Original article

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

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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.

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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

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.

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).

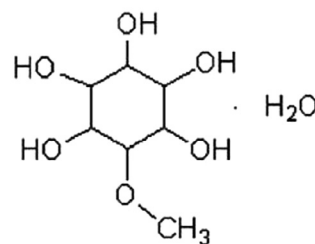### 2.4.2. Croton oil-induced ear oedema in mice

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

Fig. 1. Structure of ononitol monohydrate.

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

hind paw licking or to jump off from the surface. Before drug treatment, the response time of each mouse (jumping response or licking of the forepaws) was measured at 0 and 10 min intervals. The average values of two readings were considered as the initial reaction time. Mouse with baseline latencies of <5 s or >30 s were removed from the analysis. The initial reaction time followed by the treatment of OM (20 mg/kg p.o.), morphine (05 mg/kg s.c.), n aloxone + morphine (02 mg/kg i.p. + 05 mg/kg s.c.), naloxone + OM (02 mg/kg i.p. + 20 mg/kg p.o.) and vehicle (0.5 ml 0.5% CMC p.o.) and reaction time was measured at 30 min (Parkhouse and Pleuvry, 1979).

2.6. Statistical analysis

Data were evaluated with one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc tests. $P < .05$ was considered as statistically significant.

3. Results and discussion

In the current experimental analysis, we have assessed the anti-inflammatory and analgesic efficacy of ononitol monohydrate (OM) against different in vivo experimental models.

3.1. Carrageenan-induced hind paw oedema in rats

Carrageenan-induced hind paw oedema is an appropriate examination for appraising anti-inflammatory drugs and has commonly been used to evaluate the anti-oedematous activity of natural products (Antonisamy et al., 2017). Oral treatment of animals with OM produced considerable inhibition of carrageenan-induced hind paw oedema. As shown in Fig. 2, ononitol monohydrate (OM), at dose of 20 mg/kg evidently reduced the oedema formation of the hind paw induced by carrageenan at different time schedule. OM and reference drug indomethacin (10 mg/kg) produced an inhibitory activity on the paw oedema formation even at 6 h after treatment.

3.2. Croton oil-induced ear oedema in mice

The second method applied for the anti-inflammatory screening of OM was the croton oil-induced mouse ear oedema model, which has firm benefits for natural product analysis (Antonisamy et al., 2011). As shown in Fig. 3A, topical apply of croton oil on rat ears made significant oedema development. OM at the dose 2 mg per ear considerably inhibited the ear oedema formation.

3.3. Acetic acid-induced vascular permeability in mice

Acetic acid-induced vascular permeability is a typical capillary permeability assay in mouse model (Antonisamy et al., 2011). OM treatment significantly inhibited the increased vascular permeability demonstrating the overwhelming of the vascular response in acute inflammation process. As shown in Fig. 3B, OM displayed an inhibitory action against peritoneal capillary permeability brought by acetic acid induction in mice model. Similarly, the reference drug indomethacin (10 mg/kg) also produced inhibitory effect on peritoneal capillary permeability.

3.4. Cotton pellet-induced granuloma test in rats

Exploration of the activity of OM on the proliferative phase of inflammation exposed significant inhibition against granuloma tissue formation. The effects of OM and positive control (indomethacin) against cotton pellet induced granuloma in rats are shown in Fig. 3C. Both OM (20 mg/kg) and indomethacin (10 mg/kg) significantly withdrawn the formation of granuloma compared with control. This result proposed that OM showed significant activity against granulomatous inflammation.

3.5. Adjuvant-induced arthritis in rats

Adjuvant arthritis in rat model imitates numerous clinical and pathological characters of human rheumatoid arthritis, such as paw swelling, ankylosis and joint erosions and it is the most advanced frequently used animal model for rheumatoid arthritis (Oliver and Brahn, 1996; Antonisamy et al., 2011). In this experimental study, we have applied adjuvant-induced arthritis model in rats to validate that OM mediated inhibiting activity against adjuvant arthritis in rats. The mean paw swelling value was around 8.24 mm at day 21 in the Freund's complete adjuvant-induced control animals. OM treatment significantly abridged the paw swelling on day 21 similar to reference drug indomethacin group (Fig. 4).

3.6. Anti-nociceptive effects

The antinociceptive activity of OM was assessed with the acetic acid-induced abdominal constriction method, formalin test and hot-plate test respectively. It is well known that abdominal constriction response is very profound and is able to identify antinociceptive activity of natural compounds. OM considerably inhibited abdominal constrictions induced by acetic acid induction (Fig. 5). The protective effect of OM was 75.86% ($P < .05$) at 20 mg/kg. Indo-
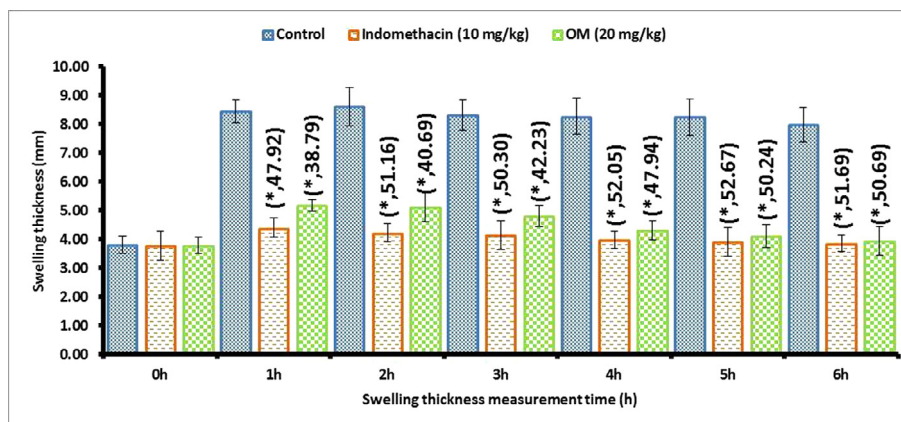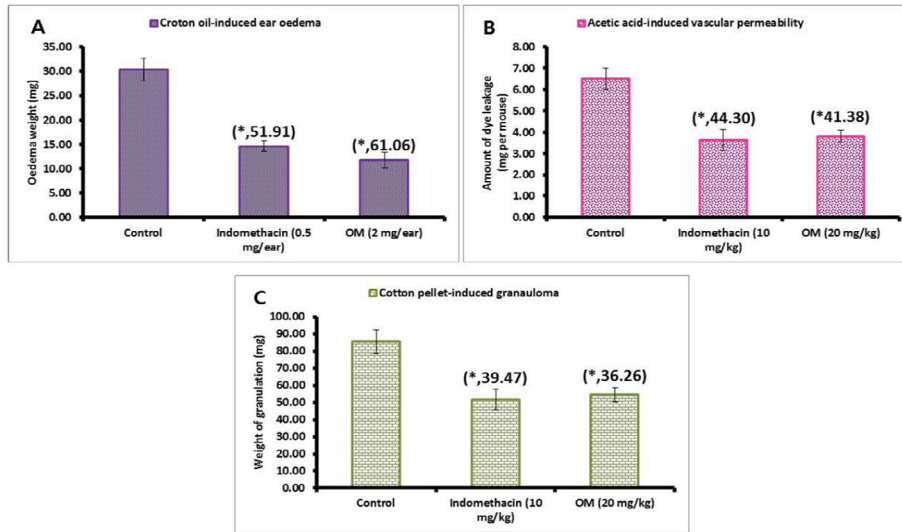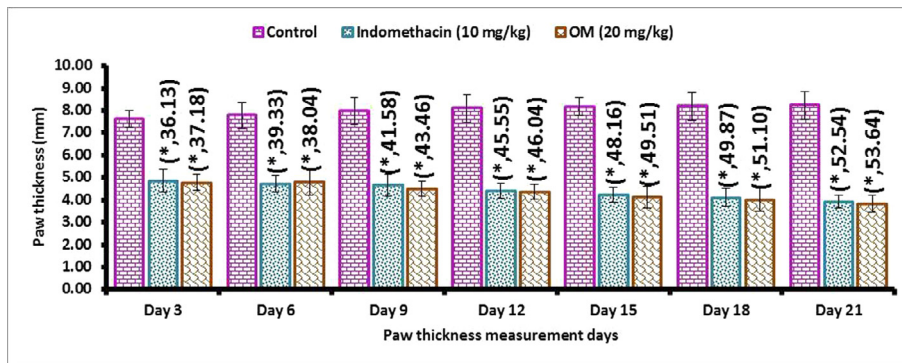

Fig. 2. Effects of OM (20 mg/kg) and indomethacin (10 mg/kg) in carrageenan-induced paw oedema in rats. Values are expressed as mean ± SD, n = 6, *p < .05 compare control with all the groups. Values in the parenthesis indicate paw oedema inhibition percentage.

Fig. 3. (A) Effects of OM (20 mg/kg) and indomethacin (10 mg/kg) in croton oil-induced ear oedema in mice. (B) Acetic acid-induced vascular permeability. (C) Cotton pellet-induced granuloma. Values are expressed as mean ± SD, n = 6, *p < .05 compare control with all the groups. Values in the parenthesis indicate inhibition percentage of croton oil-induced ear oedema, acetic acid-induced vascular permeability and cotton pellet-induced granuloma.

Fig. 4. Effects of OM (20 mg/kg) and indomethacin (10 mg/kg) in adjuvant-induced arthritis in rats. Values are expressed as mean ± SD, n = 6, *p < .05 compare control with all the groups. Values in the parenthesis indicate inhibition percentage of adjuvant-induced arthritis.
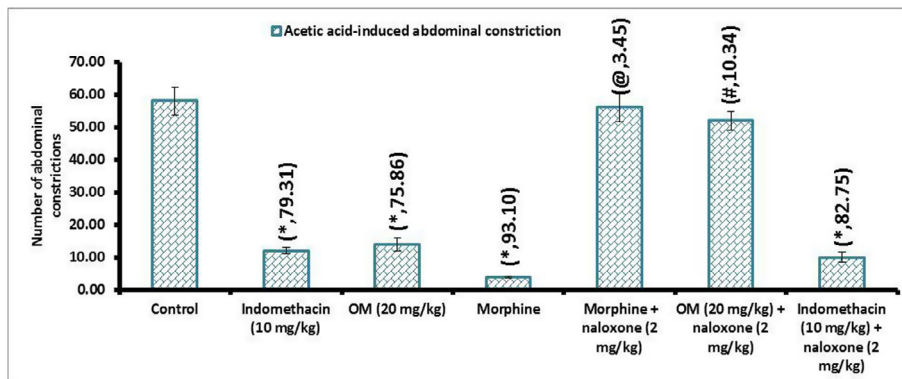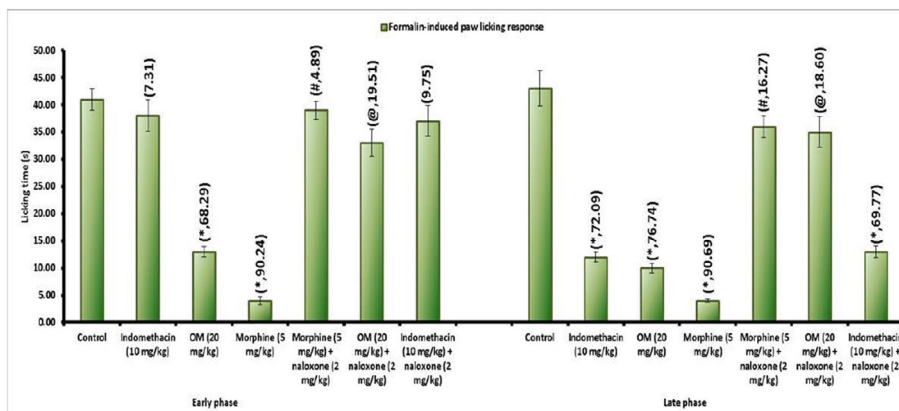

Fig. 5. Effects of OM, indomethacin, morphine and naloxone on acetic acid-induced abdominal constriction response in mice. Values are expressed as mean ± SD, comparison made between: *control with all the groups; @morphine with morphine + naloxone; #OM with OM + naloxone. *@# P < .05 was considered as statistically significant. Values in the parenthesis indicate inhibition percentage of acetic acid-induced abdominal constriction.

methacin inhibited 79.31% (P < .05) and morphine (a centrally acting analgesic) inhibited 93.10% (P < .05). Naloxone significantly blocked the protective actions of OM similar to morphine.

Formalin-induced paw licking response could be more valuable model of clinical pain wherein first phase (early phase) consist of

direct chemical stimulation of nociceptors, while the second phase (late phase) is reliant on peripheral inflammation and alterations in central mechanism (Sayyah et al., 2004; Antonisamy and Ignacimuthu, 2010). Substance P and bradykinin contribute in the neurogenic phase (early phase), whereas serotonin, histamine,

Fig. 6. Effects of OM, indomethacin, morphine and naloxone on formalin-induced paw licking response in mice. Values are expressed as mean ± SD, comparison made between: *control with all the groups; #morphine with morphine + naloxone; @OM with OM + naloxone. *@# P < .05 was considered as statistically significant. Values in the parenthesis indicate inhibition percentage of formalin-induced paw licking response.
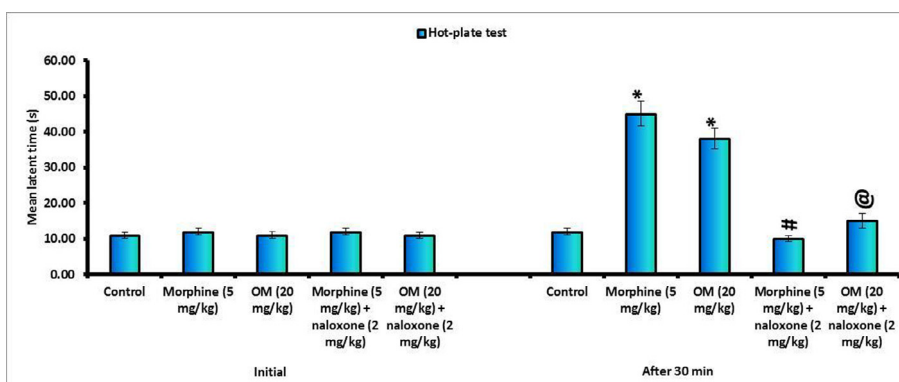
Fig. 7. Effects of OM, indomethacin, morphine and naloxone on acetic acid-induced abdominal constriction response in mice. Values are expressed as mean ± SD, comparison made between: *control with all the groups; #morphine with morphine + naloxone; @OM with OM + naloxone. *@# P < .05 was considered as statistically significant.

bradykinin, nitric oxide and prostaglandins were take part in the inflammatory phase (late phase) (García et al., 2004). OM showed analgesic activity on both phases (early and late phases) of the formalin test, proposing that both direct effect on the nocciceptor and inflammatory pain inhibition. Indomethacin significantly inhibited formalin-induced paw licking response in the second phase.

OM exerted significant activity on the first phase (0–5 min) as well as in second phase (20–30 min) of the formalin-induced nociceptive test. These different phases corresponded to neurogenic and inflammatory pains, respectively. OM at 20 mg/kg inhibited 68.29% (P < .05) in the first phase and 76.74% (P < .05) in the second phase. Reference drug indomethacin was significantly active (72.09%, P < .05) on the second phase while morphine was effective at both the phases (Fig. 6). Naloxone (opioid antagonist) inhibited the effect of morphine and OM at both the phases. The activity of indomethacin was not interrupted by naloxone on both tests (acetic acid-induced abdominal constriction and formalin-induced paw licking).

OM also able to increases the latency to nociceptive behaviour in the hot-plate test proposing that it performed as a central analgesic drug. Additionally, naloxone (a nonselective opioid receptor antagonist) significantly modifies the analgesic activity of OM; it may possibly be decided that the opioid system was involved in this effect. In the hot-plate test, OM showed significant effect compared with control. The maximum latent time (38 s) was observed at 20 mg/kg. Morphine at 05 mg/kg established its supreme latent time of 45 s (P < .05). The effect of OM and morphine was entirely blocked by the interference of naloxone (2 mg/kg) (Fig. 7).

4. Conclusions

The results of present study have intensely specified that ononitol monohydrate (OM) was vastly effective in the treatment of inflammatory diseases. OM displayed potent in vivo anti-inflammatory and analgesic activities. The foremost mechanism of action of OM could be the inhibition of synthesis or release of inflammatory mediators. As a result of the notable biological activity of OM it will be applicable to conduct additional research to improve it into a medicine.

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