The Antinociceptive and Anti-inflammatory Action of the CHCl₃-Soluble Phase and Its Main Active Component, Damnacanthal, Isolated from the Root of Morinda citrifolia

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**Morinda citrifolia** (Rubiaceae, Noni) is a traditional medicine with various pharmacological activities. We investigated if the MeOH-, CHCl₃- and BuOH-soluble phase and its main active component, damnacanthal, isolated from the Noni root, have antinociceptive and anti-inflammatory actions in mice. The CHCl₃-soluble phase (3 g/kg, per os (p.o.)) significantly reduced pain-related behavior observed in the formalin test. These effects were not suppressed by pretreatment with naloxone (1 mg/kg, intraperitoneally (i.p.)), an opioid receptor antagonist. The CHCl₃-soluble phase (3 g/kg, p.o.) significantly reduced histamine-induced paw edema. The MeOH- and BuOH-soluble phase had no effect in either test. Furthermore, damnacanthal (10—100 mg/kg, p.o.) exerted an antinociceptive effect on chemical nociceptive stimuli, and decreased histamine-induced paw edema. Damnacanthal was weakly bound to the histamine H₁ receptor. These data suggest that the CHCl₃-soluble phase of the Noni root has antinociceptive and anti-inflammatory effects. Furthermore, these effects of damnacanthal isolated from the Noni root is mediated in part by the histamine H₁ receptor.

**Key words** Morinda citrifolia; damnacanthal; histamine H₁ receptor; antinociception; anti-inflammatory

**Materials and Methods**

**Animals** Male ddY mice (age, 5—6 weeks) were obtained from SLC (Hamamatsu, Japan). Mice were housed in cages at 23—24 °C with a 12-h light–dark cycle (light on at 8 a.m. to 8 p.m.). Food and water were available ad libitum. The present study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, which has been adopted by the Japanese Pharmacological Society. Experiments were approved by Ethical Committee for Animal Experimentation at Kobe Gakuin University (approved number: A060601-9).

**Plant Materials** The dried roots of the tropical plant Noni (*Morinda citrifolia*, Rubiaceae) were collected in August 2004 at Okinawa in Japan and the plant was identified by Dr. K. Kamiya. A voucher specimen has been deposited in Department of Pharmacognosy and Natural Product Chemistry, Kobe Gakuin University.

**Extraction, Fractionation and Isolation** Dried powdered roots were extracted with MeOH ten under reflux. Extracts were then filtered and evaporated on a rotary evaporator under reduced pressure. These MeOH extracts were then suspended in H₂O. They were partitioned with chloroform (CHCl₃) and the butanol (BuOH) layer concentrated to dry. The obtained extract was freeze-dried to give the CHCl₃ extract. The CHCl₃ extract (122 g) was chromatographed on Sephadex LH-20 using CHCl₃–MeOH (1 : 1) to give an anthraquinone-containing fraction. This fraction was subjected to preparative high-performance liquid chromatography on a Symmetry C₁₈ column to isolate damnacanthal (CHCl₃−MeOH, 1 : 100). The antinociceptive activity of the CHCl₃−MeOH (1 : 100) fraction was examined using the formalin test.

**Antinociceptive Tests** Male ddY mice (age, 5—6 weeks) were housed in cages at 23—24 °C with a 12-h light–dark cycle (light on at 8 a.m. to 8 p.m.). Food and water were available ad libitum. The present study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, which has been adopted by the Japanese Pharmacological Society. Experiments were approved by Ethical Committee for Animal Experimentation at Kobe Gakuin University (approved number: A060601-9).

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fraction was subjected to repeated SiO₂ column chromatography using n-hexane–CHCl₃ solvent system. It was purified by preparative TLC using a CHCl₃–MeOH solvent system to afford compounds.

HPLC was performed with a Diol column (Inertsil Diol 5 mm, 4.6×250 mm, GL Sciences, Tokyo, Japan). The detection wavelength was 254 nm. Elution was carried out with n-hexane: CHCl₃ (a, 25 : 75), (b, 50 : 50) and (c, 75 : 25) at a flow rate of 1 ml/min. The injection volume was 10 μl (1.0 mg/ml, chloroform-soluble phase in CHCl₃). Damnacanthal contained in the chloroform-soluble phase could be separated by HPLC using three solvent systems (Fig. 1A). The yields of damnacanthal from the dried root 0.434%. The purified by HPLC using three solvent systems (Fig. 1A). The yields of damnacanthal from the dried root 0.434%. The purified damnacanthal were analysed by IR, UV, 1H- and 13C-NMR spectra, and the chemical structures of the isolated damnacanthal are shown in Fig. 1B. Damnacanthal: Yellow amorphous powder; IR νmax cm⁻¹: 3070, 1668, 1647, 1565, 1344, 1329, 1132, 980; UV λmax (MeOH) nm (log ε): 283 (4.23), 247 (4.26), 203 (4.12); 1H-NMR (400 MHz, pyridine-d₅) δ: 7.77 (1H, s, H-4), 8.24 (1H, dd, J=7.5, 1.3 Hz, H-5), 7.64 (1H, td, J=7.5, 1.2 Hz, H-6), 7.70 (1H, td, J=7.5, 1.3 Hz, H-7), 8.33 (1H, dd, J=7.5, 1.2 Hz, H-8), 10.57 (1H, s, H-11), 4.14 (3H, s, 1-OCH₃); 13C-NMR (100 MHz, pyridine-d₅) δ: 166.58 (C-1), 119.51 (C-2), 166.41 (C-3), 112.67 (C-4), 141.58 (C-4a), 126.96 (C-5), 133.67 (C-6), 134.89 (C-7), 127.39 (C-8), 135.41 (C-8a), 180.07 (C-9), 118.21 (C-9a), 182.22 (C-10), 132.92 (C-10a), 194.98 (C-11), 64.31 (1-OCH₃).

Sample Preparation and Drugs The MeOH (3 g/kg), CHCl₃ (3 g/kg) and BuOH (3 g/kg)-soluble phase and damnacanthal (10—100 mg/kg) were orally administrated to mice 30 min before each test. Samples were suspended in 1% CMC-Na. Indomethacin (50 mg/kg), a non-steroidal anti-inflammatory drug (NSAIDs), was orally administrated to mice 30 min before each test. The area under the curve (AUC) value for the effect of restraining paw edema on each mouse was then calculated.

Histamine-Induced Paw Edema Histamine (0.5 μmol, 10 μl) was injected intraplantarly (i.pl.) into the right hind paw to induce acute inflammation. The thickness of the injected hind paw was measured every 10 min or 60 min for 300 min after histamine injection (i.pl.). The intraperitoneal administration of diphenhydramine (30 mg/kg), a histamine H₁ receptor antagonist, was used as a positive control in the test. The area under the curve (AUC) value for the effect of restraining paw edema on each mouse was then calculated.

Histamine H₁ Receptor Binding Assay The histamine H₁ receptor binding affinity assay was conducted at Cerep Incorporated (Celle l’Evescault, France), according to an established method. The influence of damnacanthal (10⁻⁹—10⁻⁷ M) on histamine H₁ receptors was tested in a radioligand binding assay with HEK-293 cells transfected with the human recombinant histamine H₁ receptor using [³H]-pyrilamine (1 nmol/l; incubation time, 60 min at 22 °C). Bound radioactivity was measured with a scintillation counter. The histamine H₁ binding assay was done in duplicate. Non-specific binding was defined using 1 μM unlabeled pyrilamine.

Statistical Analyses Data were mean±S.E.M. The statistical significance of differences between the control and CHCl₃ soluble phase-treated group and damnacanthal-treated group were analyzed using one-way ANOVA followed by Scheffe’s multiple-comparison test for the acetic acid writhing test and formalin test. p<0.05 was considered significant.

RESULTS

Effect of the MeOH-, CHCl₃- and BuOH-Soluble Phase of the Noni Root on the Chemical Nociceptive Stimuli in the Formalin Test The CHCl₃-soluble phase (3 g/kg) did not reduce pain response time in the early phase, but significantly suppressed pain-related behavior in the late phase (p<0.01) of the formalin test. Similarly, indomethacin (50 mg/kg) as a positive control significantly attenuated pain-related behavior in the late phase of the formalin test, but not the early phase. However, the MeOH (3 g/kg)- and BuOH (3 g/kg)-soluble phase had no effect in the formalin tests (Fig. 2).

Effect of NLX on CHCl₃ Soluble Phase-Induced Antinociception The antinociceptive effect in the late phase of the CHCl₃-soluble phase (3 g/kg) was not affected by pretreatment with NLX (1 mg/kg). On the other hand, morphine (5 mg/kg) significantly attenuated pain-related behavior in the early and late phase in a NLX-reversible manner (Fig. 3).

Effect of the CHCl₃-Soluble Phase of the Noni Root on Histamine-Induced Paw Edema The saline-injected hind paw to induce acute inflammation. The thickness of the injected hind paw was measured every 10 min or 60 min for 300 min after histamine injection (i.pl.). The intraperitoneal administration of diphenhydramine (30 mg/kg), a histamine H₁ receptor antagonist, was used as a positive control in the test. The area under the curve (AUC) value for the effect of restraining paw edema on each mouse was then calculated.

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paws used as control showed no increase of paw volume during the entire experiment; on the other hand, hind paw edema was successfully induced by intraplantar injection of histamine. Administration of the CHCl$_3$-soluble phase showed significant inhibition of the edema. Diphenhydramine (30 mg/kg) significantly inhibited the paw edema (Fig. 4).

**Effect of Damnacanthal on Histamine-Induced Paw Edema**

The thickness of the injected hind paw was measured between 0 min and 300 min. The thickness measured every 10 min or 60 min for 300 min after the histamine injection (i.pl.) at 0.5 μmol is described in the Materials and Methods. The area under the curve (AUC) was calculated. Diphenhydramine was used as a positive control. Each column indicates mean±S.E.M. (n=5—6). **p<0.01, *p<0.05 vs. Vehicle, Scheffe test.

**Effects of Damnacanthal in the Displacement of [3H]-Pyrilamine in Human HEK293 Cells**

Damnacanthal dis-
Histamine $H_1$ radioligand ([3H]pyrilamine) displacement curves for drugs using HEK293 cells. Displacement of [3H]pyrilamine binding to the histamine $H_1$ receptor by different concentrations of [3H]pyrilamine. The data, shown as percentage of specific binding, fit according to a one-site ligand–receptor model.

![Fig. 7. Displacement of [3H]-Pyrilamine Binding by Damnacanthal or Histamine](image)

**DISCUSSION**

We examined the antinociceptive effect of the MeOH-, CHCl$_3$- and BuOH-soluble phase in a model of acute inflammatory pain induced by formalin. In general, the early phase (0—10 min) of formalin-induced pain behavior is produced by direct stimulation of primary afferent nerves, whereas pain behaviors associated with the late phase (10—30 min) are related to the sensitization of dorsal horn neurons due to the initial barrage of primary afferent input during the early phase or the formalin-induced inflammatory reaction. In the present study, we obtained evidence that the CHCl$_3$-soluble phase exhibited a significant antinociceptive effect on chemical nociceptive stimuli. In particular, the CHCl$_3$-soluble phase reduced pain-related behavior in the late phase. The antinociceptive effect of the CHCl$_3$-soluble phase may therefore be (at least in part) due to attenuation of the central sensitization induced by certain inflammatory substances.

It is well known that opioidergic nervous systems regulate the various types of pain. The activation of opioid receptors has a pivotal role in the production of analgesic effects in animal and human. Some researchers have shown that the aqueous extracts of the fruit and roots of the Noni plant have an antinociceptive effect via opioid receptors. In the present study, these effects were not inhibited by pretreatment of NLX on the antinociceptive effect of the CHCl$_3$-soluble phase. The CHCl$_3$-soluble phase may therefore produce an antinociceptive effect without acting on opioid receptors. This discrepancy may be dependent upon the main ingredients of the Noni root.

The CHCl$_3$-soluble phase may have an anti-inflammatory effect because administration of indomethacin attenuated pain-related behavior in the late phase of the formalin test. To examine the anti-inflammatory effect of the CHCl$_3$-soluble phase, we evaluated histamine-induced paw edema in mice. Histamine is a potent mediator of acute inflammation. It is produced in the early phase of acute inflammation to increase vascular permeability. The CHCl$_3$-soluble phase significantly suppressed the paw edema induced by histamine. This test was inhibited by treatment with diphenhydrine, so this response seems to be partly mediated by the histamine $H_1$ receptor. Therefore, we suggest that the CHCl$_3$-soluble phase exerts anti-inflammatory effects by mechanisms similar to those of histamine.

Histamine activates polymodal nociceptors and produces pain. The role of histamine in pain is different in the central nervous system and peripheral nervous system. In the former, histamine $H_1$ and $H_2$ receptors are involved in the modulation of pain sensations. In the peripheral nervous system, it is reported that pyrilamine (histamine $H_1$ receptor antagonist) and cimetidine (histamine $H_2$ receptor antagonist) show analgesic effects in the formalin test. In addition, anti-histaminic drugs show antinociceptive effects on formalin-induced stimuli. Thus, histamine seems to be involved in the stimulation of nociceptive fibers, and its antagonists show antinociceptive effects, considering histaminergic neurons are important for pain modulation.

We demonstrated that damnacanthal is the major component in the CHCl$_3$-soluble phase of the Noni root. Damnacanthal has been reported to inhibit: selective tyrosine kinase activity, activation of nuclear factor kappa-B, progression of cancer, and the human immunodeficiency virus (HIV). However, there is no evidence that damnacanthal has an antinociceptive and anti-inflammatory effect on chemical nociceptive stimuli. Here, we found that damnacanthal possesses antinociceptive and anti-inflammatory effects. Therefore, in the present study, damnacanthal may have been one of the active components of the CHCl$_3$-soluble phase of the Noni root. Interestingly, damnacanthal was weakly bound to the histamine $H_1$ receptor. Damnacanthal may therefore exert its antinociceptive and anti-inflammatory effects through binding to in part the histamine $H_1$ receptor.

In conclusion, we demonstrated that the CHCl$_3$-soluble phase of the Noni root has antinociceptive and anti-inflammatory effects. In addition, damnacanthal was shown to be the main active component through in part a histamine $H_1$ receptor-mediated system in the production of these effects in the CHCl$_3$-soluble phase.

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**REFERENCES**