J. Pharm. Pharmacol. 1992, 44: 456–458
Communicated August 27, 1991



# Anti-inflammatory activity of oleanolic acid in rats and mice

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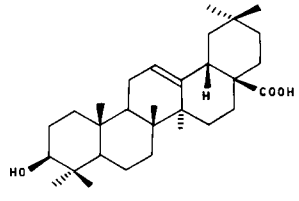
**Abstract**—Oleanolic acid displayed anti-inflammatory activity in carrageenan and dextran-induced oedema in rats. It elicited marked anti-arthritic action in adjuvant-induced polyarthritis in rats and mice and in formaldehyde-induced arthritis in rats. Oleanolic acid checked the inflammation-induced increased serum transaminase levels. It reduced exudate volume and inhibited leucocyte infiltration in carrageenan-induced pleurisy in rats. It is devoid of any analgesic, antipyretic or ulcerogenic action. Oleanolic acid did not affect the parturition time in pregnant rats or castor oil-induced diarrhoea in rats. Oral LD50 was found to be greater than 2 g $kg^{-1}$ in mice and rats.

In the course of our investigations aimed at developing anti-inflammatory agents based on plants, we studied the gum resin exudate of *Boswellia serrata*, locally known as *Salai guggal* and used in the Ayurvedic system of medicine for the treatment of rheumatism, obesity and a number of other disorders. The active principles of this gum resin were found to be β-boswellic acid, a triterpene acid, and a number of its derivatives. This study has led to the discovery of a non-steroidal anti-inflammatory agent, marketed in India under the brand name Sallaki (Singh & Atal 1986). The anti-inflammatory effect of a number of other triterpenes has been reported. These are glycyrrhetinic acid (D'Arcy & Kellett 1957; Somers 1957), α- and β-amyrin acetates (Gupta et al 1969, 1971), friedelin and friedelan-3 β-ol (Chaturvedi et al 1974) and some oleanane triterpene glycosides, papyrioside (Sugishta et al 1982). In view of the marked anti-inflammatory activity of the triterpene acids mentioned above—glycyrrhetinic acid and β-boswellic acid—it was thought desirable to study the anti-inflammatory activity of oleanolic acid (3β-hydroxyolean-12-en-28-oic acid), a triterpene acid occurring in a large number of plants in the free state, as the acetate and as glycosides in many saponins. Oleanolic acid used for this study was isolated from a known source, seeds of *Luffa cylindrica* (Barua & Bose 1960).




Oleanolic acid

## Materials and methods

The dried and powdered seeds of *Luffa cylindrica* (2·7 kg) were successively extracted with *n*-hexane and methanol in a soxhlet extractor. To the concentrated methanolic extract (500 mL) conc. hydrochloric acid (80 mL) was added and the mixture refluxed for 3 h, cooled and filtered. The residue was washed free from acid, dried and extracted with acetone in a thimble extractor. The residue obtained on removal of the solvent was crystallized from ethanol to yield oleanolic acid as fine needles (5 g) mp 309–310°C, $[\alpha]_D^{25}$ +81·1°C (C = 0·6 in $CHCl_3$); molecular ion peak ($M^+$) at m/e 456; acetate, needles from methanol, mp 267–268°C.

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*Methods.* Male albino Charles Foster rats, 110–140 g, in groups of 5 were used at a room temperature of 25 ± 1°C. In acute tests drugs were dosed orally 1 h and intraperitoneally 30 min before induction of oedema. In chronic tests dosing was orally for the duration of the experiment. Paw volume was measured with a volume differential meter model 7101 Ugo Basile (Italy). Data were analysed using Student's *t*-test. Drugs were freshly prepared as a fine homogenized suspension in gum acacia (2% w/v).

*Carrageenan-induced oedema in rats.* Carrageenan (1% w/v) in 0·9% NaCl (saline), 0·1 mL, was injected into the subplantar region of the left hind paw 1 h after oral drug treatment (Winter et al 1962). The volume of the paw was measured with a differential volume meter immediately and 3·5 h after carrageenan injection.

*Carrageenan-induced oedema in mice.* Oedema in Swiss albino mice, 22–28 g, was induced by injecting 0·25 mL of 1% carrageenan into the left hind paw, the other acting as a control (Srimal & Dhawan 1971). The animals were killed with ether after 3·5 h, both the hind paws were cut at the ankle and the difference between the weights of the paws of drug treated and control animals was calculated for the inhibitory action of test drugs.

*Dextran-induced oedema in rats.* Dextran (6% w/v) solution in normal saline, 0·1 mL, was injected into the left hind paw 1 h after drug administration (Winter 1964). Final paw volume was measured 1 h later.

*Carrageenan-induced oedema in adrenalectomized rats.* Adrenalectomy was done under ether anaesthesia as described by Schultzer (1935). Experiments were performed two days later by injecting carrageenan as described above.

*Cotton pellet test in rats.* Using the method of Winter & Porter (1957) autoclaved cotton pellets weighing 50 ± 1 mg each were implanted under each axilla and groin region of rats under ether anaesthesia. Drugs were administered orally once a day for seven days.

*Adjuvant-induced developing arthritis in rats.* Arthritis was induced by injecting 0·05 mL of (0·5%) w/v suspension of killed *Mycobacterium tuberculosis* (Difco) homogenized in a liquid paraffin into the left hind foot (Newbould 1963). Administration of drug was initiated orally one day before the injection of oedemogen and continued daily until day 13. Paw volume was measured on alternate days and per cent inhibition was determined.

*Formaldehyde arthritis in rats.* Formaldehyde (2% v/v) solution, 0·1 mL, was injected into the left hind paw just beneath the plantar aponeurosis on the first and third day of the experiment in groups of 5 rats (Selye 1949).

*Effect of serum transaminases in arthritic rats.* Formaldehyde arthritis was induced in rats (n = 5) as described. The test drug was administered orally every day for seven days and animals were killed on the 8th day. Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) levels were found by the method of Hanson (1963).

*Study on leucocyte migration. Carrageenan-induced pleurisy in rats.* Pleurisy in rats was induced by injecting 0·5 mL of 1%(w/v) carrageenan into the pleural cavity as described by Meacock & Kitchen (1979).

*Effect on gestation period, parturition, litter size and post-partum bleeding in rats.* Using the method described by Aiken (1972) female rats (n = 4) in the 18–21st day of gestation were acquired and maintained individually in separate cages with a bedding of wood shavings. Drug was administered orally twice a day with a control group given only the vehicle. Animals were kept under close observation during the period of parturition.

*Effect on castor oil-induced diarrhoea in rats.* This experiment was carried out in 24 h fasted rats in groups of 5 by the method of Awouters et al (1978).

*Ulcerogenic effect in rats.* Test compound was administered to rats according to the method of Cashin et al (1977). Animals were killed 3 h after dosing of the compounds, the stomachs removed, cut along the lesser curvature, and the gastric mucosa was washed with normal saline and scored according to the scale: 0 = no lesion, 0·5 = hyperaemia, 1 = one or two lesions, 2 = severe lesions, 3 = very severe lesions, 4 = mucosa full of lesions.

*Other pharmacological assays.* Analgesic activity was assessed using the method of Bianchi & David (1960) and the abdominal constriction response (writhing) of mice to intraperitoneal injection of acetic acid (Witken et al 1961). Antipyretic activity was tested using the technique of Brownlee (1937).

*Effect on gross behaviour and acute toxicity.* Drug was administered in increasing doses orally, as well as intraperitoneally, to groups of ten mice each kept in a transparent perspex observation chamber at a room temperature of 25 ± 1°C. Observation was according to Singh et al (1978).

## Results and discussion

The findings of the present study revealed oleanolic acid to possess dose related anti-inflammatory activity in a variety of test models (Table 1). The anti-inflammatory activity of oleanolic acid (50 and 100 mg $kg^{-1}$, p.o.) was further substantiated by its inhibitory action on serum transaminases (SGOT and SGPT) levels elevated due to prolonged inflammation in formaldehyde arthritis in rats (Table 2). Oleanolic acid does not appear to act through activation of the adrenal-pituitary axis as its anti-inflammatory activity was not altered in adrenalectomized rats. It failed to show any effect in the cotton pellet test, which is considered to be more sensitive to the steroidal type of anti-inflammatory drugs (Di Rosa 1979). Like other non-steroidal anti-inflammatory drugs (NSAIDs) (Ford Hutchinson et al 1975), oral administration of oleanolic acid (50 and 100 mg $kg^{-1}$) produced reduction of exudate volume and migration of leucocytes into the pleural cavity (Fig. 1).

A strong correlation between the potency of NSAIDs as inhibitor of prostaglandin synthesis and ulcerogenic activity has been suggested (Boyle et al 1982). Oleanolic acid lacked ulcerogenic action in doses as high as 900 mg $kg^{-1}$ (Table 3), and analgesic or antipyretic effects suggesting that it does not act by inhibiting PG synthetase. This is further supported by the fact that oral administration of oleanolic acid (100–300 mg $kg^{-1}$)

Table 1. Acute and chronic anti-inflammatory actions of oleanolic acid in groups of 5 rats.

| Treatment | Dose (mg $kg^{-1}$, p.o.) | Carrageenan oedema (mL) | Carrageenan* oedema (mL) | Dextran oedema (mL) | Formaldehyde arthritis (mL) | Adjuvant arthritis swelling (mL) | Adjuvant** arthritis swelling (mg) |
|---|---|---|---|---|---|---|---|
| Control | — | 1·30 ± 0·08 | 1·47 ± 0·09 | 1·27 ± 0·06 | 0·94 ± 0·08 | 1·40 ± 0·17 | 171·67 ± 22·42 |
| Oleanolic acid | 25 | — | 1·02 ± 0·04$^c$ (31) | 1·03 ± 0·09 (19) | 0·72 ± 0·04$^a$ (23) | 1·12 ± 0·13 (20) | — |
| | 50 | 1·05 ± 0·05$^a$ (19) | 0·80 ± 0·07$^d$ (46) | 0·95 ± 0·08$^b$ (25) | 0·66 ± 0·06$^a$ (30) | 0·90 ± 0·11$^a$ (36) | 117·00 ± 14·83 (32) |
| | 100 | 1·00 ± 0·06$^b$ (23) | 0·62 ± 0·07$^d$ (58) | 0·78 ± 0·07$^d$ (39) | 0·57 ± 0·08$^a$ (39) | 0·72 ± 0·10$^c$ (49) | 91·87 ± 21·01$^a$ (46) |
| | 300 | 0·91 ± 0·08$^c$ (30) | — | — | — | — | — |
| Acetyl salicylic acid | 100 | 0·88 ± 0·04$^c$ (32) | 0·05 ± 0·03$^d$ (63) | 0·75 ± 0·10$^c$ (41) | 0·62 ± 0·09$^a$ (34) | 0·92 ± 0·08$^a$ (34) | 0·90 ± 18·37$^b$ (48) |

* Intraperitoneal administration. ** Effect in mice. Each value represents the mean ± s.e. with % inhibition shown in parenthesis. $^aP < 0·05$, $^bP < 0·02$, $^cP < 0·01$, $^dP < 0·001$.

Table 2. Effect of oleanolic acid on serum glutamic oxaloacetic acid transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) in formaldehyde arthritis in groups of 5 rats.

| Treatment | Dose (mg $kg^{-1}$, p.o.) | Level of SGOT (int. units) | Level of SGPT (int. units) |
|---|---|---|---|
| Normal control (without arthritis) | — | 26·00 ± 1·01 | 21·15 ± 1·50 |
| Control with arthritis | — | 35·50 ± 1·02 | 32·35 ± 1·24 |
| Oleanolic acid | 50 | 33·60 ± 1·52 (20) | 28·45 ± 1·07$^b$ (35) |
| | 100 | 32·30 ± 0·91$^a$ (34) | 27·65 ± 1·17$^c$ (42) |
| Acetyl salicylic acid | 50 | 32·50 ± 1·33 (32) | 28·15 ± 1·03$^b$ (37) |

One units of the enzyme activity was equivalent to the formation of 0·047 μg unit of pyruvic acid $min^{-1}$ $mL^{-1}$. Each value represents mean ± s.e. with % inhibition of arthritis induced increase values given in the parenthesis. $^aP < 0·05$, $^bP < 0·02$, $^cP < 0·01$.

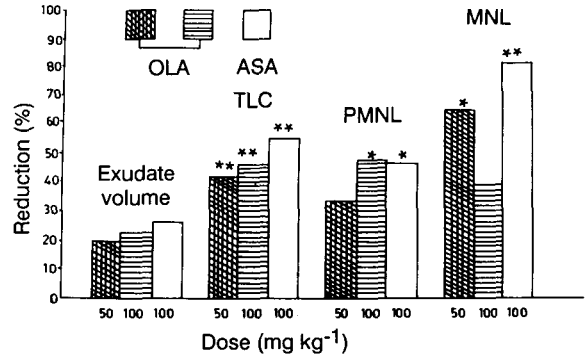


FIG. 1. Inhibitory action of oleanolic acid (OLA) and acetyl salicylic acid (ASA) on pleural exudate volume, total leucocyte count (TLC), polymorphonuclear leucocytes (PMNL) and mononuclear leucocytes (MNL), in carrageenan-induced pleurisy in rats. Each point represents the mean of 5 animals in each group.

Table 3. Ulcerogenic action of oleanolic acid orally administered in groups of six rats.

| Treatment | Dose (mg kg$^{-1}$) | Ulcer score (mean ± s.e.) |
|---|---|---|
| Control | — | 0 ± 0·00 |
| Oleanolic acid | 300 | 0 ± 0·00 |
| | 600 | 0·16 ± 0·10 |
| | 900 | 0·25 ± 0·17 |
| Acetylsalicylic acid | 100 | 0·63 ± 0·11* |
| | 300 | 1·03 ± 0·17* |

*$P < 0·001$ compared with control (Mann-Whitney U-test).

unlike other NSAIDs failed to prolong gestation and did not affect the onset time of castor oil-induced diarrhoea in rats which is reported to be due to inhibition of prostaglandin synthesis (Aiken 1972; Awouters et al 1978). Graded doses of oleanolic acid up to 2 g kg$^{-1}$ orally, showed no significant change in the gross general behaviour in rats and mice. The oral LD50 was found to be greater than 2 g kg$^{-1}$ in mice and rats and the intraperitoneal LD50 was 1500 mg kg$^{-1}$ in mice.

It may be concluded that oleanolic acid, whose spectrum of anti-inflammatory activity appears to be different from classical NSAIDs, would be of therapeutic value.

The authors are grateful to Mr M. L. Sharma for his useful suggestions.

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