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RESEARCH ARTICLE

Experimental Study on the Analgesic and Arresting Bleeding Effects of Curcuma Aromatica Salisb

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Abstract: Objective: To explore and analyze the analgesic and arresting bleeding effects of Curcuma aromatica Salisb. **Methods**: The aqueous extract and alcoholic extract with the analgesic and arresting bleeding effects come from the rhizome of C. aromatica. These effects can be analyzed by writhes of the abdominal cavity on mice, hot-plate test, bleeding time by cutting the mouse's tail and recovery time of decalcifying plasma clotting. **Results**: The aqueous extract and alcoholic extract of the rhizome of C. aromatica can effectively inhibit the writhing reaction of mice, and the effect intensity is similar. The high-dose group (10 g/kg) of aqueous extract solution of C. aromatica could significantly shorten the bleeding time by cutting the mouse's tail and the recovery time of decalcifying plasma clotting. There was a significant difference in pain by heat stimulation on mice before and after aqueous extract and alcoholic extract ($P < 0.05$). **Conclusion**: The Curcuma aromatica has analgesic and arresting bleeding effects. The arresting bleeding effect is related to blood coagulation factors, and hemostatic activity is water-soluble.

Keywords: Curcuma aromatica Salisb, analgesia, arresting bleeding, writhes of the abdominal cavity, pain by heat stimulation

1 Introduction

As the dried rhizome of Curcuma longa L, C. aromatica is mainly produced in Guangdong, Guangxi and other places. Wild C. aromatica is widely distributed among them, concentrated primarily in the north, middle and northwest of Guilin, most of which are distributed at 33–565 m above sea level, showing a sporadic and isolated distribution law [1]. Its root used as medicine has the functions of promoting blood circulation and alleviating blood stasis, promoting circulation of qi and relieving pain. The relevant research results of Huang Yongqi et al. [2,3] show that the Curcuma aromatica has analgesic, arresting bleeding and anti-inflammatory effects. To provide a specific reference for the related products research, this study will take the aqueous extract and alcoholic extract of C. aromatica produced in Guilin, Guangxi as the research object and analyze the effects of the two in analgesia and arresting bleeding. The report is as follows.

2 Materials and methods

2.1 General information and extracts

The Chinese medicine Curcuma aromatica is produced in Guilin City and Heng County, Guangxi Province. The dried rhizomes of C. aromatica are taken and excavated after the stems and leaves of C. aromatica withered in winter. The sediment and fibrous roots are removed, washed, and boiled until thoroughly cooked. Under the dry environment, dry or fresh sliced, dry. The Curcuma aromatica in this article is irregular longitudinal and oblique sections, length: 3–6 cm, width 1–3 cm, thickness 1–3 mm. The outer skin of C. aromatica was light yellow-brown or gray-yellow, and the surface was rough and showed longitudinal wrinkles, and part of it had fibrous root residues. The slices are earthy yellow and slightly powdery, with more longitudinal fibers. The material is solid, and the cross-section is pale yellow. It is identified as authentic by researchers of medicinal plants in the Chinese medicine department.

Preparation of aqueous extract of rhizome of C. aromatica: chop the Curcuma aromatics into about 1cm small pieces, stove it at 60°C, and use ten times the amount of 70% ethanol for reflux extraction three times, each time for 1.5h, add 8 times the amount for the first time, six times the amount for the second time, and six times the amount for the third time, filter, and recover ethanol at low temperature, merge the filtrates, reduce pressure and concentrate to contain 1g/1ml drug solution under reduced pressure at 50°C, use ethyl acetate for continuous extraction two times at 50–55°C, adding three times the amount for the first time, and adding two times the amount for the second time, merge into the ethyl acetate layers, concentrate under reduced pressure to made dry extract, extraction yield: 4%. For temporary use, it was prepared into a 0.4% concentration, Tween-80 alcoholic extract aqueous suspension for this research experiment.

Preparation of alcoholic extract: chop the Curcuma aromatics into small pieces of about 1cm, stove it at 60°C, accurately weigh, decoct three times continuously in water, filter and keep it in the refrigerator overnight ($\geq$ 7 h). After stationary, it was centrifuged and concentrated to make a dry extract with a 12.5% extraction yield. It was prepared into a 0.4% aqueous concentration solution for this research experiment for temporary use. The dosage of aqueous extract and alcoholic extract in this research experiment was calculated according to the amount of dry raw medicinal materials.

2.2 Medicine

The medicine used in this research is Heat-clearing and pain-relieving powder (manufacturer: Nanjing Baijingyu Pharmaceutical Co., Ltd.; National Drug Administration Approval number: H32024636; Specification: 0.4238g $\times$ 10 bags); Yunnan Baiyao: (manufacturer: Yunnan Baiyao Group Co., Ltd. Company; National Drug Administration Approval number: Z53020798; Specification: 4g/bottle + 1 fuse).

2.3 Experimental animals

Analysis of the experimental animals used in this research: mice; grade: cleaning level.; body weight 18–22 g, half male and half female, a total of 70, provided by Shanghai Xingang Experimental Animal Farm, production license number: SCXK (Shanghai) 2017-0006.

2.4 Observation indicators

In this study, the Arresting Bleeding Effects (bleeding time by cutting the mouse's tail and recovery time of decalcifying plasma clotting) and the Analgesic Effects (writhes of abdominal cavity: number of writhing, inhibition rate of writhing response; pain by heat stimulation on mice) are observed in different groups of mice. Inhibition rate of writhing response = (average writhing times in the control group – average writhing times in the treatment group)/average writhing times in the control group $\times$ 100% [4]. The increase in pain by heat stimulation on mice before and after administration = (post-administration pain threshold - pre-administration pain threshold) / pre-administration pain threshold $\times$ 100% [5].

2.5 Statistical analysis

The data in this paper were all entered in SPSS26.0 to expand the statistics. The measurement content conformed to the normal distribution was expressed as mean $\pm$ standard deviation (SD) ($\pm$ S). The t-test was performed, and the difference was statistically significant($P < 0.05$).

3 Experimental methods and results

3.1 Analgesic effect

3.1.1 Comparison of writhing response

Divided into control group (equal volume), clearing heat and relieving pain group (Heat-clearing and pain-relieving powder 0.1g/kg), high-dose group of aqueous extract solution of C. aromatica (10g/kg), low-dose group (5g/kg), high-dose group (10g/kg) of alcoholic extract solution of C. aromatica (10g/kg), low-dose group (5g/kg). There were ten mice in each group, five female mice and five male mice. According to the corresponding test, 0.2mL/20g ig was administered continuously three times within 24 hours and once on the next day. Forty minutes after the last dose, each mouse was given 0.2ml of 0.7% acetic acid solution, and after 5min administration, the number of the writhing of each mouse within 10min were closely observed.

Compared with the control group, 0.4% concentration group, and Tween-80 aqueous extract solution group, the number of writhing was significantly decreased in the aqueous extract and alcoholic extract solution groups, and the difference is significant ($P < 0.05$). Compared with the control group, 0.4% concentration group, and Tween-80 aqueous extract solution group, there were substantial decreases in the clearing heat and relieving pain group, and the difference was significant ($P < 0.05$). There was no significant difference between the aqueous extract and alcoholic extract solution of C. aromatica groups at different doses ($P > 0.05$). The specific comparison results are shown in Table 1.

Table 1 Comparison of the effects of each group on the number of writhing and inhibition rate of mice induced by acetic acid [$\bar{X} \pm S$, times (n = 10)]

Group	Writhing Times	Inhibition Rate
Control group	23.86 ± 9.97	/
Clearing heat and relieving pain group	8.86 ± 7.46	62.86%
High-dose group of aqueous extract solution of C. aromatica	10.68 ± 10.75	55.23%
Low-dose group of aqueous extract solution of C. aromatica	14.39 ± 12.47	39.68%
High-dose group of alcoholic extract solution of C. aromatica	11.40 ± 11.52	52.22%
Low-dose group of alcoholic extract solution of C. aromatica	16.89 ± 11.75	29.21%

3.1.2 Comparison of pain thresholds by heat stimulation

Mice were divided into groups, and the dose used in each group was the same as in the writhing reaction experiment. This experimental study uses the ZH-6C intelligent hot and cold plate pain measuring instrument, a total of 70 mice, 10 in each group. The mice were tested qualified in advance, and each mouse's pain threshold by heat stimulation was measured before administration. The administration method of the writhing reaction experiment is that the mice were administered thrice within 24 hours and once on the next day. The pain threshold of each mouse in each group was measured 50 minutes after the last administration. There were significant differences before and after administration in the clearing heat and relieving pain group, the high-dose group of aqueous extract solution of C. aromatica (low-dose group), and a high-dose group of alcoholic extract solution (low-dose group)($P < 0.05$). The specific comparison results are shown in Table 2.

Table 2 Comparison of the effects of each group on the pain thresholds by heat stimulation in mice [$\bar{X} \pm S$, (n = 10)]

Group	Pain threshold before administration (s)	Pain threshold after administration (s)	χ^2 value	P value	Increase in pain threshold before and after administration (%)
Control group	12.85 ± 3.69	13.81 ± 4.78	0.503	0.621	6.95%
Clearing heat and relieving pain group	13.19 ± 3.68	21.44 ± 4.45*	4.518	0.000	62.55%
High-dose group of aqueous extract solution of C. aromatica	12.76 ± 4.11	19.58 ± 3.56*	3.966	0.001	53.45%
Low-dose group of aqueous extract solution of C. aromatica	13.04 ± 3.86	17.85 ± 3.71*	2.841	0.011	36.89%
High-dose group of alcoholic extract solution of C. aromatica	12.38 ± 3.06	19.18 ± 3.76*	4.436	0.000	54.93%
Low-dose group of alcoholic extract solution of C. aromatica	13.66 ± 3.58	17.15 ± 4.17*	2.008	0.060	25.55%

Note: Compared with the control group after administration, * $P < 0.05$.

3.2 Arresting bleeding effects

3.2.1 Comparison of bleeding time by cutting the mouse′s tail

Divided into control group (equal volume), high-dose group of aqueous extract solution of C. aromatica (10g/kg), low-dose group (5g/kg), high-dose group (10g/kg) of alcoholic extract solution of C. aromatica (10g/kg), low-dose group (5g/kg), Yunnan Baiyao group (0.2g/kg), a total of 6 groups. According to the above dosage, each group was continuously given 0.2 mL/20 g ig three times within 24 hours, and 1 hour after the last administration, the mice were cut off 3 mm from the tail tip. Mice were placed in water to keep a disposal state, and the bleeding time was recorded.

Compared with the control group, the bleeding time of the high-dose group of aqueous extract solution of C. aromatica was significantly different ($P < 0.05$) and shortened considerably. But there was no significant difference between the high-dose group of alcoholic extract solution of C. aromatica group (low dose) and the control group ($P < 0.05$). The specific time comparison is shown in Table 3.

Table 3 Comparison of bleeding time by cutting the mouse's tail in each group [$\bar{X} \pm$ S, s (n = 10)]

Group	Bleeding time by cutting the mouse's tail
Control group	164.58 ± 31.44
Yunnan Baiyao Group	98.25 ± 30.46
High-dose group of aqueous extract solution of C. aromatica	106.98 ± 35.59
Low-dose group of aqueous extract solution of C. aromatica	142.87 ± 32.76
High-dose group of alcoholic extract solution of C. aromatica	184.25 ± 41.67
Low-dose group (10g/kg) of alcoholic extract solution of C. aromatica	166.25 ± 36.18

3.2.2 Comparison of recovery time of decalcifying plasma clotting

They were divided into a control group (equal volume), aqueous extract solution of C. aromatica (10g/kg), and Yunnan Baiyao group(0.2g/kg), a total of 3 groups. According to the above dosage, each group was given continuously 0.2 mL/20 g ig three times within 24 hours, and 40min after the last administration, 1mL blood was taken after the neck was severed, and 0.1mL Sodium Citrate Solution(37mg/mL) was added. Centrifuge at 900 r/min for 10 min, take 0.2 mL of the supernatant and place it in a test tube, place the test tube in 37°C water for 60 s, and add 0.2 mL of calcium chloride solution (2.7 mg/mL) and mix well. Every 10s, the tube is tilted and then placed upright every 10 seconds to observe the plasma clotting time. Compared with the control group, the bleeding time of the mice in the high-dose group of aqueous extract solution of C. aromatica was significantly different ($P < 0.05$) and shortened considerably, as shown in Table 4.

Table 4 Comparison of recovery time of decalcifying plasma clotting in each group [$\bar{X} \pm$ S, s (n = 10)]

Group	Recovery time of decalcifying plasma clotting
Control group	174.68 ± 18.25
Yunnan Baiyao Group	130.27 ± 20.68
High-dose group of aqueous extract solution of C. aromatica	138.17 ± 32.66

4 Discussion

Curcuma aromatica is a wild plant of Curcuma Genus that grows in Guangxi and belongs to the local characteristic medicinal material. Guangxi Zhuang doctors believe that the Curcuma aromatica has the effects of Tonglonglu, Tonghuolu, analgesic, and arresting bleeding [6]. It is mainly used in treating angina pectoris, amenorrhea, abdominal pain, uterine fibroids, injuries, and other diseases. In traditional Chinese medicine, the Curcuma aromatica has the curative effect of activating blood circulation and qi-flowing, inducing menstruation and relieving menalgia. It can treat Xiong Xie's pain and rheumatism, and other diseases. The properties, flavour, and meridian tropism of C. aromatica are pungent, bitter, warm, Liver and spleen meridians [7]. The rhizome of C. aromatica is mainly used to treat distending pain in the stomach and abdomen, haematemesis, hematuria, Menstrual disorder, and other symptoms. The Curcuma aromatica contains many chemical constituents. According to the relevant experimental research reality of Lin Juan, Wang Shu, Zhou Fujun, et al. [8], A total of 22 compounds were isolated and identified from C. aromatica. Among them, 14 sesquiterpenoids and four curcuminoids are the representative compounds. The research on its chemical composition also provides a more scientific and reliable basis for product development. According to relevant statistical results, since the 1970s, many patients have been treated with C. aromatica, reducing inflammation and relieving pain, and the disease has been effectively cured. However, at this stage, the research on C. aromatica-related products is still in the research and development setting.

According to the survey results of relevant visits, the Curcuma aromatica is used relatively small in the folk, mainly for treating rheumatism, postpartum weakness, increased leucorrhea, and other symptoms, or as an animal medicinal to treat diarrhea and other symptoms [9]. The treatment effect of other aspects still needs further research. In the results of this study, for different algogenic models of mice, both the aqueous extract and the alcoholic extract solution of C. aromatica can play a good fighting effect. It is shown that C. aromatica has an analgesic effect, and the analgesic activity can be extracted by water or ethanol. In terms of arresting bleeding,

after cutting the mouse's tail, the application of the aqueous extract solution of C. aromatica of 10g/kg can significantly shorten the bleeding time and recovery time of decalcifying plasma clotting. It shows that the Curcuma aromatica has an analgesic effect and the analgesic effect has a specific relationship with the blood coagulation factors in the internal coagulation system. The application effect of alcoholic extract solution is not apparent, which shows that the hemostatic activity of C. aromatica is water-soluble.

To sum up, as a characteristic of Zhuang medicine in Guangxi, after this experimental study confirmed, the Curcuma aromatica has analgesic and arresting bleeding effects, which provides a scientific and reliable pharmacological reference for the research and development of related products.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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