model group, the high dose unprocessed group, medium dose processed group, high dose processed group and aspirin group were significantly different ($P<0.05$). The body temperature decreased most significantly in the aspirin group, and the body temperature of high dose unprocessed group, medium dose processed group and high dose processed group increased slightly higher than that in the aspirin group, and also had a good effect on inhibiting the rise of body temperature in rats.

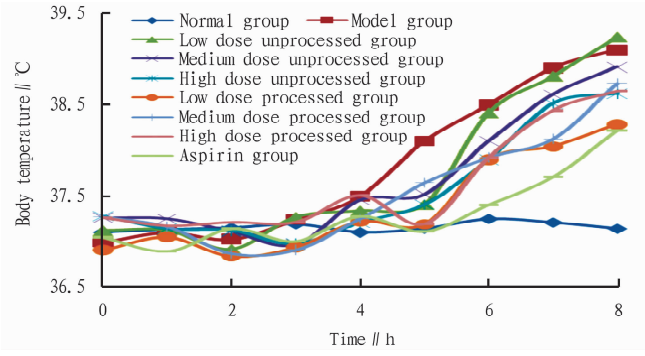
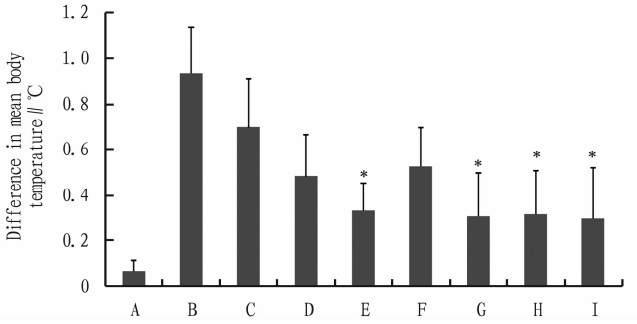


Fig. 3 Temperature change curve of rats at each time point



NOTE A. normal group; B. model group; C. low dose unprocessed group; D. medium dose unprocessed group; E. high dose unprocessed group; F. low dose processed group; G. medium dose processed group; H. high dose processed group; I. aspirin group. Compared with the model group, $P<0.05$.

Fig. 4 Change in difference of mean body temperature in each medication group

4 Discussion and conclusions

4.1 Discussion Because the temperature of different positions of the rat's intestine is different, it is necessary to insert the animal thermometer into the rat's anus at a fixed depth each time to reduce the error caused by the operation. The dry yeast fever model was slow, and the lipopolysaccharide fever model was tried, but failed, so only one rat fever model was investigated. The reason for the increase in body temperature after 6 h in the aspirin group may be that the potency of the drug decreases after 6 h, and the half-life of aspirin is 5.6 – 7.2 h[6]. If aspirin is supplemented in time, better results may be obtained.

The resources of Arnebiae Radix in China are increasingly exhausted, and Inner Mongolian *Lithospermum erythrorhizon* has become rare. The milk processed Arnebiae Radix of that low dose group and the unprocessed Arnebiae Radix of the medium dose group has the similar effect of lowering the body temperature. It can be seen that the efficacy of Arnebiae Radix is enhanced after processing, and the dosage of Arnebiae Radix can be reduced, which has certain significance in protecting the resources of Arnebiae Radix.

4.2 Conclusions In conclusion, the efficacy of Arnebiae Radix is enhanced after milk processing, and the fever of rats in each group is inhibited to different degrees, and the milk processed Arnebiae Radix has a "synergistic" effect. Specifically, the milk processed Arnebiae Radix of the high dose group and the medium dose group can well inhibit the rising of the body temperature of the rats, and the milk processed Arnebiae Radix of the low-dose group also can delay the rising time of the body temperature of the rats. Compared with the unprocessed group, the processed group with the same administration dose shows better experimental results, indicating that the efficacy of the processed Arnebiae Radix is enhanced, and the dosage of the Arnebiae Radix can be reduced under the condition that the milk processed Arnebiae Radix is used to achieve the same efficacy.

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