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Research Paper

Anti-inflammatory, analgesic and antipyretic effects of friedelin isolated from *Azima tetracantha* Lam. in mouse and rat models

Paulrayer Antonisamy, Veeramuthu Duraipandiyan and Savarimuthu Ignacimuthu

Division of Ethnopharmacology, Entomology Research Institute, Loyola College, Chennai, Tamil Nadu, India

Abstract

Objectives Friedelin was isolated from *Azima tetracantha* Lam. leaves collected from Kallakurichi, Villuppuram district, Tamil Nadu, India. The anti-inflammatory, analgesic and antipyretic activities of friedelin have been investigated in Wistar rats and mice.

Methods Friedelin was isolated from the hexane extract of leaves of *A. tetracantha* using column chromatography. The effects of friedelin on inflammation were studied by using carrageenan-induced hind paw oedema, croton oil-induced ear oedema, acetic acid-induced vascular permeability, cotton pellet-induced granuloma and adjuvant-induced arthritis. The analgesic effect of friedelin was evaluated using the acetic acid-induced abdominal constriction response, formalin-induced paw licking response and the hot-plate test. The antipyretic effect of friedelin was evaluated using the yeast-induced hyperthermia test in rats.

Key findings In the acute phase of inflammation, maximum inhibitions of 52.5 and 68.7% ($P < 0.05$) were noted with 40 mg/kg friedelin in carrageenan-induced paw oedema and croton oil-induced ear oedema, respectively. Administration of friedelin (40 mg/kg) significantly ($P < 0.05$) decreased the formation of granuloma tissue induced by cotton pellet at a rate of 36.3%. In the adjuvant-induced arthritis test friedelin inhibited 54.5% of paw thickness. Friedelin inhibited acetic acid-induced vascular permeability in mice. Friedelin also produced significant ($P < 0.05$) analgesic activity in the acetic acid-induced abdominal constriction response and formalin-induced paw licking response. In the hot-plate test, friedelin did not show any significant results when compared with control. Treatment with friedelin showed a significant ($P < 0.05$) dose-dependent reduction in pyrexia in rats.

Conclusions The results suggested that friedelin possessed potent anti-inflammatory, analgesic and antipyretic activities.

Keywords analgesic; anti-inflammatory; antipyretic; *Azima tetracantha*; friedelin

Introduction

Inflammation or phlogosis is a pathophysiological response of mammalian tissues to a variety of hostile agents including infectious organisms, toxic chemical substances, physical injury or tumour growth leading to local accumulation of plasmic fluid and blood cells. Although inflammation is a defence mechanism, the complex events and mediators involved in the inflammatory reaction can induce, maintain and aggravate many disorders. Hence, the employment of anti-inflammatory agents may be helpful in the therapeutic treatment of those pathologies associated with inflammatory reactions.[1] The clinical treatment of inflammatory diseases is dependent on drugs which belong either to the nonsteroidal or steroidal chemical therapeutics. The nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin, indometacin and ibuprofen inhibit the early steps in the biosynthesis pathway of prostaglandins by inhibition of cyclooxygenase (COX) enzymes and are the main drugs used to reduce the untoward consequences of inflammation. However, the side effects of the currently available anti-inflammatory drugs pose a major problem in their clinical use. The use of steroidal drugs as anti-inflammatory agents is also becoming highly controversial due to their multiple side effects.[2] Therefore, a need arises for the development of newer anti-inflammatory agents from natural sources with more powerful activity and with fewer side effects as substitutes for chemical therapeutics.

Correspondence: Savarimuthu Ignacimuthu, Division of Ethnopharmacology, Entomology Research Institute, Loyola College, Chennai-600 034, Tamil Nadu, India.
E-mail: entolc@hotmail.com

Azima tetracantha Lam. (Salvadoraceae) is known as 'Mulsangu' in Tamil and 'Kundali' in Sanskrit. Its root, root bark and leaves are used with food as a remedy for rheumatism. It is a powerful diuretic given in rheumatism, dropsy, dyspepsia, chronic diarrhoea and as stimulant tonic after childbirth.[3] *A. tetracantha* is an efficient acute phase anti-inflammatory drug traditionally used by Indian medical practitioners. *A. tetracantha* is used to treat cough, phthisis, asthma, small pox and diarrhoea. The decoction of the stem bark is considered astringent, expectorant and antiperiodic. Williams and Nagarajan[4] reported the compounds azimine, azecarpin, carpine and isorhamnetin-3-O-rutinoside from the leaves, whilst Rao and Prasada Rao[5] isolated friedelin, lupeol, glutinol and β-sitosterol from the leaves of *A. tetracantha.* Daulatabad *et al*.[6] isolated novel fatty acids from seeds of this plant and antifungal activity of *A. tetracantha* has been reported.[7]

Friedelin isolated from *Ageratum conyzoides* inhibited crystalline-induced inflammation in rabbit eyes.[8] Friedelin isolated from *Aucuba jabonica* showed anti-inflammatory activity in acute studies such as carrageenan-induced paw oedema, histamine and compound 48/80-induced paw oedema.[9] In this study, we have investigated whether friedelin from *A. tetracantha* was able to reduce murine inflammation, nociception and hyperthermia.

Materials and Methods

Plant collection

The leaves of *A. tetracantha* Lam. were collected in the month of February 2009, from Kalvarayan hills (altitude 200 m), in Kallakurichi, Villupuram district, Tamil Nadu, South India. The plant was identified and authenticated by Dr M. Ayyanar, Taxonomist, Department of Botany, Pachaiyappa's College, University of Madras, Chennai, India. A voucher specimen (ERI-569) has been deposited at the Entomology Research Institute, Loyola College, Chennai, India.

Isolation and identification of friedelin

Leaves of *A. tetracantha* were dried at room temperature and powdered using an electric blender (500 g). The dried powder was extracted with hexane (3 l) by cold percolation for 48 h. The extraction was performed twice. The solvent was removed under reduced pressure using a rotary evaporator; 40 g of crude extract was obtained. Organic qualitative analysis was carried out.[10] Hexane extract (20 g) was subjected to an initial separation on silica gel (60 × 5 cm) using a short glass column (90 × 3 cm) and eluted with n-hexane and n-hexane–ethyl acetate mixtures with increasing amounts of ethyl acetate. Twenty five fractions were collected, each 100 ml. Fractions 13 and 14 were eluted using hexane/ethyl acetate (95 : 5) and yielded 2.20 g white powder. This showed three spots on TLC. This was sub-fractionated using a small silica gel column (30 × 1.5 cm). The column was eluted with n-hexane–ethyl acetate mixtures and 25 ml fractions were collected. Fraction 18 eluted with hexane : ethyl acetate (98 : 2) gave friedelin which crystallized from hexane–ethyl acetate mixtures (yield 900 mg, mp 263–265°C).

Animals

Adult Wistar albino rats (200–220 g) and mice (24–28 g) of either sex were used for the experiments. Animals were maintained on 12 h light/dark cycle at approximately 25 ± 1°C and relative humidity 60–70%; they had access to diet and water was freely available. The animals were acclimatized for at least two weeks before starting the experiments. All studies were carried out using six animals in each group. All the animal experiments were conducted according to the ethical norms approved by Ministry of Social Justice and Empowerment, Government of India and Institutional Animal Ethics Committee guidelines.

Chemicals and drugs

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

Anti-inflammatory studies

Carrageenan-induced paw oedema in rats

Friedelin (10, 20 and 40 mg/kg) and indometacin (10 mg/kg) dissolved in 0.5% carmellose were administered orally 1 h before carrageenan application. After that 1% carrageenan in saline (0.1 ml) was injected subcutaneously into the rat right-hind paw. Paw thickness was measured initially and then at 1, 2, 3, 4, 5 and 6 h after the carrageenan injection using a digital vernier calliper.[11]

Croton oil-induced ear oedema in mice

Oedema was induced according to the method of Tubaro *et al*.[12] Cutaneous inflammation was induced in mice by application of 10 µl freshly prepared croton oil (5% in acetone) to the inner surface of the right ear. Friedelin (1.0, 2.0 or 4.0 mg per ear) was applied topically to the right ear approximately 60 min before the croton oil. The left ear received an equal volume of acetone. As a reference, indometacin (0. 5 mg per ear) was used. Four hours after the application of the irritant agent, the mice were killed by cervical dislocation and the plugs (6 mm Ø) were removed from the treated and untreated ears. The oedematous response was measured as the weight difference between the two plugs.

Acetic acid-induced vascular permeability in mice

To evaluate the effect of friedelin on vascular permeability adult albino mice of both sexes were evaluated by the method of Whittle.[13] Briefly, one hour after oral administration of friedelin (10, 20 or 40 mg/kg), 0.2 ml Evans blue dye (0.25% in normal saline) was intravenously administered through the tail vein. Control animals received either indometacin (10 mg/kg) or an equivalent volume of vehicle (0.5% carmellose). Thirty minutes later animals received an intraperitoneal injection of 1 ml/100 g acetic acid (0.6%, v/v). Treated animals were killed by cervical dislocation 30 min after acetic acid injection and the peritoneal cavity was washed with

normal saline (3 ml) into heparinized tubes and centrifuged. The dye content in the supernatant was measured at 610 nm using a spectrophotometer.

Cotton pellet-induced granuloma in rats

The effect of friedelin on chronic or proliferative phase of inflammation was assessed in the cotton pellet granuloma rat model as described by Winter and Porter.[14] Autoclaved cotton pellets weighing 35 ± 1 mg each were implanted subcutaneously through a small incision made along the axilla region of the rats, which had been anaesthetized with ether. Different groups of rats were administered friedelin (10, 20 or 40 mg/kg) or indometacin (10 mg/kg) once daily for seven consecutive days from the day of cotton pellet insertion. The control group received vehicle (1 ml/kg). On the eighth day, the cotton pellets covered by the granulomatous tissue were excised and dried in a hot air oven at 60°C until a constant weight was achieved. Granuloma weight was obtained by subtracting the weight of the cotton pellet on 0 day (before start of the experiment) from the weight of the cotton pellet on the eighth day.

Adjuvant-induced chronic arthritis in rats

To assess the effect on chronic inflammation, the method described by Newbould[15] was employed with some modification. Friedelin (10, 20 or 40 mg/kg) or indometacin (10 mg/kg) were administered orally to rats once daily for 14 consecutive days. On the third day, 0.1 ml Freund's complete adjuvant was injected into subplantar tissue of each rat. Swelling of the right hind paw in the control and treated animals was measured on day 3, 6, 9, 12, 15, 18 and 21 using digital vernier calipers. The differences in severity of arthritis between the experimental groups and the control group were statistically analysed.

Analgesic tests

Acetic acid-induced abdominal constriction response in mice

Mice weighing 24–28 g were divided into nine groups of six animals each. The study was carried out by a modified method of Mungantiwar *et al*.[16] Each mouse was given an injection of 0.75% acetic acid aqueous solution in a volume of 0.1 ml/10 g body weight into the peritoneal cavity and the animals were then placed in a transparent plastic box. The number of abdominal constrictions was counted for 15 min beginning from 5 min after the acetic acid injection. Test drugs friedelin (10, 20 or 40 mg/kg p.o.), indometacin (10 mg/kg p.o.), morphine (05 mg/kg s.c.), morphine + naloxone ((05 mg/kg s.c. + 02 mg/kg i.p., respectively), friedelin + naloxone (40 mg/kg p.o. + 02 mg/kg i.p., respectively), indometacin + naloxone (10 mg/kg p.o. + 02 mg/kg i.p., respectively) and control vehicle (0.5 ml 0.5% carmellose p.o.) were administered 1 h before the acetic acid injection.

Formalin-induced paw licking response in mice

The test was performed according to the method of Reisine and Pasternack.[17] Mice weighing 24–28 g were divided into two sets of nine groups of six animals each. Test drugs friedelin (10, 20 or 40 mg/kg p.o.), indometacin (10 mg/kg p.o.), morphine (05 mg/kg s.c.), morphine + naloxone (05 mg/kg s.c. + 02 mg/kg i.p., respectively), friedelin + naloxone (40 mg/kg p.o. + 02 mg/kg i.p., respectively), indometacin + naloxone (10 mg/kg p.o. + 02 mg/kg i.p., respectively) and control vehicle (0.5 ml 0.5% carmellose p.o.) were administered 1 h before formalin injection to animals in the first set (for early phase) and 40 min before formalin injection to animals in the second set (for late phase), respectively. Mice were injected subcutaneously with 50 µl 1% formalin in normal saline solution into the right dorsal hind paw. The time animals spent in licking the injected paw was determined during 0–5 min (the first set of mice for early phase) and during 20–30 min (the second set of mice for late phase) after the injection of formalin.

Hot-plate test in mice

Experiments were carried out according to the method of Parkhouse and Pleuvry.[18] Mice weighing 24–28 g were divided into seven groups of six animals each. For testing, mice were placed on a hot plate maintained at 55 ± 5°C. The time that elapsed until occurrence of either a hind paw licking or a jump off from the surface was recorded as the hot plate latency. Before treatment, the reaction time of each mouse (licking of the forepaws or jumping response) was done at 0 and 10 min intervals. The average of the two readings was obtained as the initial reaction time. Mice with baseline latencies of <5 s or >30 s were eliminated from the study. The initial reaction time following the administration of friedelin (10, 20 or 40 mg/kg p.o.), morphine (05 mg/kg s.c.), naloxone + morphine (02 mg/kg i.p. + 05 mg/kg s.c., respectively), naloxone + friedelin (02 mg/kg i.p. + 40 mg/kg p.o., respectively) and vehicle (0.5 ml 0.5% carmellose p.o.) was measured at 30 min.

Antipyretic activity in rats

Hyperthermia was induced in rats by the method of Vogel and Vogel.[19] Rats were given 10 ml/kg 20% aqueous suspension of brewer's yeast subcutaneously. Initial rectal temperature was recorded. When the temperature was at peak i.e. 18 h after yeast injection, only rats which developed satisfactory pyrexia (1°C or more increase in rectal temperature) were used. The thermometer was inserted approximately 3 cm into the rectum of each rat. Friedelin (10, 20 or 40 mg/kg) was administered to three groups. The control group received 0.5 ml vehicle. Paracetamol (150 mg/kg) was used as a reference drug. Rectal temperature was determined at 30, 60, 90 and 120 min after drug administration.

Statistical analysis

Data were statistically analysed by analysis of variance followed by Student's *t*-test. A probability level lower than 0.05 was considered statistically significant.

Results

Identification of friedelin

The hexane extract of leaves of *A. tetracantha* showed the presence of triterpenoid, steroid, phenol, flavonoid, glycoside and alkaloid. When fraction 18 was subjected to Naller's test

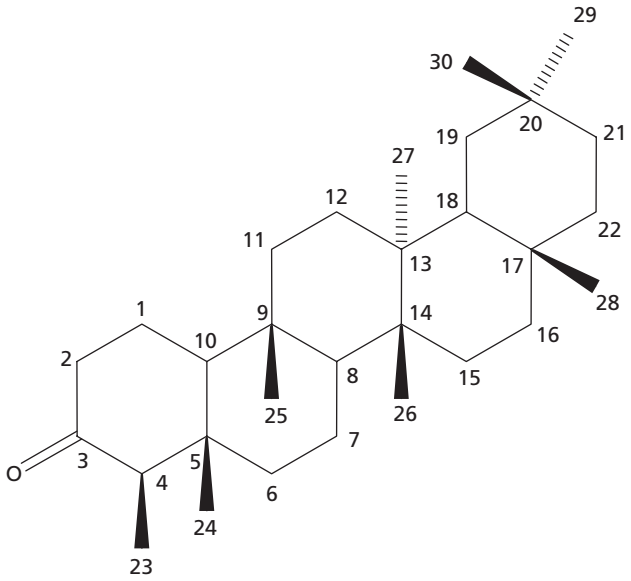

Figure 1 Chemical structure of friedelin.

(tin and thionyl chloride) it gave a pink colour for triterpenoid. It showed a single spot (Rf = 0.33) on TLC with hexane : ethyl acetate (9 : 1). The purity of the compound was 96%. This compound was identified as friedelin (Figure 1) from its spectroscopic data (IR, ^{1}H NMR, ^{13}C NMR, MS) and literature values.[20]

Carrageenan-induced hind paw oedema in rats

As shown in Table 1, friedelin, at doses of 10, 20 or 40 mg/kg markedly reduced the oedema formation of the hind paw induced by carrageenan at all assessment times. Friedelin and indometacin elicited an inhibitory effect on the oedema formation even at 6 h after drug treatment.

Croton oil-induced ear oedema in mice

As shown in Table 2, application of croton oil topically on rat ears produced marked oedema formation. Friedelin at the dose of 2 or 4 mg per ear significantly inhibited formation of ear oedema.

Acetic acid-induced vascular permeability in mice

As shown in Table 3, friedelin exhibited a dose-related inhibitory effect on peritoneal capillary permeability induced by acetic acid in mice. The positive drug, indometacin (10 mg/kg) also markedly inhibited peritoneal capillary permeability.

Cotton pellet-induced granuloma test in rats

The effects of friedelin and indometacin on cotton pellet-induced granuloma in rats are shown in Table 4. Friedelin (10, 20 and 40 mg/kg) and indometacin (10 mg/kg) markedly inhibited formation of granuloma surrounding the pellets when compared with vehicle control.

Adjuvant-induced arthritis in rats

The mean change in paw swelling was approximately 8.28 mm in the Freund's complete adjuvant-induced control

Table 1 Anti-inflammatory activity of friedelin using the carrageenan-induced paw oedema model in rats

Test samples	Dose (mg/kg)	Swelling thickness (mm)						
		0 h	1 h	2 h	3 h	4 h	5 h	6 h
Control	–	3.68 ± 0.28	8.24 ± 0.63	8.39 ± 0.64	8.21 ± 0.63	8.26 ± 0.63	8.00 ± 0.61	7.91 ± 0.60
Indometacin	10	3.62 ± 0.28	3.82 ± 0.29 (53.6)*	3.77 ± 0.29 (55.0)*	3.78 ± 0.29 (53.9)*	3.69 ± 0.28 (55.3)*	3.67 ± 0.28 (54.1)*	3.64 ± 0.28 (53.9)*
Friedelin	10	3.67 ± 0.28	6.87 ± 0.52 (16.6)	6.81 ± 0.52 (18.8)	6.82 ± 0.52 (16.9)	6.76 ± 0.51 (18.1)	6.69 ± 0.51 (16.3)	6.65 ± 0.51 (15.9)
	20	3.61 ± 0.27	5.36 ± 0.41 (34.9)*	5.23 ± 0.40 (37.6)*	5.18 ± 0.39 (36.9)*	5.13 ± 0.39 (37.8)*	5.07 ± 0.39 (36.6)*	4.91 ± 0.37 (37.9)*
	40	3.68 ± 0.28	3.96 ± 0.30 (51.9)*	3.99 ± 0.30 (52.4)*	3.91 ± 0.30 (52.3)*	3.86 ± 0.29 (53.2)*	3.80 ± 0.29 (52.5)*	3.75 ± 0.29 (52.5)*

Data represent mean ± SD (standard deviation) ($n = 6$). *$P < 0.05$ significant from the control.

Table 2 Anti-inflammatory activity of friedelin against croton oil-induced ear oedema in mice

Test samples	Dose (mg per ear)	Oedema weight (mg)
Control	–	32.65 ± 2.49
Indometacin	0.5	12.39 ± 0.94 (62.0)*
Friedelin	1.0	27.18 ± 2.07 (16.7)*
	2.0	18.60 ± 1.42 (43.0)*
	4.0	10.21 ± 0.78 (68.7)*

Data represent mean ± SD (standard deviation) ($n = 6$). *$P < 0.05$ significant from the control.

Table 3 Effect of friedelin on the acetic acid-induced vascular permeability test in mice

Test samples	Dose (mg/kg)	Amount of dye leakage (μg per mouse)
Control	–	6.31 ± 0.48
Indometacin	10	3.72 ± 0.28 (41.0)*
Friedelin	10	5.28 ± 0.40 (16.3)*
	20	4.21 ± 0.32 (33.2)*
	40	3.63 ± 0.28 (42.4)*

Data represent mean ± SD (standard deviation) ($n = 6$). *$P < 0.05$ significant from the control.

Table 4 Anti-inflammatory effect of friedelin on cotton pellet-induced granuloma in rats

Test samples	Dose (mg/kg)	Weight of granulation (mg)
Control	–	83.47 ± 6.36
Indometacin	10	50.33 ± 3.83 (39.7)*
Friedelin	10	70.63 ± 5.38 (15.3)*
	20	61.24 ± 4.66 (26.6)*
	40	53.12 ± 4.04 (36.3)*

Data represent mean ± SD (standard deviation) ($n = 6$). *$P < 0.05$ significant from the control.

group (day 21). Friedelin significantly reduced the paw swelling on day 21 in a dose-dependent manner (Table 5).

Anti-nociceptive effects

Evidence of analgesic activity of friedelin was detected by three different models for nociception used to investigate the antinociceptive effect.

Friedelin significantly reduced abdominal constrictions and stretching induced by acetic acid (Table 6). The protective effect of friedelin was dose dependent with 80.0% ($P < 0.05$) reduction observed with 40 mg/kg. Indometacin (10 mg/kg) inhibited 81.8% ($P < 0.05$) and morphine (a centrally acting analgesic) inhibited 96.3% ($P < 0.05$). Naloxone never blocked the protective actions of friedelin whilst it completely arrested morphine activity. The effect exerted by friedelin on the first phase (0–5 min) was less when compared with the second phase (20–30 min) of the formalin test. These phases corresponded to neurogenic and inflammatory pains, respectively. The 40 mg/kg dose inhibited 21.0% ($P < 0.05$) in the first phase and 74.3% ($P < 0.05$) in the second phase.

Table 5 Anti-inflammatory effect of friedelin on adjuvant-induced arthritis in rats

Test samples	Dose (mg/kg)	Paw thickness (mm)						
		Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21
Control	–	7.93 ± 0.60	7.99 ± 0.61	8.16 ± 0.62	8.14 ± 0.62	8.23 ± 0.63	8.23 ± 0.63	8.28 ± 0.63
Indometacin	10	4.93 ± 0.38 (37.8)*	4.68 ± 0.36 (41.4)*	4.63 ± 0.35 (43.2)*	4.32 ± 0.33 (46.9)*	4.11 ± 0.31 (50.0)*	3.96 ± 0.30 (55.1)*	3.87 ± 0.29 (53.2)*
Friedelin	10	6.52 ± 0.50 (17.7)	6.56 ± 0.50 (17.8)	6.53 ± 0.50 (19.9)	6.55 ± 0.50 (19.5)	6.49 ± 0.49 (21.1)	6.41 ± 0.49 (22.1)*	6.36 ± 0.48 (23.1)*
	20	5.35 ± 0.41 (32.5)*	5.31 ± 0.40 (33.5)*	5.27 ± 0.40 (35.4)*	5.20 ± 0.40 (36.1)*	5.20 ± 0.40 (36.8)*	5.19 ± 0.40 (36.9)*	5.10 ± 0.39 (38.4)*
	40	4.56 ± 0.35 (42.4)*	4.47 ± 0.34 (44.0)*	4.49 ± 0.34 (44.9)*	4.26 ± 0.32 (47.6)*	4.03 ± 0.31 (51.0)*	3.82 ± 0.29 (53.5)*	3.76 ± 0.29 (54.5)*

Data represent mean ± SD (standard deviation) ($n = 6$). *$P < 0.05$ significant from the control.

Table 6 Effects of friedelin, indometacin, morphine and naloxone on acetic acid-induced abdominal constriction response and formalin-induced paw licking in mice

Test samples	Dose (mg/kg)	Acetic acid	Formalin test	
		Number of abdominal constrictions	Early phase licking time (s)	Late phase licking time (s)
Control	–	55.01 ± 4.19	38.01 ± 2.89	39.01 ± 2.97
Indometacin	10	10.00 ± 0.76 (81.8)*#	31.01 ± 2.36 (18.4)*#	8.00 ± 0.61 (79.4)*#
Friedelin	10	48.01 ± 3.66 (12.7)	33.01 ± 2.51 (13.1)	27.00 ± 2.06 (30.7)*
	20	22.00 ± 1.68 (60.0)*	30.01 ± 2.28 (21.0)*	18.00 ± 1.37 (53.8)*
	40	11.00 ± 0.84 (80.0)*#	30.01 ± 2.28 (21.0)*#	10.00 ± 0.76 (74.3)*#
Morphine	5	2.00 ± 0.15 (96.3)*	3.00 ± 0.23 (92.1)*	3.00 ± 0.23 (92.3)*
Morphine + naloxone	5 + 2	57.01 ± 4.34	36.01 ± 2.74 (5.2)	37.01 ± 2.82 (5.1)
Friedelin + naloxone	40 + 2	12.00 ± 0.91 (78.1)*†	32.01 ± 2.44 (15.7)	10.00 ± 0.76 (74.3)*†
Indometacin + naloxone	10 + 2	9.00 ± 0.69 (83.6)*†	33.01 ± 2.51 (13.1)	9.00 ± 0.69 (76.9)*†

Data represent mean ± SD (standard deviation) ($n = 6$). Comparison made between: *control with all the groups; †morphine + naloxone with friedelin + naloxone and indometacin + naloxone; #morphine with indometacin and friedelin (40 mg/kg). *†#$P < 0.05$ significant from the control.

Table 7 Effect of the friedelin, morphine and naloxone on the pain threshold of mice in the hot-plate test

Test samples	Dose (mg/kg)	Mean latent time (s)	
		Initial	After 30 min
Control	–	10.00 ± 0.76	11.00 ± 0.84
Morphine	5	10.00 ± 0.76	43.01 ± 3.27*
Friedelin	10	10.00 ± 0.76	10.00 ± 0.76
	20	11.00 ± 0.84	12.00 ± 0.91
	40	11.00 ± 0.84	12.00 ± 0.91
Morphine + naloxone	5 + 2	10.00 ± 0.76	9.00 ± 0.69
Friedelin + naloxone	40 + 2	10.00 ± 0.76	14.00 ± 1.07

Data represent mean ± SD (standard deviation) ($n = 6$). Comparison made between: *control with all the groups. *$P < 0.05$ significant from the control.

Table 8 Effect of friedelin and paracetamol in the yeast-induced hyperthermia test in rats

Test samples	Dose (mg/kg)	Rectal temperature (°C)					
		Before yeast	18 h after yeast	Time after treatment (min)			
				30	60	90	120
Control	–	37.22 ± 2.83	39.25 ± 2.99	39.32 ± 2.99	39.27 ± 2.99	39.27 ± 2.99	39.19 ± 2.98
Paracetamol	150	37.19 ± 2.83	39.20 ± 2.98	38.22 ± 2.91*	37.63 ± 2.87*	37.42 ± 2.85*	37.27 ± 2.84*
Friedelin	10	37.31 ± 2.84	39.29 ± 2.99	39.15 ± 2.98	39.11 ± 2.98	38.72 ± 2.95	38.64 ± 2.94
	20	37.19 ± 2.83	39.31 ± 2.99	38.75 ± 2.95	38.41 ± 2.92	38.26 ± 2.91*	38.11 ± 2.90*
	40	37.21 ± 2.83	39.17 ± 2.98	38.29 ± 2.92*	37.71 ± 2.87*	37.54 ± 2.86*	37.43 ± 2.85*

Data represent mean ± SD (standard deviation) ($n = 6$). *$P < 0.05$ significant from the control.

Indometacin was significantly active (79.4%, $P < 0.05$) on the second phase whereas morphine acted at both the phases (Table 6). The opioid antagonist naloxone inhibited the action of morphine at both the phases, but naloxone did not inhibit friedelin. In both tests (acetic acid-induced abdominal constriction and formalin-induced paw licking) the activity of indometacin was not disrupted by naloxone.

In the hot-plate test, friedelin did not show any significant results when compared with control. The maximum latent time (12 s) was observed with 40 mg/kg. Morphine sulphate at 05 mg/kg manifested its maximum latent time of 43.01 s ($P < 0.05$). The action of morphine was completely arrested by naloxone (2 mg/kg) (Table 7).

Yeast-induced hyperthermia in rats

The results of the antipyretic effect of friedelin are presented in Table 8. Administration of brewer's yeast to rats produced a significant increase in rectal temperature 18 h after yeast injection ($P < 0.05$). The results of the antipyretic study showed that oral administration of friedelin at 20 or 40 mg/kg caused a significant ($P < 0.05$) inhibition of pyrexia induced by yeast.

Discussion

We have evaluated the anti-inflammatory, analgesic and antipyretic effects of friedelin employing various experimental

test models. Carrageenan-induced rat paw oedema is a suitable test for evaluating anti-inflammatory drugs and has frequently been used to assess the anti-oedematous effect of natural products. Oral pretreatment of animals with friedelin resulted in a significant inhibition of carrageenan-induced hind paw oedema. This kind of test induces an inflammatory reaction in two different phases. The initial phase, which occurs between 0 and 2.5 h after injection of the phlogistic agent, has been attributed to the action of histamine, serotonin and bradykinin on vascular permeability. The oedema volume reaches its maximum approximately 3 h post-treatment and then begins to decline. The late phase, which is also a complement-dependent reaction, has been shown to be a result of over production of prostaglandins in tissues.[21] Regarding the possible mechanisms involved, it has been suggested that several inflammatory mediators play a role e.g. complement, histamine, kinins, prostaglandins (PGs) and pro-inflammatory cytokines. It is well known that leucocyte migration to the injured tissues is an important aspect of the inflammatory process.[22] It is assumed that at least some of these mediators are the subject of inhibition by friedelin. Histamine and serotonin are responsible for the immediate inflammation response, whereas kinins and prostaglandins mediate prolonged response.[23]

The second method employed for the screening was the croton oil-induced mouse ear oedema test, which has certain advantages for natural product testing. First, the response is local and involves the skin of the ear, thus, the topical application avoids drug metabolism and excretion. Secondly, this model uses a very small amount of drug. In this case, friedelin demonstrated a clear dose-response relation. In this oedema the most important mediators involved were prostaglandins, histamine and serotonin, whereas the lypoxygenase pathway did not play an important role.[24]

Acetic acid-induced increased vascular permeability in the mouse model is a typical capillary permeability assay.[11] Friedelin significantly reduced the increased peritoneal vascular permeability indicating the suppression of the vascular response in the process of acute inflammation. The injection of carrageenan into a subcutaneous air pouch on the dorsal surface of rats initiated an inflammatory process. The carrageenan-induced air-pouch model in rats was used to examine acute anti-inflammatory activity of friedelin on fluid extravasation, leucocyte accumulation and various biochemical parameters in the exudate involved in the inflammatory response.

The repair phase of the inflammatory process begins with proliferation of fibroblasts as well as multiplication of small blood vessels. Such proliferating cells penetrate and the exudates produce a highly vascularized and reddened mass known as granulation tissue.[25] Investigation of the effect of friedelin on the proliferative phase of inflammation revealed significant inhibition of the granuloma tissue formation. This effect suggested that friedelin exerted significant effect on the reactions mediating granulomatous inflammation.

Rheumatoid arthritis is a chronic inflammatory disease affecting approximately 1% of the population in developed countries. It is a chronic, cytokine-mediated destructive inflammatory polyarticular joint disease. It is characterized by massive synovial proliferation, systemic and local inflammation resulting in cartilage and bone destruction. Adjuvant arthritis in rat mimics many of the clinical and pathological features of human rheumatoid arthritis, such as paw swelling, joint erosions and ankylosis, and it is the most commonly used animal model for rheumatoid arthritis.[26] It seems that bacterial peptidoglycan and muramyl dipeptide are responsible for its induction. Since the composition of bacterial adjuvant is complex and the immune response is a multi-stage process of intercellular cooperation, the mechanism is unclear. The model of adjuvant-induced arthritis in rats has been extensively used in the study of inflammatory processes and validated as a model of chronic pain.[27] In this study, we used adjuvant-induced arthritis in rats to demonstrate that friedelin had good inhibiting effects on adjuvant arthritis in rats.

The antinociceptive activity of friedelin was evaluated using the acetic acid-induced abdominal constriction, formalin test and hot-plate test. The compound significantly inhibited the acetic acid-induced abdominal constrictions in mice. It is known that the abdominal constriction response is very sensitive and is able to detect antinociceptive effects of compounds. Local peritoneal receptors are postulated to be partly involved in the abdominal constriction response. The mechanism of the reaction to this nociceptive stimulus seems to be related to the prostanoid system. Experimental results obtained by several researchers indicated increased levels of lipoxygenase product as well as increased peritoneal fluid level of PGE_2 and $PGF_{2\alpha}$.[28] Friedelin might play a role in the inhibition of prostaglandin synthesis.

The formalin test may be more useful as a model of clinical pain in which the first phase seems to be due to direct chemical stimulation of nociceptors, whereas the second phase is dependent on peripheral inflammation and changes in central processing.[29] Substance P and bradykinin participate in the neurogenic phase, while serotonin, histamine, bradykinin, nitric oxide and prostaglandins are involved in the inflammatory phase.[28] The significant inhibitory effect of friedelin on nociceptive response in the late phase of the formalin test suggested that the antinociceptive effect might have been due to its peripheral (or anti-inflammatory) action. Indometacin also inhibited pain induced by formalin significantly in the second phase. Friedelin did not increase the latency to nociceptive behaviour in the hot-plate model suggesting that it acted as an anti-inflammatory drug. Moreover, naloxone (a nonselective opioid receptor antagonist) failed to modify the analgesic effect of friedelin; it could be concluded that the opioid system was not involved in this effect.[28]

Antipyretic activity is commonly mentioned as a characteristic of drugs or compounds which have an inhibitory effect on prostaglandin-biosynthesis. The yeast-induced hyperthermia in rats was employed to investigate the antipyretic activity of friedelin. It was found that friedelin caused a significant decrease in rectal temperature similar to paracetamol. This result seems to support the view that friedelin had some influence on prostaglandin biosynthesis, because prostaglandin is believed to be a regulator of body temperature.[30]

Conclusions

Azima tetracantha has been used traditionally as an anti-inflammatory drug. Based on our results we believe that the

compound friedelin present in *A. tetracantha* may be responsible for this. The results of this study have empirically indicated that friedelin was effective in the treatment of inflammatory disease. Friedelin showed potent in-vivo anti-inflammatory, analgesic and antipyretic effects. Inhibition of the synthesis or release of inflammatory mediators may be the main mechanism of action of friedelin. Due to the remarkable biological activity of friedelin it will be appropriate to conduct further research to develop it into a medicine.

Declarations

Conflict of interest

The authors declare that they have no conflicts of interest to disclose.

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