# Anti-inflammatory and Analgesic Effects of Extract from Roots and Leaves of *Citrullus lanatus*

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**Abstract:** **Objective** To study anti-inflammatory and analgesic effects of extract from the roots and leaves of *Citrullus lanatus* and assess their acute toxicity in animals. **Methods** The mouse model with ear edema induced by xylene and the rat model with paw edema or granuloma by carrageenin or cotton pellet were used for anti-inflammatory effects of the extract. Effects of the extract on analgesia was tested respectively by measuring the latency of mice licking hind foot from hot plates and by counting the times of body twisting in response to acetic acid. The acute toxicity of the extract was determined with the method of Bliss. **Results** The extract significantly inhibited the ear edema, granuloma hyperplasia, and paw edema. It significantly lifted the pain threshold on mouse hot-plate responses and reduced their writhing times. During the 7 d observation period in its acute toxicity assay, no apparent toxic reaction was shown and all mice survived at a dose of 87 g extract per kg body weight. **Conclusion** The extract could protecte mice/rates from inflammation and analgesia, and may be safe as an orally administered natural product for humans.

**Key words:** acute toxicity; analgesia; anti-inflammation; *Citrullus lanatus*
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## Introduction

The roots and leaves of *Citrullus lanatus* (Thunb.) Matsum, et Nakai have the properties of bitter, cool, and acting on the large intestine; And their effects: Clearing away heat evil and promoting diuresis. The main indication is watery, diarrhea, dysentery, scald, and atrophic rhinitis (Jiangsu Medical College, 1997). Although a large number of pharmacological studies on *C. lanatus* (mainly its rind and flesh) were carried out worldwide, anti-inflammatory and analgesic effects of extract from roots and leaves of *C. lanatus* still remain unexplored. The present work is to carry out the anti-inflammatory and analgesic efficacy studies of the roots and leaves.

## Materials and methods

### Experimental animals

Experiments were performed using Kunming albino mice (18.2 ± 2.1) g and SD rats (180 ± 20) g, procured from the Laboratory Animal Resource Section of Guangxi Province (China), and the certificate number is SCXK (GUI) 2008-0002. All the animals were maintained in an air conditioned environment: Temperature kept at (22 ± 2) ℃, and humidity kept at 55% ± 15% with a 12 h-light and 12 h-darkness cycle. They were housed in colony cages (five animals per cage) and had free access to water and food. The animals were acclimatized to the laboratory environment for 2 d before experiments. Unless otherwise specified, there were ten animals per group comprising five male and five female in each experiment. The animals need to be fasted overnight just prior to the experiment but had free access to drinking water.

### Chemicals and reagents

Carrageenin and xylene from Taizhou Hongtaiyang Chemical Reagent Co., dexamethasone (DXM, 0.75 mg/tablet) from Zhejiang Xianju Pharmaceutical Co., acetic acid from Nanning Precision Instruments Co., and Aspirin (220 mg/tablet) from Hubei Jianyuan Chemical Co.

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**Preparation of extract from roots and leaves of *C. lanatus***

The extract prepared from roots and leaves of *C. lanatus* was collected and identified by CAI Yi in Nanning (Guangxi, China) in March 20, 2008. The preparation was as follows: The roots and leaves (2 kg) were first ground and soaked three times with water, changing water for every 2 h. The mixture was then filtered and concentrated by evaporation at 60 ℃ and got 0.156 kg extract. For animal studies, the extract and other control drugs were dissolved in a 0.5% CMC-Na solution (Guoyao Chemical Company, China, F2005-718). All drugs or controls were ig administered orally to mice in a dose volume of 20 mL/kg of mouse body weight and 15 mL/kg of rat body weight.

**Acute toxicity study**

The animals were divided into control, low, and high dose groups. Each group included 10 mice (5 male and 5 female). The manifestation, mortality and main viscera pathological morphology of mice were evaluated after ig administration of the extract.

**Effects of extract from roots and leaves of *C. lanatus* on acute inflammation-model of xylene-induced ear edema**

In this experiment, fifty male and female mice were divided into five groups randomly with 5 male and 5 female in each group. Three groups of them were used for pharmacological studies at doses of 5, 10, and 20 g extract per kg of mouse body weight; One group was administered with 0.02 g DXM/kg of mouse body weight as a positive control and one group administered with the same volume of medium as a blank control. Drugs and controls were administered once per day for 7 d continuously. Sixty minutes after the seventh administration, xylene (0.05 mL) was applied to the surface of mouse's right ear. Mice were killed 15 min later and both ears of each mouse were removed. Xylene applied area was taken out by a cork borer with a 8 mm diameter, and its weight was measured. The increase in weights (indicator of ear swelling caused by the irritant) was obtained by subtracting the weight of untreated left ear sections from that of xylene-treated right ear sections.

**Effects of extract from roots and leaves of *C. lanatus* on chronic inflammation**

**Model of cotton pellet inducing granuloma in rats** Autoclaved cotton pellets weighing (30 ± 1) mg were implanted subcutaneously through small incision made along the flank region of the rats anesthetized with ether. Different groups (ten rats for each group) were ig administered with different doses of the extract (3.5, 7, and 14 g/kg, ig), or DXM (0. 002 g/kg, ig) once per day for ten consecutive days, counting from the day when cotton pellets were inserted. The control group received medium alone. On the day 11, all the rats were sacrificed and cotton pellets were recovered/excised from the granulomatous tissues. They were dried in a hot air oven at 60 ℃ until a constant weight was achieved. Granuloma weight was obtained by subtracting the weight of cotton pellets on 0 d (before implantation) from the one at day 11 when experiments were finished (Xu, Bian, and Chen, 2002).

**Model of carrageenin-induced rat paw edema**

SD rats were randomly divided into five experiment groups and two control groups ($n$ = 10). The experiment groups were ig treated with the extract at doses of 3.5, 7, and 14 mg/kg, respectively, thirty minutes prior to injection of carrageenin (Sigma) (1% × 0.1mL) into rat's right hind paw. One hour after the injection, paw edema of rats was determined every hour for four consecutive hours. The other two groups either receiving DXM at a dose of 0.002 g/kg or medium alone, used as a positive and a blank control, respectively.

**Hot-plate experiment**

The temperature of metal surface was maintained at (55.0 ± 0.5) ℃. Latency to a discomfort reaction (licking hind paws) was taken as a pain threshold in female mice. The cut-off time was 60s. The fifty female mice were selected with the pain threshold within 5 to 30 s, and they were divided into five groups. Different groups of mice were administrated either the extract (5, 10, and 20 g/kg), or Aspirin (0.2 g/kg) once daily for seven consecutive days. In the day 7, the pain threshold was determined before administration, 30, 60, and 90 min post administration, respectively.

**Writhing experiment**

Mice were kept singly in a clear plastic observational cage (35 cm × 25 cm × 15 cm) and ig pretreated with the extract or Aspirin 60 min prior to ip injection of 0.6% acetic acid in a volume of 0.1 mL/10.0 g per mouse. After ip injection of acetic acid, the number of writhes exhibited for 15 min were

counted. Aspirin (0.2 g/kg) was used as a positive control. The writhe times and prolongation of the latency were compared between the extract treated and control mice.

**Statistical analysis**

All experimental data were expressed by mean ± standard deviation. SPSS 10.0 statistical software was used to analyze the differences between the extract, positive control treated groups, and those treated with vehicles (blank). A $P$ value ($P \leq 0.05$) from Students' test was considered statistically significant.

## Results

**Extract from roots and leaves of *C. lanatus* significantly inhibited xylene-induced ear edema in mice**

Using the xylene-induced ear edema model, we tested if the extract could protect mice from the inflammatory reaction by xylene. The increase in ear edema (mean from ten mice) was compared between the extract-treated and control (vehicle) groups (Table 1). The control group had an increase of (10.11 ± 3.63) mg for ear edema. The extract groups (treated with 5, 10, and 20 g/kg, ig) exhibited anti-inflammatory effects in a dose-dependent manner. The inhibition rate of ear edema by the extract was 55.39%, 63.90%, and 71.22%, respectively. Statistical analysis revealed that the extract treatment indeed significantly ($P < 0.01$) reduced the ear edema weights at 1 h after xylene application. The inhibition of the extract to xylene inducing inflammation was comparable to DXM, a positive control drug typically used for anti- inflammation, which had a percentage of inhibition of 66.17% compared with control group (Table 1).

**Table 1 Effects of extract from roots and leaves of *C. lanatus* on xylene-induced ear edema in mice ($\bar{X} \pm s$, $n = 10$)**

| Group | Dose / ($g \cdot kg^{-1}$) | Xylene-induced ear edema / mg | Inhitition rate / % |
|---|---|---|---|
| Control | — | 10.11 ± 3. 65 | — |
| DXM | 0.002 | 3.42 ± 2.83** | 66. 17 |
| Extract | 20.0 | 2.91 ± 1.64** | 71.22 |
| | 10.0 | 3.65 ± 3.50** | 63.90 |
| | 5.0 | 4.51 ± 2.19** | 55.39 |

**$P < 0.01$ *vs* control group

**The extract prevented the formation of granuloma triggered by cotton pellet implantation**

The examination of granuloma at the end of the treatments showed significant inhibition of granuloma formation in rats administered with the extract in comparison with control groups. The inhibition rate was 16.56%, 22.40% ($P < 0.05$), and 23.58% ($P < 0.01$), respectively. Again, such inhibition by the extract was comparable with DXM, which had a inhibition rate of 26.49% ($P < 0.01$) for granulaoma formation (Table 2).

**Table 2 Effects of extract from roots and leaves of *C. lanatus* on cotton pellet granuloma in rats ($\bar{X} \pm s$, $n = 10$)**

| Group | Dose / ($g \cdot kg^{-1}$) | Weight of cotton pellet granuloma / mg | Inhibition rate / % |
|---|---|---|---|
| Control | — | 49.41 ± 9. 64 | — |
| DXM | 0.002 | 36.32 ± 7.39** | 26.49 |
| Extract | 3.5 | 41.23 ± 6.55* | 16.56 |
| | 7.0 | 38.34 ± 5.49** | 22.40 |
| | 14.0 | 37.76 ± 6.54** | 23.58 |

*$P < 0.05$ **$P < 0.01$ *vs* control group

**Effects on carrageenin-induced paw edema of rats**

Theoretically, carrageenin-induced paw edema belongs to sub-acute inflammation. The paw edema of the rats treated by medium alone rose from 0.94 mL at 1 h to 1.32 mL at 3 h following the exposure to carrageenin and began to scale down from 1.32 mL at 3 h to 1.23 mL at 4 h. In contrast, the paw edema of the rats treated by the extract at used doses did not exceed 0.94 mL at 1 h and 1.32 mL at 3 h. The extract (3.5, 7.0, and 14.0 g/kg, ig) significantly ($P < 0.05, 0.01$) reduced the paw edema of the rats with inhibition rate of 8.36%, 14.60%, and 30.92%, compared with the control group. However, the standard positive drug, DXM (0.002 g/kg, ig) showed highly significant ($P < 0.01$) inhibition on granuloma formation with the inhibition rate of 44. 71% (Tables 3 and 4).

**Effect of extract from roots and leaves of *C. lanatus* on pain threshold of mice in the hot-plate experiment**

The extract (5.0, 10.0, and 20.0 g/kg, ig) significantly ($P < 0.05, 0.01$) increased the pain threshold at 30, 60, and 90 min after administration as compared with the blank control. Also, Aspirin at a dose of 0.2 g/kg showed significant ($P < 0.05, 0.01$) increase in the pain threshold (Table 5).

**The analgesic effect of extract from roots and leaves of *C. lanatus* of pain threshold on acetic acid-induced writhing experiment in mice**

The extract from roots and leaves of *C. lanatus*

**Table 3 Effects of extract from roots and leaves of *C. lanatus* on carrageenin-induced paw edema of rats ( $\bar{X} \pm s$, $n$ = 10)**

| Group | Dose / (g·kg$^{-1}$) | Edema volume / mL | | | |
|---|---|---|---|---|---|
| | | 1 h | 2 h | 3 h | 4 h |
| Control | — | 0.94 ± 0.11 | 1.03 ± 0.17 | 1.32 ± 0.20 | 1.23 ± 0.18 |
| DXM | 0.002 | 0.41 ± 0.21$^{**}$ | 0.58 ± 0.27$^{**}$ | 0.75 ± 0.28$^{**}$ | 0.68 ± 0.21$^{**}$ |
| Extract | 3.5 | 0.70 ± 0.31$^{*}$ | 0.83 ± 0.21$^{*}$ | 1.14 ± 0.19$^{*}$ | 1.12 ± 0.20 |
| | 7.0 | 0.72 ± 0.26$^{*}$ | 0.81 ± 0.26$^{*}$ | 1.06 ± 0.27$^{*}$ | 1.05 ± 0.24 |
| | 14.0 | 0.56 ± 0.19$^{**}$ | 0.70 ± 0.18$^{**}$ | 0.99 ± 0.18$^{**}$ | 0.85 ± 0.17$^{**}$ |

$^{*}P < 0.05$ $^{**}P < 0.01$ *vs* control group

**Table 4 Inhibitory rate of extract from roots and leaves of *C. lanatus* on carrageenin-induced paw edema of rats ( $\bar{X} \pm s$, $n$ = 10)**

| Group | Dose / (g·kg$^{-1}$) | Inhibitory rate / % | | | |
|---|---|---|---|---|---|
| | | 1 h | 2 h | 3 h | 4 h |
| DXM | 0.002 | 56.42 | 43.74 | 43.27 | 44.71 |
| Extract | 3.5 | 25.56 | 19.47 | 13.65 | 8.36 |
| | 7.0 | 23.41 | 21.33 | 19.73 | 14.60 |
| | 14.0 | 40.49 | 32.02 | 25.11 | 30.92 |

**Table 5 Effects of extract from roots and leaves of *C. lanatus* on pain threshold in the hot-plate experiment of mice ( $\bar{X} \pm s$, $n$ = 10)**

| Group | Dose / (g·kg$^{-1}$) | Pain threshold / s | | | |
|---|---|---|---|---|---|
| | | 0 min | 30 min | 60 min | 90 min |
| Control | — | 16.3 ± 5.43 | 15.6 ± 5.16 | 14.3 ± 5.54 | 15.9 ± 4.17 |
| Aspirin | 0. 2 | 15.7 ± 6.63 | 20.1 ± 7.76$^{*}$ | 25.6 ± 4.12$^{**}$ | 32.8 ± 9.71$^{**}$ |
| Extract | 5.0 | 16.1 ± 6.21 | 17.8 ± 4.34 | 19.8 ± 5.80$^{*}$ | 23.1 ± 3.66$^{*}$ |
| | 10.0 | 15.8 ± 26.01 | 25.8 ± 9.22$^{**}$ | 33.2 ± 9.98$^{**}$ | 31.4 ± 11.34$^{**}$ |
| | 20.0 | 15.2 ± 5.65 | 27.5 ± 7.53$^{**}$ | 36.9 ± 11.71$^{**}$ | 37.4 ± 12.41$^{**}$ |

$^{*}P < 0.05$ $^{**}P < 0.01$ *vs* control group

(5.0, 10.0, and 20.0 g/kg, ig) significantly ($P < 0.05$ or $P < 0.01$) increased the pain threshold in 15 min after ip injection of 0.6% acetic acid. The extract from roots and leaves of *C. lanatus* (5.0, 10.0, and 20.0 g/kg, ig) exhibited analgesic activity in a dose-dependent manner with the inhibition rate of 29.41%, 50.26%, and 59.89%, respectively, compared with the control group. Meanwhile, the standard positive drug, Aspirin (0.2g/kg, ig) showed highly significant ($P < 0.01$) analgesic activity with the inhibition rate of 79. 14% (Table 6).

**Table 6 Analgesic effects of extract from roots and leaves of *C. lanatus* on pain threshold of acetic acid-induced writhing experiment in mice ( $\bar{X} \pm s$, $n$ = 10)**

| Group | Dose / (g·kg$^{-1}$) | Writhes numbers | Inhibition rate / % |
|---|---|---|---|
| Control | — | 18.7 ± 7. 54 | — |
| Aspirin | 0. 2 | 3.9 ± 3.64$^{**}$ | 79.14 |
| Extract | 5.0 | 12.2 ± 5.12$^{*}$ | 29.41 |
| | 10.0 | 9.3 ± 6.65$^{**}$ | 50.26 |
| | 20.0 | 7.5 ± 5.57$^{**}$ | 59.89 |

$^{*}P < 0.05$ $^{**}P < 0.01$ *vs* control group

**Acute toxicity study**

None of mice died during the observation period. That is to say $LD_{50}$ can not be measured in this experiment. So the maximum tolerance experiment was done and the maximum tolerance dose (MTD) was determined according to GLP 2003 by the State Food and Drug Administration (SFDA). Twenty mice were orally perfused with the maximum single-dose (43.5 g/kg) at two time points (7 am, 5 pm) for the first day, then their manifestations were observed for 7 d. No mice died in the process. So the MTD was 87 g/kg. Besides, degeneration or necrosis was not found in stomach.

## Discussion

Different parts of *C. lantaus* have been recommended as remedies for various ailments in China. The seed and rind of this plant have been used for treating various inflammatory diseases (Xu and Ma, 1998; Peng, 2004; Zou, Li, and Zhang, 1999; Bei and Bei, 2001; Yang, 2002; Zhou, 2007; Gao, Ma, and Guo, 2004; Zou, Wang, and Pan, 2003). So, we have been studying its pharmacological action, in order to develop the extract from the roots and leaves of *C. lantaus* into one of the affluent medicine resources. Although a large number of pharmacological studies on *C. lantaus* were

carried out worldwide, anti-inflammatory and analgesic effect of extract from roots and leaves of *C. lantaus* still remain unexplored both domesticly and abroad. So there is a need to investigate its anti-inflammatory and analgesia activity and toxicity. The present study is aimed to evaluate the possible anti-inflammatory and analgesic activity of extract from roots and leaves of *C. lantaus*, keeping in view to the cardinal signs of acute and chronic inflammation and analgesia. Five experimental models, xylene-induced ear edema, cotton pellet granuloma in rats, carrageenin-induced rat paw edema, hot-plate experiment, and writhing experiment had been chosen in order to ascertain the anti-inflammatory and analgesic effect.

The results show that extract from roots and leaves of *C. lantaus* is a safe tradition Chinese medicine. Some parameters，like $LD_{50}$ and the MTD, are used to evaluate the drug toxicity. In this experiment, the permissible maximum dose of extract from roots and leaves of *C. lantaus* did not kill the mice. So the maximum tolerance experiments were done and the MTD were investigated which was 87 g/kg. It is generally agreed that it is secure if the MTD in mice once per day is higher than one hundred times of the adult clinical dose (Xie, 2000). Therefore, its acute toxicity is very small, for clinical application and experimental study. We will study the long-term toxicity in order to preferably evaluate its safety for medication. It could significantly prolong the pain threshold on hot-plate in mice and reduce the writhing times in mice. Inflammation, which involves both innate and adaptive immune mechanisms, is the response of living tissue to cell injury (Zheng and Chen, 2003). In acute and chronic inflammation models, The extract from roots and leaves of *C. lantaus* shows significant anti-inflammatory activity. The mechanism of anti-inflammatory activity of extract from roots and leaves of *C. lantaus* is not exactly known and needs further study. In this research, standard deviation is larger likely due to the gap between the weight of animals.

In conclusion, the present study clearly shows that extract from roots and leaves of *C. lantaus* possesses good anti-inflammatory activity and analgesic effect. The anti-inflammatory and analgesic activity deserve further studies to identify the possible mechanism of action as well as establish the therapeutic value in the treatment of inflammatory and pain diseases.

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