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Original article

Anti-inflammatory and analgesic activity of ononitol monohydrate isolated from *Cassia tora* L. in animal models

Paulrayer Antonisamy [a,1], Muniyappan Dhanasekaran [b,1], Ha-Rim Kim [a], Sung-Gang Jo [a], Paul Agastian [c], Kang-Beom Kwon [a,*]

[a] Department of Korean Physiology, Wonkwang University School of Korean Medicine, 460 Iksan-daero, Iksan City, Jeonbuk 570−749, Republic of Korea
[b] Division of Ethnopharmacology, Entomology Research Institute, Loyola College, Chennai, Tamil Nadu, India
[c] Department of Plant Biology and Biotechnology, Loyola College, Chennai 600 034, Tamil Nadu, India

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ABSTRACT

Ononitol monohydrate (OM) was isolated from *Cassia tora* L. leaves. The anti-inflammatory and analgesic activities of OM have been examined in male Wistar rats and mice. The efficacy of OM against inflammation was studied by using carrageenan-induced paw oedema, croton oil-induced ear oedema, acetic acid-induced vascular permeability, cotton pellet-induced granuloma and adjuvant-induced arthritis. The analgesic activity of OM was assessed using the acetic acid-induced abdominal constriction response, formalin-induced paw licking response and the hot-plate test. In acute type inflammation models, maximum inhibitions of 50.69 and 61.06% ($P < .05$) were noted with 20 mg/kg of OM in carrageenan-induced hind paw oedema and croton oil-induced ear oedema, respectively. Treatment of OM (20 mg/kg) meaningfully ($P < .05$) reduced the granuloma tissue formation by cotton pellet study at a rate of 36.25%. OM (20 mg/kg) inhibited 53.64% of paw thickness in adjuvant-induced arthritis model. OM has also been produced significant ($P < .05$) analgesic activity in acetic acid-induced abdominal constriction response, formalin-induced paw licking response and in hot-plate test suggesting its peripheral and central analgesic potential. The outcomes of the present study proposed that OM influenced on the anti-inflammatory and analgesic activities.

1. Introduction

Inflammation is complicated biological and bio-chemical processes consist of vascular tissues and nonspecific reactions activated by natural immune responses against irritants, infection, injury and injured cells. The microcirculation is the central playground where the course of inflammatory events was assessed and examined. Inflammation contains a lengthy sequence of molecular reactions and cellular actions, which are intended to renovate a tissue from simple skin cut or to cure numerous burn wounds. An inflammatory process in cellular and tissue levels consist of a chain of events with dilation of arterioles and venules, increased blood vessel permeability, and blood flow with infiltration of leukocytes into the tissues (Antonisamy et al., 2017; Schmid-Schonbein, 2006). Medicinal plants showed essential roles as foundations of effective anti-inflammatory agents. According to the World Health Organization (WHO), nearby three-quarters of the world's inhabitants depend on traditional medicines for their healthiness.

Cassia tora Linn. (family Leguminosae) is an undershrub which found all over the tropical Asian countries and grows well in wasteland. It is usually known as 'Sicklepod'. The leaves of *C. tora* have numerous anthraquinone glycosides which are well recognized for their therapeutic importance. The present study was undertaken to evaluate the anti-inflammatory and analgesic potential of ononitol monohydrate (OM) isolated from *C. tora* against inflammation and pain induced in animal models.

2. Materials and methods

2.1. Animals

Male Wistar albino rats (200–220 g) and mice (24–28 g) were used for the experimentations. Animals were maintained on 12 h

* Corresponding author.
E-mail address: desson@wku.ac.kr (K.-B. Kwon).
[1] These authors contributed equally to this work.

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light/dark rotation at nearly 25 ± 1 °C with the humidity of 60–70% and have free access to diet and water. All animals were adapted to new environment minimum two weeks before initiating the studies. All experiments were carried out using six animals in each respective group. All the animal studies were directed agreeing to the ethical norms permitted by Ministry of Social Justice and Empowerment, Government of India and Institutional Animal Ethics Committee guidelines.

2.2. Chemicals and drugs

Indomethacin, Freund's complete adjuvant, croton oil, formalin, morphine and naloxone were obtained from Sigma- Aldrich (St. Louis, MO, USA). Carrageenan, carmellose (carboxymethyl cellulose (CMC)) and Evans blue dye were obtained from Himedia (Mumbai, Maharashtra, India).

2.3. Ononitol monohydrate identification and characterization

The isolation and identification of ononitol monohydrate (OM) has been previously reported (Dhanasekaran et al., 2009). The chemical structure of OM is shown in Fig. 1.

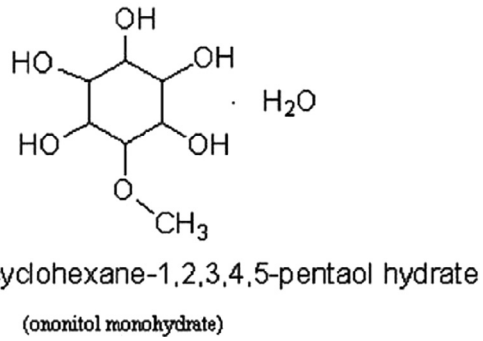

Fig. 1. Structure of ononitol monohydrate.

2.4. Anti-inflammatory studies

2.4.1. Carrageenan-induced paw oedema in rats

OM (20 mg/kg) and indomethacin (10 mg/kg) dissolved in 0.5% CMC and administered orally 1 h before carrageenan induction. After that 1% carrageenan dissolved in saline (0.1 ml) was injected subcutaneously into the right hind paw of each rat. Each hind paw thickness was measured initially and then at 1, 2, 3, 4, 5 and 6 h after the injection of carrageenan using a digital vernier calipers (Winter et al., 1962).

2.4.2. Croton oil-induced ear oedema in mice

Ear oedema was induced to the inner surface of the right ear in mice by 10 ml of croton oil (5% in acetone) application. OM (2.0 mg per ear) was treated topically to the right ear around 60 min before the croton oil application. Equal volume of acetone applied to the left ear. Indomethacin (0. 5 mg per ear) was used as a reference drug. Four hours after the treatment of the croton oil, animals were sacrificed by cervical dislocation and the ear plugs (6 mm Ø) were detached from each group. The oedematous level was quantified through the weight variance between the two plugs (Tubaro et al., 1985).

2.4.3. Acetic acid-induced vascular permeability in mice

All the mice from different groups were intravenously injected with 0.2 ml Evans blue dye (0.25% in normal saline) to the tail vein one hour after oral administration of OM (20 mg/kg). Control animals treated with an equal volume of vehicle (0.5% CMC) or indomethacin (10 mg/kg). Intraperitoneal injection of 1 ml/100 g of acetic acid (0.6%, v/v) was treated thirty minutes later. Thirty min after the acetic acid injection, animals were sacrificed by cervical dislocation and each peritoneal cavity was washed with normal saline (3 ml) and collected into heparinized tubes. After centrifugation, supernatant containing dye content was measured at 610 nm with spectrophotometer (Whittle, 1964).

2.4.4. Cotton pellet-induced granuloma in rats

Autoclaved cotton pellets (35 ± 1 mg) induced granuloma was created on the axilla region of the rats after anaesthetized with ether. Different groups of rats were treated by OM (20 mg/kg) or indomethacin (10 mg/kg) once every day for seven successive days from the day of cotton pellet implantation. The control group treated with vehicle (1 ml/kg). On the eighth day, cotton pellets were removed from each group of animals and dried in a hot air oven at 60 °C until solid weight received. Granuloma level was measured by subtracting the cotton pellet weight on 0 day (before start of the experiment) from the cotton pellet weight on the eighth day (end of the experiment) (Winter and Porter, 1957).

2.4.5. Adjuvant-induced chronic arthritis in rats

OM (20 mg/kg) or indomethacin (10 mg/kg) was treated orally to rats once daily for 14 successive days. Freund's complete adjuvant (0.1 ml) was injected into subplantar region of each rat on third day. Right hind paw swelling of the control and treated animals was monitored on day 3, 6, 9, 12, 15, 18 and 21 with digital vernier calipers (Newbould, 1963).

2.5. Analgesic tests

2.5.1. Acetic acid-induced abdominal constriction response in mice

Mice were divided into seven groups ($n = 6$). Each mouse injected with 0.75% acetic in a volume of 0.1 ml/10 g body weight into the peritoneal cavity and animals were placed in a clear plastic box. Five minutes after the acetic acid injection, the number of abdominal constrictions was counted for 15 min. Test drugs OM (20 mg/kg p.o.), indomethacin (10 mg/kg p.o.), morphine (05 mg/kg s.c.), morphine + naloxone ((05 mg/kg s.c. + 02 mg/kg i.p.), OM + naloxone (20 mg/kg p.o. + 02 mg/kg i.p.), indomethacin + naloxone (10 mg/kg p.o. + 02 mg/kg i.p.) and control vehicle (0.5 ml 0.5% CMC p.o.) were treated 1 h before the acetic acid injection (Mungantiwar et al., 1999).

2.5.2. Formalin-induced paw licking response in mice

Mice were distributed into two sets of seven groups ($n = 6$). Test drugs OM (20 mg/kg p.o.), indomethacin (10 mg/kg p.o.), morphine (05 mg/kg s.c.), morphine + naloxone (05 mg/kg s.c. + 02 mg/kg i.p.), OM + naloxone (20 mg/kg p.o. + 02 mg/kg i.p.), indomethacin + naloxone (10 mg/kg p.o. + 02 mg/kg i.p.) and control vehicle (0.5 ml 0.5% CMC p.o.) were treated 1 h before formalin injection to animals in the first set (early phase) and 40 min before formalin injection to animals in the second set (late phase), respectively. Mice were subcutaneously injected with 50 μl of formalin (1% in normal saline) into the right dorsal hind paw. The time period of animals spent to licking the injected paw was examined during 0–5 min (early phase for first set of animals) and during 20–30 min (late phase for second set of animals) after formalin injection (Reisine and Pasternack, 1996).

2.5.3. Hot-plate test in mice

Mice were divided into five groups ($n = 6$). A mouse from each group was placed on a hot plate maintained at 55 ± 5 °C in order to assess central analgesic activity of drugs. Hot plate latency was recorded based on the time elapsed by the animal either to

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