Mesaconitine, One of the Aconitine-type Alkaloids, Plays the Major Role in the Antinociceptive and Anti-inflammatory Activities of Radix Aconiti Carmichaeli (Chuan Wu)

MEI CHOU LAI[1], I-MIN LIU[2], SHORONG-SHII LIOU[2], YUAN-SHIUN CHANG[1]

[1]*Graduate Institute of Chinese Pharmaceutical Sciences, College of Pharmacy, China Medical University, Taichung, Taiwan, R.O.C.*

[2]*Department of Pharmacy & Graduate Institute of Pharmaceutical Technology, Tajen University, Yen-Pou, Ping Tung Shien, Taiwan, R.O.C.*

* Corresponding author. Tel.: +886-4-22030380-5502; fax: +886-4-22083362

E-mail address: yschang@mail.cmu.edu.tw (Y.S. Chang)

ABSTRACT

The present study was undertaken to investigate the antinociceptive and anti-inflammatory activities of the aqueous extracts of Radix Aconiti Carmichaeli (Chuan Wu) by oral gavage. Carrageenan-induced paw edema was served as an acute inflammation model in mice. The acetic acid-induced writhing test and formalin-induced nociceptive responses in the early and late phases of mice were used to assess the analgesic activity. The contents of *Aconitum* alkaloids were analyzed by high-performance liquid chromatography. The order of concentration of aconitine-type alkaloids in Radix Aconiti Carmichaeli was mesaconitine > aconitine > hypaconitine. Radix Aconiti Carmichaeli at the dosage of 60 mg/kg not only effectively inhibited carrageenan-induced paw edema, but also significantly attenuated the abdominal constriction induced by an intraperitoneal injection of acetic acid and late phase of pain response caused by a subplantar injection of formalin in mice. Mesaconitine and aconitine are effective in the decreasing the painful sensation elicited by acetic acid in mice. The aqueous extracts of Radix Aconiti Carmichaeli exhibits a greater antinociceptive activity and a more expressive anti-inflammatory effect probably due to the presence of high content of mesaconitine.

Keywords: Aconitum alkaloids; antinociceptive activity; anti-inflammatory activity; Radix Aconiti Carmichaeli (Chuan Wu)

INTRODUCTION

Plants of *Aconitum* genus (Ranunculaceae) are widely distributed across North Asia and North America. This perennial plant grows to a height of 3 ft (1 m) and has tuberous roots, palmately lobed leaves, blue or white flowers with large hoodlike upper sepals, and an aggregate of follicles[(1)]. It is stated that there are 167 species of *Aconitum* grown in China and among 44 kinds of them have been used in folk medicine administered by oral or topical route to treat rheumatic pain, paralysis due to stroke, carbuncle and furuncle[(2)]. The tubers and roots of *Aconitum* contain many diterpenoid alkaloids which have analgesic, antipyretic and local anaesthetic properties, but they are highly toxic and there is a narrow margin of safety between therapeutic and toxic dose[(3)]. Hence, it was considered that investigations for these medicinal properties might give scientific authentication to the traditional claims.

The main root of *A. carmichaelii* Debeaux (Radix Aconiti Carmichaeli) is an aconite-containing herb that was named Chuan Wu in Mandarin. Several studies have been devoted to confirm antinociceptive activities of plants that are part of traditional medicine. Mediation of μ-opioid receptor in the antinociception of lateral roots of *A. carmichaelii* Debeaux are identified[(5)]. Since diterpenoid alkaloids are important biologically active secondary metabolites and the main constituents of the genus *Aconitum*, their amounts could be an important index in quality evaluation and biological activities of these crude drugs[(4)]. The antinociceptive and anti-inflammatory effects are examined, in order to determine which alkaloids may be a basis for the medicinal properties attributed to *Aconitum* plants.

MATERIALS AND METHODS

I. *Materials*

Radix Aconiti Carmichaeli were collected from a pharmacy in Chengdu, the capital of Sichuan Province in China, and identified by Prof. Y. C. Wu, China Medical University, Taiwan. Voucher

specimens of Radix Aconiti Carmichaeli have been deposited at the Herbarium of Graduate Institute of Chinese Pharmaceutical Sciences, College of Pharmacy, China Medical University, Taichung, Taiwan, under the reference numbers of CMC7021 and CMC7022, Aconitine, acetic acid, formalin, λ-carrageenan and indomethacin were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Mesaconitine and hypaconitine were purchased from Boppard (H. K. Co., Ltd., Hong Kong). All other reagents were obtained from standard sources.

Ⅱ. *Aqueous Extract Preparation*

One kilogram of powdered samples (100 mesh) as immersed in 5000 mL distilled water for 30 min, and then boiled for 1 h. Filtration and collection of the extracts were triplicated. The combined decoctions were evaporated to dryness. The yield percentage of the crude aqueous lyophilized extract from Radix Aconiti Carmichaeli is 17.62% (w/w). For the pharmacological tests, the lyophilized extract was dissolved in saline solution prior to its use.

Ⅲ. *Detection of Aconitum Alkaloids*

Aconitine, hypaconitine and mesaconitine were each accurately weighed (1 mg) and dissolved with 10 mL absolute methanol to prepare stock solutions. One gram of aqueous extract of Radix Aconiti Carmichaeli was mixed with 30 mL 100% MeOH and then extracted ultrasonically for 35 min. This extraction process was repeated twice. The extract solutions were combined and evaporated under a stream of nitrogen gas to dryness. The dried residues were then dissolved in 10 mL 100% MeOH and filtered through a membrane filter (0.45 μm) prior to injection.

HPLC experiments were carried out on a preparative HPLC system equipped with a Waters 2695 separation module, a Waters 2996 photodiode array detector (Waters; Milford, MA, USA), and a XBridge Shield RP-18 column (4.6 I.D. ×250 mm, particle size 5 μm). The eluents were A (aqueous 0.03 M ammonium hydrogen carbonate, pH 9.5 adjusted with concentrated ammonia) and B (100% acetonitrile). The flow rate was maintained at 0.8 mL/min with an isocratic system consisting of 85%

solvent A and 15% solvent B. The detection wavelength was set at 235 nm. The column temperature was 35°C and the sample injection volume was 10 μL[6].

Expressed as relative standard deviations (RSDs), precision was evaluated by HPLC runs with standard solutions at three concentrations under the optimal condition five times in one day for intra-day variation test and twice a day on three consecutive days for inter-day variation test.

Ⅳ. *Animals*

Male BALB/c albino mice weighing 18 to 22 g were obtained from the National Laboratory Animal Center (Taipei, Taiwan). The animals were fed with standard chow (Purina Mills, Inc.), given water *ad libitum*, and maintained under well-ventilated conditions with a 12 - 12 light-dark cycle (light on at 6:00 am) in our animal center. All animal procedures were performed according to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, as well as the guidelines of the Animal Welfare Act. All tests were conducted under the guidelines of the International Association for the Study of Pain [7].

Ⅴ. *Drug Administration*

Extract of tested *Aconitum* plants as well as alkaloids were orally administered in an equivalent volume of 0.5 mL/100 g body weight of the animal. Vehicle-treated animals received only normal saline by oral gavage in the same volume. Positive control animals received indomethacin (10 mg/kg) intraperitoneally (i. p.). Drugs were freshly prepared on the day of the experiments.

Ⅵ. *Acute Oral Toxicity Studies*

Acute oral toxicity studies were performed according to OECD-423 guidelines (acute toxic class method) [8]. Male mice (n = 10) were fasted for 4 h with free access to water only. The aqueous extracts of tested Radix Aconiti Carmichaeli and alkaloids were administered orally at the desired doses and mortality was observed for 3 days.

Ⅶ. *Anti-Inflammatory Activity*

The carrageenan-induced paw edema test, an acute inflammatory model first proposed by Winter *et al*.[9], was used with modifications in the present study. 0.05 mL of 1% (w/v) carrageenin in normal saline solution was injected intradermally into the plantar side of the right hind paw of the mouse. Animals were administered with *Aconitum* extracts (p. o.) or indomethacin (i. p.) 1 h prior to carrageenin injection. Paw volumes were measured using a plethysmometer (model PP01, Diagnostic & Research Instruments Co., Ltd., Taiwan) at hourly interval for four hours after the injection of the carrageenan.

Ⅷ. *Antinociceptive Activity*

(Ⅰ) *Acetic Acid-Induced Writhing Test*

This test was performed by the modified method described by Koster *et al.*[10]. Animals were first pretreated with either aqueous extracts or alkaloids via peritoneum administration whilst for the standard drug; indomethacin was given intraperitoneally at 10 mg/kg. Test compounds were administered 1 h before the i. p. injection of 0.1 mL/10 g body weight of 0.75% (v/v) acetic acid to induce the typical stretching response. After induction, pairs of mice were placed in separate boxes and the writhings or stretchings per animal were counted for 5 min under a double blind observation for 15 min. The antinociceptive effect was measured by calculating the mean reduction in the number of abdominal constriction for *Aconitum* extracts or indomethacin as compared with the vehicle-treated group.

(Ⅱ) *Formalin Test*

The method used was similar to that described by Hunskaar and Hole[11]. To induce nociception, mice were injected under the dorsal surface skin of the right hind paw with 20 μL of diluted formalin (1%) using a needle of 30 gauge. Immediately after, each mouse was placed into a glass cylinder

equipped with mirrors to enable a total panorama of the nociceptive behavior. The number of shakings and the time duration spent in licking the injected paw was taken as nociceptive response. Two periods of high licking and shaking activity were considered: the first one was obtained right after injection and lasted for 5 min, as the early phase (neurogenic phase). The second period was observed 20-30 min after formalin injection and denominated late phase (inflammatory phase). Animals were administered with *Aconitum* extracts (p. o.) or indomethacin (i. p.) 1 h before formalin test. Limb-licking time during each of the phases was recorded and used as an indicator of pain.

Ⅸ. *Statistical Analysis*

Data are shown in the mean ± S.E.M. for each group of animals at the number (*n*) indicated in Tables and Figures. Statistical differences among groups were determined using two-way repeated-measures ANOVA. The Dunnett range post-hoc comparisons were used to determine the source of significant differences where appropriate. A *P*-value < 0.05 was considered statistically significant.

RESULTS

Ⅰ. *Quantitative Analysis*

HPLC chromatograms of standards and the sample solution are shown in Figure 1. Linear regression analysis for each alkaloid was performed by the external standard method. The calculated results are given in Table 1, where *a* and *b* were the coefficients of the regression equation $y = ax + b$ (*x* referred to the concentration of the alkaloid (μg/mL) ; *y* denoted the peak area) and r presented the correlation coefficient of equation. All the alkaloids showed good linearity (r = 0.99) in a relatively wide concentration range. The average recovery rate for the alkaloids was nearly 98% (Table 2). The precision of the intra-day and inter-day data was indicated by RSD which were less than 3.2% for each alkaloid at three concentrations (Table 3). The order of concentration of three aconitine-type alkaloids in Radix Aconiti Carmichaeli was mesaconitine (292.5 ± 2.1 μg/g) > aconitine (49.4 ± 0.8

μg/g) > hypaconitine (18.8 ± 0.6 μg/g).

Ⅱ. *Acute Oral Toxicity Test*

Radix Aconiti Carmichaeli did not cause any mortality in mice even at the highest oral dose (60 mg/kg) employed. Radix Aconiti Carmichaeli at the oral dosages of 20, 40 and 60 mg/kg were chosen for further pharmacological studies. Additionally, the mortality was not observed in mice either receiving aconitine or mesaconitine and hypoaconitine at an oral dose of 0.5 mg/kg for 3 days.

Ⅲ. *Carrageenin-Induced Paw Edema*

Subplantar injection of carrageenan in mice showed a time-dependent increase in paw thickness (Figure 2A); this increase was observed at 1 h and was maximal at 3 h after administration in the vehicle-treated groups. It was found that indomethacin (10 mg/kg) markedly reduced the paw edema induced by carrageenin at all assessment times (Figure 2A). Radix Aconiti Carmichaeli reduced the edema formation of the paw induced by carrageenin at all phases of the experiments.

The percentage of inhibition of Radix Aconiti Carmichaeli and 10 mg/kg indomethacin were calculated at 3 h as compared to the optimum edema effect in control group as showed in Figure 1B. Radix Aconiti Carmichaeli at oral dosage of 60 mg/kg exerted 33.9 ± 5.1% inhibition of edema while indomethacin (10 mg/kg, i. p.) showed 60.1 ± 4.7% inhibition.

Ⅳ. *Acetic Acid-Induced Writhing Response*

The effect of Radix Aconiti Carmichaeli on writhing response in mice was shown in Table 4. Indomethacin (10 mg/kg, i. p.) showed marked inhibition of writhes by 83.2%. It was found that Radix Aconiti Carmichaeli exibited an inhibition of the writhing response induced by acetic acid. An increase in the doses of Radix Aconiti Carmichaeli resulted in a greater inhibition and this inhibition approached to 50% at the highest dose (60 mg/kg).

Table 4 also showed that aconitine and mesaconitine at the oral dose of 0.5 mg/kg were effective

in inhibiting the writhings in mice by 75.3 ± 2.5 and 80.4 ± 3.2%, respectively; while oral treatment mice with 0.5 mg/kg of hypaconitine slight led to 15.1 ± 2.1% reduction of number of writhing.

Ⅴ. *Formalin-Induced Paw Licking in Mice*

In the early phase of pain response, a dose-dependent reduction in paw licking time was observed in mice treated with Radix Aconiti Carmichaeli (Table 5). However, indomethacin (10 mg/kg, i. p.) could not change significant the paw licking time in the early phase of pain response but markedly reduced the licking time in the late phase of the formalin test (Table 5).

The oral administration of animals with Radix Aconiti Carmichaeli produced a dose-dependent inhibition on licking time in formalin-induced late phase. Radix Aconiti Carmichaeli at the highest dose (60 mg/kg) showed an activity similar to the reference drugs used, indomethacin (10 mg/kg, i. p.) in the reduction of the licking time period in the formalin-induced late phase (Table 5).

DISCUSSION

Carrageenan-induced paw edema is a suitable test for evaluating anti-inflammatory drugs, which has frequently been used to assess the anti-edematous effect of natural products[9]. Although not as effective as indomethacin, Radix Aconiti Carmichaeli was shown to be effective in inhibiting the carrageenan-induced paw edema in mice at an oral dose of 60 mg/kg. Actually, development of edema in the paw after injection of carrageenan is a biphasic event[12]. The early phase (1-2 h) involves the release of serotonin and histamine; kinins play a role in the middle phase[13], while prostaglandins(PG) appear to be the most important mediators in the final phase (3-5 h) of the postcarrageenan response[14]. Based on this, it could be speculated that the suppression of the first phase may be due to inhibition of the release of early mediators, such as histamine and serotonin, and the action in the second phase may be explained by an inhibition of cyclooxygenase. It is suggested that Radix Aconiti Carmichaeli probably exerted anti-inflammatory activity through the inhibition of those inflammatory mediators of the acute inflammation. The antinociceptive and anti-inflammatory

activities of Radix Aconiti Carmichaeli are thus worthy of further identification.

The abdominal constriction response induced by acetic acid is widely used for antinociceptive screening, which enables the detection of peripheral antinociceptive activity of compounds. Acetic acid causes pain by liberating endogeneous substances and many others that excite pain nerve endings[15]. In mice there is an increase in the peritoneal fluid levels of PGE2 and PGF2α, as well as in lipooxygenase products[16], liberation of sympathetic nervous system mediators [17] and the nociceptive activity of acetic acid might be due to the release of cytokines, such as tumor necrosis factor-α, interleukin (IL)-1β, and IL-8, by resident peritoneal macrophages and mast cells[18]. Indomethacin and other nonsteroidal anti-inflammatory drugs (NSAIDs) can inhibit number of writhes in this model by inhibiting of cycoloxygenase enzyme in peripheral tissues, thus interfering with the mechanism of transduction in primary afferent nocciceptors by blocking the effect or the synthesis and/or the release of inflammatory mediators[19]. Therefore, anti-inflammatory substances may also be involved in the peripheral analgesic activity. Although not as effective as indomethacin, that Radix Aconiti Carmichaeli was found to present a significant antinociceptive as well as an expressive anti-inflammatory activities, indicating that the anti-inflammatory mechanism of Radix Aconiti Carmichaeli, likewise NSAIDs, could probably be due to the blockade of the effect or the release of endogenous substances that excite pain nerve endings.

The formalin test produced a distinct biphasic response and different analgesics may act differentially in the early and late phases of this test. There are lines of evidence that suggest centrally acting drugs such as opioids inhibit both phases equally[20]; peripheral inflammatory processes are involved in the late phase and are blocked by NSAIDs while the first phase seems unaffected [11,21]. Therefore, the formalin test was employed to further clarify the possible antinociceptive activity of Radix Aconiti Carmichaeli. Radix Aconiti Carmichaeli was found able to exert an inhibitory effect on the early phase of formalin test, suggesting that pain modulation by Radix Aconiti Carmichaeli was partly mediated by opioid pathway. Additionally, the strong inhibition of Radix Aconiti Carmichaeli on the late phase of formalin test is similar to those obtained from the acetic acid-induced writhing

test and carrageenan-induced edematogenic test. Antinociceptive effects of Radix Aconiti Carmichaeli in both phases of the formalin test suggest an involvement at both central and peripheral levels.

In our present study, Radix Aconiti Carmichaeli was delivered by oral route; in consequence inflammation was effectively inhibited at 40 and 60 mg/kg doses. Although the unprocessed roots are too toxic for internal use, it is important to mention that no mortality was observed from acute oral toxicity studies even at the highest oral dose (60 mg/kg) of both *Aconitum* plants. It can be considered as the reference of safe strategy for the internal application of Radix Aconiti Carmichaeli to relieve acute inflammation.

As shown in our experiments, mesaconitine was the most abundant while hypaconitine is less in Radix Aconiti Carmichaeli. Actually, mesaconitine has been indicated as the predominant alkaloid in the unprocessed aconitine-type diterpene alkaloids[22]. Besides, mesaconitine and aconitine are effective in the decreasing the painful sensation elicited by acetic acid, clearly indicating the critical role of mesaconitine in the greater antinociceptive and anti-inflammatory potency of Radix Aconiti Carmichaeli.

Radix Aconiti Carmichaeli (Chuan Wu) has been long served as the main source of the traditional medicinal aconite drugs in China. The present work is highly relevant since it demonstrated the *in vivo* anti-inflammatory activity of the oral administration of Chuan Wu and indicates c*armichaeli* species should be used to relieve acute inflammation

It conclusions, the aqueous extracts of Radix Aconiti Carmichaeli exhibits an antinociceptive activity at both central and peripheral levels and an expressive anti-inflammatory effect. Mesaconitine is the most prevalent alkaloid of Radix Aconiti Carmichaeli participating in peripheral pathway for inflammatory pain relief.

ACKNOWLEDGMENTS

The present study was supported by grants (CCMP91-CT-104, CCMP95-TP-014) from the

Committee on Chinese Medicine and Pharmacy, Department of Health, Executive Yuan, Taiwan, R.O.C.

REFERENCES

1. Akeroyd, J. R. and Charter, A. O. 1993. *Aconitum* L. In Flora Europaea. Second ed. pp. 254-256, Tutin, T. G., Burges, N. A., Charter, A. O., Edmondson, J. R., Heywood, V. H., Moore, D. M., Valentie, D. H., Walters, S. M., Webb, D. A. (Eds.), Cambridge University Press, Cambridge.
2. Bisset, N. G. 1981. Arrow poisons in China (Part II)**:** *Aconitum*-botany, chemistry, and pharmacology. J. Ethnopharmacol. 4 : 247-336.
3. Hikino, H., Konno, C., Takata, H., Yamada, Y., Yamada, C., Ohizumi, Y., Sugio, K. and Fujimura, H. 1980. Anti-inflammatory principles of *Aconitum* roots. J. Pharmacobio-Dyn. 3: 514-525.
4. Wang, Z., Wen, J., Xing, J. and He, Y. 2006. Quantitative determination of diterpenoid alkaloids in four species of *Aconitum* by HPLC. J. Pharm. Biomed. Anal. 40: 1031-1034.
5. Liou, S. S., Liu, I. M., Lai, M. C. and Cheng, J. T. 2005. Comparison of the antinociceptive action of crude Fuzei, the root of *Aconitum*, and its processed products. J. Ethnopharmacol. 99: 379-383.
6. Wang, Z., Wen, J., Xing, J. and He, Y. 2006. Quantitative determination of diterpenoid alkaloids in four species of Aconitum by HPLC. J. Pharm. Biomed. Anal. 40: 1031-1034.
7. Zimmermann, M. 1983. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16: 109-110.
8. Ecobichon, D. J. 1997. The basis of toxicology testing. CRC Press, New York, pp. 43-86.
9. Winter, C. A., Risley, E. A. and Nuss, G. W. 1962. Carrageenan-induced oedema in hind paw of the rats as an assay for anti-inflammatory dugs. In: Proceedings of The Society for Experimental Biology and Medicine, Vol. III, pp. 544-547.
10. Koster, R., Anderson, M. and Beer, E. J. 1959. Acetic acid for analgesic screening. Federal Proceedings 18:412-417.
11. Hunskaar, S. and Hole, K. 1987. The formalin test in mice: dissociation between inflammatory

and non-inflammatory pain. Pain 30:103-114.

12. Vinegar, R., Schreiber, W. and Hugo, R. 1969. Biphasic development of carrageenan oedema in rats. J. Pharmacol. Exp. Ther. 166: 96-103.
13. Di Rosa, M. and Sorrentino, L. 1968. The mechanism of the inflammatory effect of carrageenan. Eur. J. Pharmacol. 4: 340-342.
14. Di Rosa, M. 1972. Biological properties of carrageenan. J. Pharm. Pharmacol. 24: 89-102.
15. Collier, H. O. J., Dinneen, J. C., Johnson, C. A. and Schneider, C. 1968. The abdominal constriction response and its suppression by analgesic drugs in the mouse. Br. J. Pharmacol. Chemother. 32: 295-310.
16. Deraedt, R., Jouquey, S., Delevallee, F. and Flahaut, M. 1980. Release of prostaglandins E and F in an algogenic reaction and its inhibition. Eur. J. Pharmacol. 61: 17-24.
17. Duarte, I. D. G., Nakamura, M. and Ferreira, S. H. 1988. Participation of the sympathetic system in acetic acid induced writhing in mice. Braz. J. Med. Biol. Res. 21: 341-343.
18. Ribeiro, R. A., Vale, M. L., Thomazzi, S. M., Paschoalato, A. B. P., Poole, S., Ferreira, S. H. and Cunha, F. Q. 2000. Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic acid in mice. Eur. J. Pharmacol. 387: 111-118.
19. Nakamura, H., Shimoda, A., Ishii, K. and Kadokawa, T. 1986. Central and peripheral analgesic action of non-acidic non-steroidal anti-inflammatory drugs in mice and rats. Arch. Int. Pharmacodyn. Ther. 282: 16-25.
20. Shibata, M., Ohkubo, T., Takahashi, H. and Inoki, R. 1989. Modified formalin test: characteristic biphasic pain response. Pain 38: 347-352.
21. Rosland, J. H., Tjolsen, A., Maehle, B. and Hole, K. 1990. The formalin test in mice: effect of formalin concentration. Pain 42: 235-242.
22. Csupor, D., Wenzig, E. M., Zupkó, I., Wölkart, K., Hohmann, J. and Bauer, R. 2009. Qualitative and quantitative analysis of aconitine-type and lipo-alkaloids of *Aconitum carmichaelii* Roots. J.

Chromatogr. A 1216: 2079-2086.

Table 1

Linear regression equation of the alkaloids

Compounds	Regression equation	r
Aconitine	y = 10067 x -1084.71	0.9999
Mesaconitine	y = 15998 x -126.15	0.9998
Hypaconitine	y = 13642 x -1092.34	0.9999

Values were obtained from 4 determints of each group.

Table 2

Contents of aconitum alkaloids in Radix Aconiti Kusnezoffii and recovery

	Contents (μg/g)		
Samples	Aconitine	Mesaconitine	Hypaconitine
Radix Aconiti Carmichaeli	49.4 ± 0.8	292.5 ± 2.1	18.8 ± 0.6
Recovery (%)	98.47%	98.72%	99.37%

Values (mean ± S.E.M.) were obtained from 4 determints of each group.

Table 3

Precision of the intra-day and inter-day measurements

Compounds	Concentration (μg/mL)	RSD %	
		Intra-day	Inter-day
Aconitine	20.00	1.13	2.67
	2.50	1.03	1.89
	0.31	3.12	2.51
Mesaconitine	20.00	2.83	2.44
	2.50	1.19	1.59
	0.31	3.03	2.84
Hypaconitine	20.00	1.24	1.92
	2.50	1.39	1.49
	0.31	3.06	2.28

Values were obtained from 4 determints of each group.

Table 4

Effects of Radix Aconiti Carmichaeli and *Aconitum* alkaloids on acetic acid-induced writhing response in mice

Treatments	Number of writhes (15 min)	Inhibition (%)
Vehicle	30.4 ± 1.8	—
Indomethacin (10 mg/kg, i.p.)	5.1 ± 1.0[b]	83.2 ± 2.4
Radix Aconiti Carmichaeli (mg/kg, p.o.)		
20	26.3 ± 2.1	13.4 ± 2.2
40	25.1 ± 0.8[a]	17.2 ± 1.8
60	14.2 ± 1.7[a]	53.2 ± 2.6
Aconitine (0.5 mg/kg, p.o.)	7.6 ± 0.9[b]	75.3 ± 2.5
Mesaconitine (0.5 mg/kg, p.o.)	5.9 ± 0.8[b]	80.4 ± 3.2
Hypaconitine (0.5 mg/kg, p.o.)	25.8 ± 1.2[b]	15.1 ± 2.1

Values (mean ± S.E.M.) were obtained from each group of 8 animals. [a]$P < 0.05$ and [b]$P < 0.01$ compared to the values of vehicle-treated group, respectively.

Table 5

Effects of Radix Aconiti Carmichaeli on the early phase and late phase of formalin test in mice

	First phase		Second phase	
	Licking time (sec)	Inhibition (%)	Licking time (sec)	Inhibition (%)
Vehicle	96.4 ± 7.9	—	164.6 ± 9.2	—
Indomethacin (10 mg/kg, i.p.)	90.2 ± 8.4	8.1 ± 1.2	49.2 ± 5.6[b]	70.2 ± 3.2
Radix Aconiti Carmichaeli (mg/kg, p.o.)				
20	65.4 ± 5.2[a]	32.4 ± 2.9	81.7 ± 4.8[a]	50.6 ± 2.7
40	48.1 ± 4.3[b]	50.1 ± 3.4	70.6 ± 5.1[a]	57.1 ± 3.1
60	35.6 ± 3.8[b]	63.5 ± 2.7	55.9 ± 3.6[b]	65.9 ± 2.4

Values (mean ± S.E.M.) were obtained from each group of 8 animals. [a]$P < 0.05$ and [b]$P < 0.01$ compared to the values of vehicle-treated group, respectively.

Figure Legends

Figure 1. HPLC chromatograms of (A) aconitine, (B) mesaconitine, (C) hypaconitine and (D) Radix Aconiti Carmichaeli.

Figure 2. (A) Effects of the oral administration of aqueous extract of Radix Aconiti Carmichaeli on carrageenin-induced paw edema in mice. (B) Percentage inhibition of carrageenin-induced paw edema at 3 h in mice on aqueous extract of Radix Aconiti Carmichaeli. Indomethacin (10 mg/kg) was administered intraperitoneally to the separate group of mice. The vehicle (normal saline) used to dissolve the tested medications was given at the same volume. Values (mean ± S.E.M.) were obtained from each group of 8 animals. $^{a}P < 0.05$ and $^{b}P < 0.01$ compared to the values of vehicle-treated group at the indicated times, respectively.

Figure 1

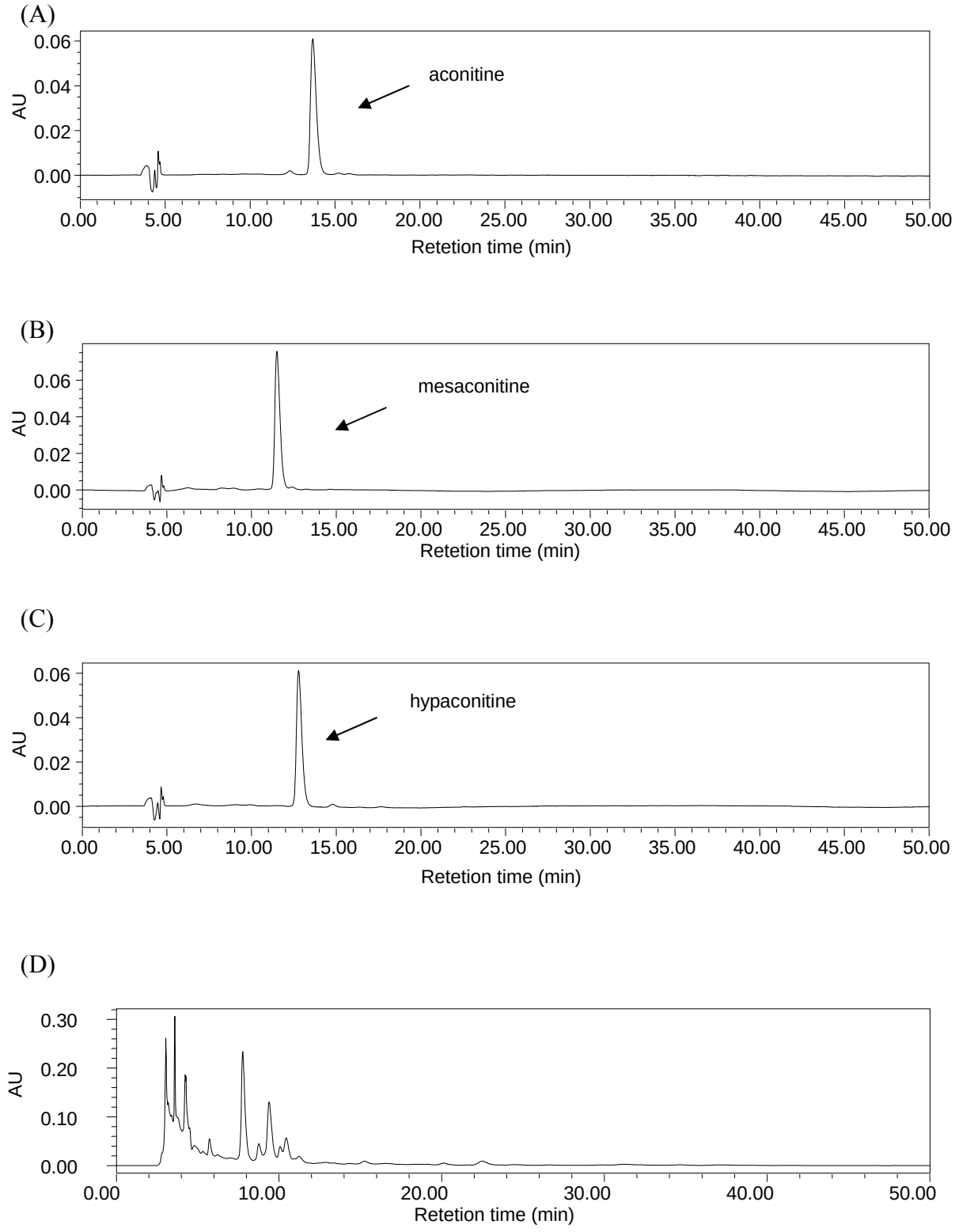
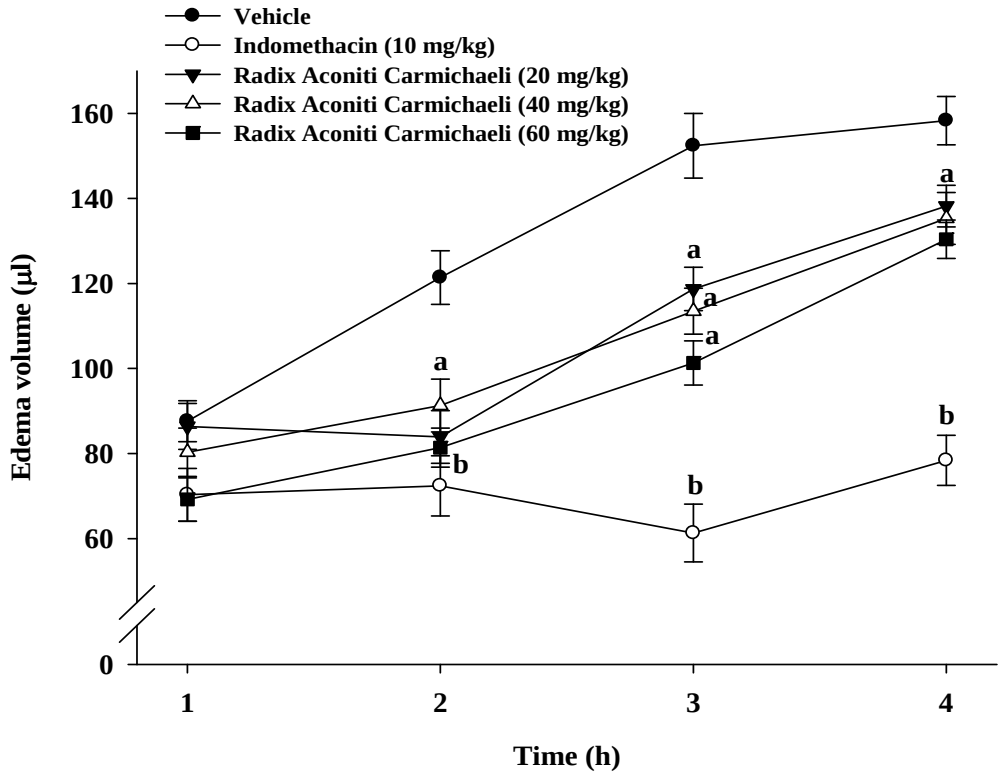
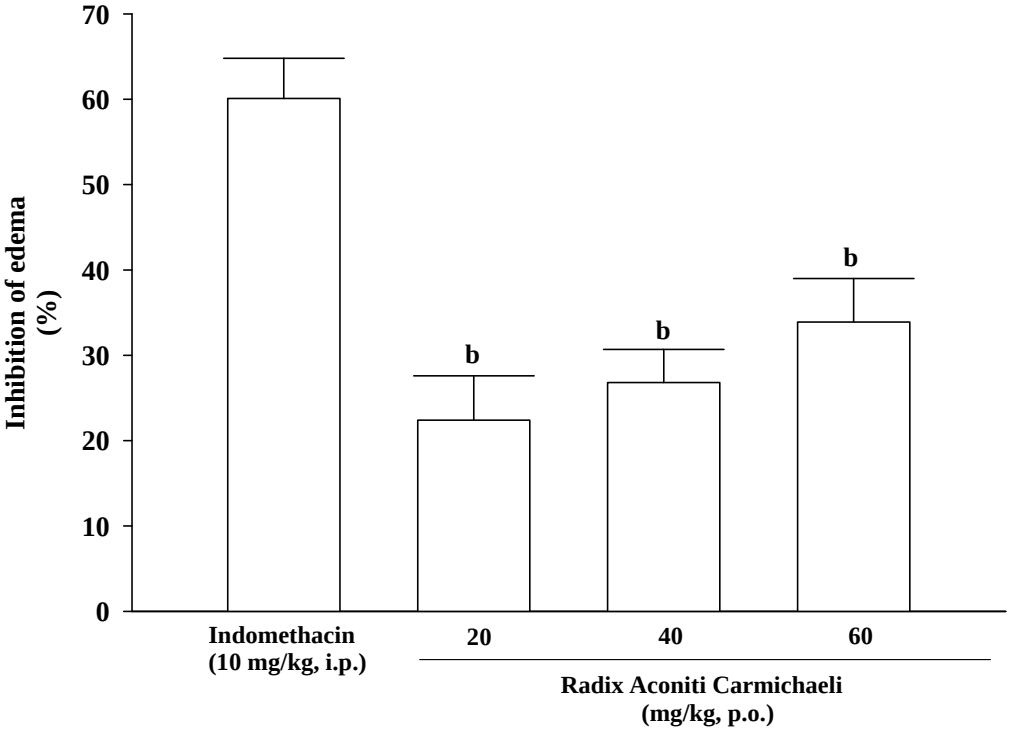

(A)
aconitine
AU
0.06
0.04
0.02
0.00
0.00 5.00 10.00 15.00 20.00 25.00 30.00 35.00 40.00 45.00 50.00
Retetion time (min)
(B)
mesaconitine
AU
0.06
0.04
0.02
0.00
0.00 5.00 10.00 15.00 20.00 25.00 30.00 35.00 40.00 45.00 50.00
Retetion time (min)
(C)
hypaconitine
AU
0.06
0.04
0.02
0.00
0.00 5.00 10.00 15.00 20.00 25.00 30.00 35.00 40.00 45.00 50.00
Retetion time (min)
(D)
AU
0.30
0.20
0.10
0.00
0.00 10.00 20.00 30.00 40.00 50.00
Retetion time (min)

Figure 2

(A)


Vehicle
Indomethacin (10 mg/kg)
Radix Aconiti Carmichaeli (20 mg/kg)
Radix Aconiti Carmichaeli (40 mg/kg)
Radix Aconiti Carmichaeli (60 mg/kg)
Edema volume (μl)
160
140
120
100
80
60
0
1
2
3
4
Time (h)
a
a
a
a
b
b
b

(B)


Inhibition of edema (%)
70
60
50
40
30
20
10
0
b
b
b
Indomethacin (10 mg/kg, i.p.)
20
40
60
Radix Aconiti Carmichaeli (mg/kg, p.o.)

中文摘要

本研究川烏的水萃取物，經由口服管灌方式小鼠予以投藥，研究止痛及抗發炎的活性。鹿角菜膠誘導小鼠足腫脹，相當於急性發炎的模式。醋酸引起的扭體實驗和福馬林致痛反應的早期和晚期階段的小鼠皆被用來評估鎮痛活性。烏頭屬中生物鹼的含量，藉由高效液相層析儀進行分析。在川烏中，烏頭鹼型生物鹼的含量順序為新烏頭鹼>烏頭鹼>次烏頭鹼。川烏在 60 mg/kg，在小鼠上不僅有效抑鹿角菜膠導致足腫脹，而且顯著減輕，因腹腔注射醋酸所引起腹部的收縮，及足蹠下注射福馬林引起後期的疼痛反應。新烏頭鹼和烏頭鹼都能有效地減少醋酸所誘發小鼠的疼痛感覺。川烏的水萃取物由於存在高含量的新烏頭鹼，因此表現出更好的鎮痛及抗發炎作用。

關鍵字：烏頭類生物鹼; 鎮痛活性；抗發炎活性;川烏