

ANTI-INFLAMMATORY ACTIVITY OF NANOGEL FORMULATION OF 3-ACETYL-11-KETO- β -BOSWELLIC ACID

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Summary

The gum of *Boswellia serrata* has been widely used in ayurvedic and traditional system of medicine for the treatment of inflammation. 3-acetyl-11-keto- β -boswellic acid (AKBA) is the most potent pentacyclic triterpenic acid present in gum of *Boswellia serrata* for anti-inflammatory and antiarthritic activity. The objective of the present investigation was to study the anti-inflammatory activity of nanogel formulation of AKBA against carrageenan induced rat paw edema. Topical gel for in-vivo study of AKBA and AKBA polymeric nanoparticles was formulated by using 1% Carbopol 940. Results of in-vivo comparison study showed much higher anti-inflammatory activity of AKBA nanogel compared to AKBA gel of equivalent concentration.

Keywords: 3-acetyl-11-keto- β -boswellic acid, carrageenan, anti-inflammatory, nanogel.

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Introduction

Boswellia serrata, family Burseraceae, is a medium to large branching tree, generally found in dry hilly areas of India, North Africa, and the Middle East. It has been mentioned in the ancient ayurvedic texts Sushruta Samhita and Charak Samhita [1]. The gum resins of *Boswellia serrata* has been used for a variety of therapeutic purposes such as cancer [2-4], analgesia [5-6], asthma [7-8], inflammation [9-10], arthritis [11-14], colitis [15], Crohn's diseases[16] and hyperlipidemia[17]. The main biologically active principles of *Boswellia serrata* for anti-inflammatory and antiarthritic activity are boswellic acids. 3-acetyl-11-keto- β -boswellic acid (AKBA) with an IC₅₀ value of 1.5 μ M proved to be the most potent pentacyclic triterpenic acid present in *Boswellia serrata*.

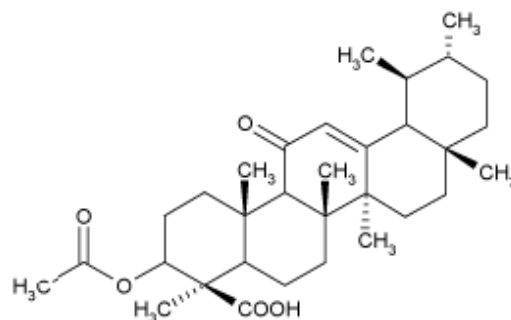


Figure 1. Chemical structure of AKBA

The current therapeutic treatment of arthritis with modern anti-inflammatory drugs is associated with side-effects. AKBA act by 5-lipoxygenase directed, non-redox, non-competitive mechanism [18-20] and therefore possess little toxicity and limited side effects compared to other anti-inflammatory drugs. However AKBA possess poor oral bioavailability [21-24] and elimination half life of 4.5 ± 0.55 hours [21]. For treatment of arthritis and reduction of inflammation higher concentration is required at the site of inflammation. Furthermore, topical application proved to be highly beneficial to the rheumatic patients because it can be directly applied to the target tissues for more effective results. So topical delivery of AKBA seems to be a preferred alternative to the oral dosage form, which provide sustained and constant plasma level and reduce frequency of administration.

In the present study topical gel of AKBA and AKBA polymeric nanoparticles has been formulated and comparison study between two was done to evaluate their anti-inflammatory activity.

Material and Methods

Materials

Creamish dry powdered methanolic extract of the resin of *Boswellia serrata* was procured from Sanat Products, Delhi, India. All chemicals and reagents used were of analytical grade and were purchased from Merck, Mumbai, India.

Extraction of pure AKBA

Methanolic extract of the resin of *Boswellia* was subjected to silica column chromatography using 5% to 30% ethyl acetate/hexane mixtures. The fractions were monitored by TLC and those containing AKBA (30% - 60%) were combined and subjected to crystallization in hexane and ethyl acetate mixtures to obtain pure AKBA.

Identification of AKBA

Identification of pure AKBA was done by HPTLC and DSC:

HPTLC of AKBA: HPTLC analysis of AKBA was achieved using silica gel coated aluminium plates as stationary phase and toluene-ethyl acetate 7:3 (v/v) as mobile phase. Samples were applied as 5-mm bands, 10 mm apart, by means of a Camag (Switzerland) Linomat V sample applicator fitted with a Camag microlitre syringe. Densitometric scanning at 250 nm was performed with a Camag TLC scanner III in absorbance mode operated by winCATS software (version 1.2.0).

DSC of AKBA: Isolated AKBA of weight of 5 ± 2 mg were sealed in aluminum hermetic pans and analysed by DSC (Pyris 6, Perkin Elmer, Germany). Scanning rate was $10^{\circ}\text{C}/\text{min}$ over the temperature range of $10\text{-}300^{\circ}\text{C}$.

Formulation of Topical Gel

For the preparation of topical gel the specified quantity of different polymers (Table1) were taken and dispersed in the distilled water. The polymers alone as well as in the combination were tried for the gel preparation. Gels containing different concentrations of polymers and combination of polymers were evaluated on the basis of different parameters to find out the most suitable polymer for the formulation of gel for comparison study.

Homogeneity test

A small quantity of gel was pressed between the thumb and the index finger and the consistency of the gel was noticed (whether homogeneous or not).

Organoleptic characteristics

Gel was tested for color, odor, texture, as well as the feel upon application (stiffness, grittiness, greasiness and tackiness) ones the preparation was on the skin and also after two minutes of application.

pH

The pH of the gel was measured using digital pH meter (HI84240, Microcomputer pH meter, Italy). One gram of gel was dissolved in 100 ml distilled water and stored for 2 hours. The measurement of pH of each formulation was done in triplicate and average values were calculated.

Viscosity

The viscosity of gel was studied by using Brookfield Viscometer (RTV model) at 100 rpm with spindle no. 7.

Extrudability

A simple method was adopted for determination of extrudability in terms of weight in grams required to extrude a 0.5 cm ribbon of gel in 10 sec from a collapsible tube.

Spreadability

Spreadability was measured on the basis of “slip” and “drag” characters of gel. A modified apparatus consisting of two glass slides containing gel in between with the lower side fixed to a wooden plate and the upper one attached to a balance by a hook was used to determine spreadability, which was calculated using the following formula,

$$S = m \times 1/t$$

Where S represents spreadability (g/sec), m is the weight in pan (g), and t is time (sec).

Formulation of final topical gel

Final topical gel of AKBA and AKBA loaded nanoparticles was prepared using Carbopol 940. 1% gel of Carbopol 940 was prepared for the study by dispersing 1 gm of Carbopol 940 in 100 ml of distilled water. After complete dispersion equivalent quantity of AKBA and AKBA loaded nanoparticles was added to the aqueous dispersion under overhead stirring at 800 rpm. Carbopol dispersion was then neutralized using 0.05 % (w/w) triethanolamine to form the gel.

Skin irritancy test

Skin irritation test was carried out on male Swiss albino mice, weighing 25-30 g. The animals were kept under standard laboratory conditions, temperature (25±1°C) and relative humidity (55±5%). The animals were housed in polypropylene cages, six per cage, with free access to standard laboratory diet (Lipton Feed, India) and water *ad libitum*. A single dose of 10 mg of the gel was applied to the left ear of the mice, with the right ear as a control. The development of erythema was monitored for 6 days.

In vivo anti-inflammatory activity on carrageenan induced rat paw edema

Approval to carry out in vivo studies was obtained from the Institutional Animal Ethics Committee (approval no: 173/CPCSEA-28/01/2000) and their guidelines were followed throughout the studies. The anti-inflammatory activity of the optimized formulations was evaluated by the carrageenan-induced hind paw edema method developed by Winter et al. [25] in wistar rats. Young male wistar rats, weighing 180-220g were used for the study. The animals were housed in polypropylene cages, five per cage, with free access to standard laboratory diet (Lipton Feed) and water *ad libitum* under standard laboratory conditions (temperature: 25±2°C; relative humidity: 55±5%). Paw edema was induced by injecting 0.1ml of the 1% w/w homogenous suspension of carrageenan in saline. A total of three groups were used: Group 1 injected with carrageenan only and serve as control, Group 2 and Group 3 received carrageenan + topically applied AKBA gel and carrageenan + topically applied AKBA nanogel respectively. Volume of the paw was measured with a digital plethysmometer (UGO basile 7140 Plethysmometer).

The edema rate and percentage inhibition of each group was calculated as follows:

$$\text{Edema rate (E)} = V_t - V_o / V_o$$

$$\text{Inhibition (\%)} = E_c - E_t / E_c \times 100$$

where V_o is the mean paw volume before carrageenan injection, V_t the mean paw volume after the carrageenan injection at time t , E_c is the edema rate of the control group and E_t is the edema rate of the treated group at time t .

Results of anti-inflammatory activity were compared using the Dunnett test of one-way analysis of variance (ANOVA).

Results and discussion

Present study was aimed at evaluating nanogel of 3-acetyl-11-keto- β -boswellic acid for its anti-inflammatory activity. Isolation of pure AKBA was achieved by column chromatography using hexane-ethyl acetate mixture. Identification of 3-acetyl-11-keto- β -boswellic acid was done by HPTLC and DSC analysis. A single sharp peak at R_f value 0.52 by HPTLC (Figure 2) and sharp crystalline peak at 274.347°C by DSC (Figure 3) confirmed the purity of 3-acetyl-11-keto- β -boswellic acid extracted from *Boswellia serrata* extract.

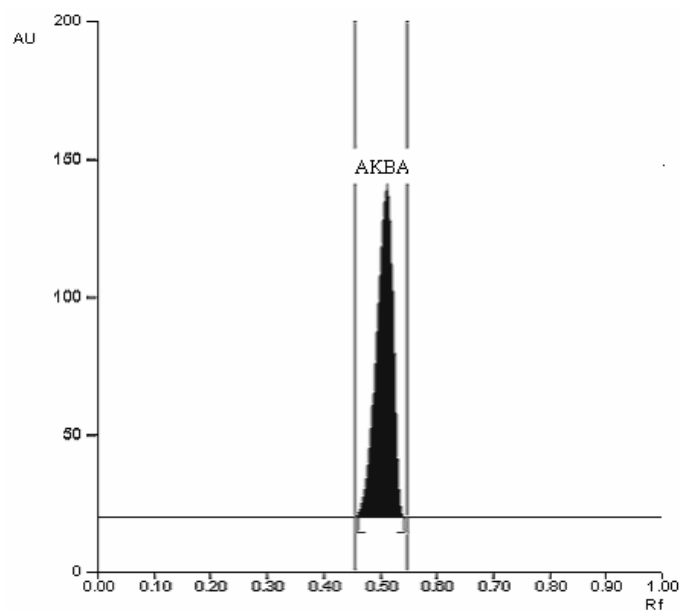


Figure2. HPTLC chromatogram of AKBA (R_f 0.52).

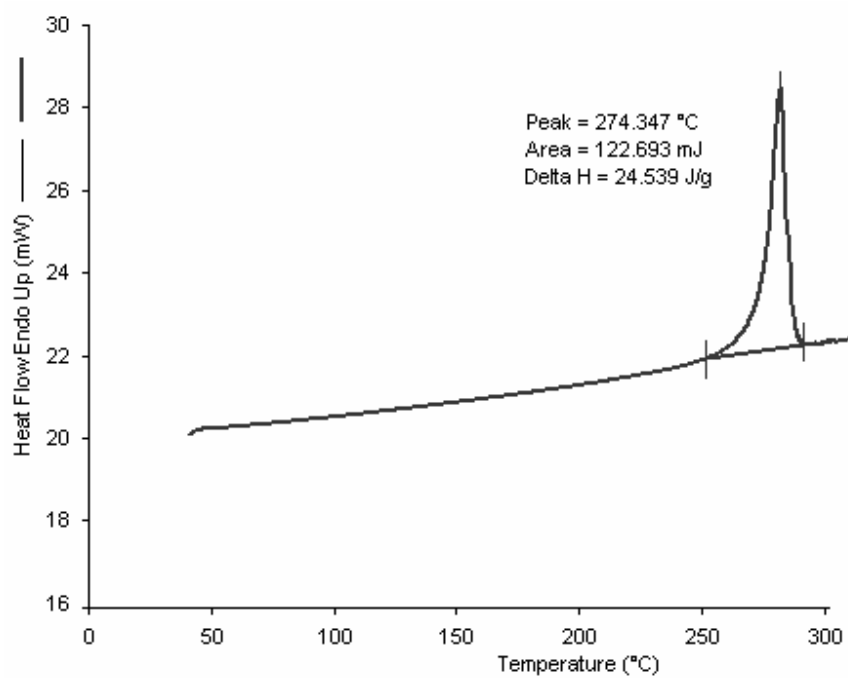


Figure3. DSC graph of AKBA

AKBA gels of different polymers in different concentrations were made as shown in Table1. The viscosity of the formulation ranges from 3200 to 5760 cps. The pH of formulation ranges from 6.8-7.4. From all the developed formulations, F₁ showed excellent homogeneity and extrudability and there were no lumps in the formulation. On the basis of results of different evaluating parameters formulation F₁ containing 1% Carbopol-940 was finally selected for gel formation.

Table1. Evaluation of AKBA gel of different polymers.

Formulation	Viscosity (cps)	Extrudability	Spreadability (gm/sec)	Homogeneity	pH
1% Carbopol-940	3250	***	35.6	***	7.2
20% PVA	5250	*	30.4	**	6.9
4%HPMC-K ₄ M + 0.5%Carbopol-940	5040	*	34.5	*	7.3
5% HPC-M+ 0.5% Carbopol-940	3200	*	31.2	*	6.8
6% SCMC+ 0.5% Carbopol-940	5760	*	31.4	**	7.4

The skin irritancy test was performed to confirm the safety of the optimized gel formulation. Van Abbe et al [26] mentioned that a value of skin irritancy score between 0 and 9 indicates that the applied formulation is non-irritant to human skin. The skin irritancy score for nanogel formulation (Table 2) indicates that the applied formulation is non-irritant to human skin.

Table2. Skin irritancy studies of developed gel

S.No.	Mice group	Score after (days)						Mean score
		1	2	3	4	5	6	
1	I	1	1	0	0	0	0	0.29
2	II	0	1	0	0	0	0	0.14
3	III	0	1	0	0	0	0	0.14

In-vivo anti-inflammatory activity studied by carragenan induced rat paw oedema method showed that nanogel formulation has maximum anti-inflammatory activity (Table 3). The enhanced anti-inflammatory effect of AKBA nanogel could be due to the enhanced permeation of AKBA through the skin.

Table 3. In-vivo anti-inflammatory activity of developed gel.

Formulation	Edema rate (%)					
	1h	2h	3h	4h	6h	12h
Control	42.1±1.2	56.3±1.3	82.2±1.2	67.2±1.5	60.3±1.5	52.1±1.3
AKBA gel	37.4±1.0 (11.2)	45.4±1.3* (19.4)	56.6±1.4** (31.1)	42.9±1.5** (36.2)	35.4±1.7** (41.3)	29.1±1.8** (44.1)
AKBA nanogel	31.9±1.1* (24.2)	35.2±1.2** (37.5)	45.1±1.4** (45.1)	26.1±2.2** (61.2)	16.8±2.3** (72.1)	9.8±2.1** (81.1)

Values represent the mean \pm S.D. of five animals for each group. Each value in parenthesis indicates the percentage inhibition rate. Statistically significant from control: *P < 0.01 and **P < 0.05.

Conclusions

AKBA and AKBA nanogel are biocompatible and do not cause any skin irritation. In-vivo anti-inflammatory activity of AKBA nanogel was much higher compared to AKBA gel of equivalent concentration. This could be attributed to the ultra small size of the polymeric nanoparticles as well as their mucoadhesiveness. From in-vivo data it can be concluded that the developed nanogel has great potential for transdermal drug delivery.

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