Original Article

Effect of a Selection of Skin Penetration Enhancers on Topical Anti-Inflammatory Effect of Boswellic Acids in Carrageenan-Induced Paw Edema in Rats

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Abstract

Background: Boswellia species have been used for treatment of chronic inflammatory disease. Several studies have documented the anti-inflammatory effect of Boswellic acids (BAs) after systemic administration. This study was aimed to evaluate the effect of some skin penetration enhancers on topical anti-inflammatory effect of BAs in rats. **Materials and Methods:** Male Wistar rats weighting 180–220 were used. Anti-inflammatory activity was assessed using carrageenan test. BAs dissolved in ethanol, propylene glycol 2%, 5%, olive oil and applied topically. Menthol, D-limonene, or eucalyptus oil 0.5%, 1% were also tested as other skin penetration enhancers and applied topically 30 min prior to subplantar injection of carrageenan into the right hind paw of rats. The volume of the paw was measured at 0 and 4 h after carrageenan with a digital plethysmometer and the difference was used as an index of inflammation. Piroxicam gel was used as a standard drug. **Results:** A 4% ethanolic solution of BAs showed significant anti-inflammatory effect. Propylene glycol (2% and 5%) in alcohol did not change the effect. Olive oil also enhanced penetration of BAs. Menthol 0.5%, 1% and D-limonene 0.5%, 1% did not show any significant change compared to olive oil alone. In the present study, eucalyptus oil 1% in olive oil was known as the best carrier for transdermal delivery of BAs. **Conclusion:** BAs have considerable topical anti-inflammatory effects and olive oil alone or especially in combination with eucalyptus oil can be promising vehicles for skin penetration of topical BAs.

Keywords: *Boswellia, carrageenan, inflammation*

Introduction

Inflammation is a pattern of defensive response to tissue injury, which allows protection from further damage. It is a complex process with involvement of histamine, serotonin, prostaglandins, nitric oxide, some kinines, and several pro-inflammatory cytokines including interleukin (IL)-1a, IL-1b, IL-6, and tumor necrosis factor-α.[1] Cyclooxygenase (COX) is the key enzyme in biosynthesis of prostaglandins and has two isoforms namely COX-1 and COX-2.[2] Non-selective inhibitors of these enzymes (traditional non-steroidal anti-inflammatory drugs) and selective COX-2 inhibitors are commonly used in inflammatory diseases. Glucocorticoids are also widely used to treat many inflammatory diseases, and despite their side effects, glucocorticoids remain a mainstay for reducing inflammation.[3] Nonsteroidal anti-inflammatory drugs (NSAIDs') significant side effects are gastrointestinal symptoms, gastritis, ulceration, hemorrhage, renal damage, and exacerbation of asthma. Glucocorticoids especially following chronic use are also associated with various adverse effects.[2,3]

Because of the significant side effect profiles of steroidal and NSAID medications, there is a greater interest in natural compounds, which have been used for reducing pain and inflammation.[3,4]

In the last decade, *Boswellia* species also called frankincense or olibanum have become more popular for the treatment of chronic inflammatory disease.

Boswellia serrata Roxb. (Burseraceae) has been used in traditional Ayurvedic medicine for the treatment of inflammatory diseases in India.[5,6] Many formulations of this plant are available on the market in the form of ointments, creams, and capsules.[7]

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Gum resin extracts of *B. serrata* contains resin acids (60%–70%), water soluble gum (about 20%) and monoterpene essential oil (3%–10%).[8] The analysis of the ingredients of these extracts revealed that its triterpenoids are effective against rheumatoid arthritis, chronic colitis, ulcerative colitis, skin allergies and ulcers, tumors, osteoarthritis, and inflammation.[8,9]

Boswellic acids (BAs) of *Boswellia serrata* are a mixture of pentacyclic triterpenes acids: Beta-BA, 3-acteyl beta BA, 11-ketobeta-BA, and 3-acetyl-11-keto-beta-BA, and have important role in its anti-inflammatory activity.[10,11]

BAs have been reported as inhibitors of 5-lipoxygenase, the key enzyme for leukotriene biosynthesis in inflammatory disorders. Human leukocyte elastase, toposiomerase I and II, as well as IκB kinases are the other molecular targets of BAs.[10-12]

Although BAs have been widely investigated for their systemic anti-inflammatory effects, only a few researches have been performed on their topical delivery.[13,14] Krüger *et al*. reported that topical delivery of BAs is difficult due to its high lipophilicity.[14]

An approach to enhance the skin permeability is addition of skin penetration enhancers to the topical formulations. These enhancers changes partition coefficient and enhance skin permeability.[15,16]

Ethanol and isopropyl alcohol are the two most common short chain alcohols used in dermal and transdermal products as a co-solvent and penetration enhancer which increase flux.[17]

Essential oils and their volatile constituents are safe and suitable permeation enhancers to promote the percutaneous absorption of hydrophilic and lipophilic drugs from topical formulation into the lower skin layers.[18]

Based on the above-mentioned subjects, this study was designed to evaluate the effect of some well-known skin enhancers on topical anti-inflammatory effect of BAs on carrageenan-induced rat paw edema in rats.

Materials and Methods

Chemicals

Carrageenan lambda (Sigma, USA) was used for the induction of paw edema. Piroxicam topical gel (Iran-najo Pharmaceutical Co.,) was used as a reference drug. Eucalyptus oil and D-limonene were provided by Tabib Darou company (Kashan, Iran). Menthol and other chemicals were purchased from Merck Company (Germany).

Animals

Male Wistar rats weighing 180–220 g were used in groups of six. Animals were maintained in standard laboratory conditions at animal house of School of Pharmacy, Isfahan University of Medical Sciences (Isfahan, Iran) with free access to food and water. All experiments were carried out in accordance with guidelines for the care of laboratory animals of Iran National Committee for Ethics in Biomedical Research (Ethics code: IR.MUI.RESEARCH.REC.1397.438).

Methods

Carrageenan-induced hind paw edema was used as the model of inflammation.[19] Male Wistar rats (180–220 g) in groups of six animals per each group were used. These animals were housed in polypropylene cages, with free access to standard laboratory diet and water and kept under standard laboratory conditions of temperature and light/dark cycle. Paw edema was induced by injecting 0.1 mL of the 1% lambda carrageenan in saline. BAs (4% w/v) were dissolved in the following vehicles: 75% and absolute ethanol, 2% and 5% propylene glycol, olive oil, olive oil contained 0.5 and 1% D-limonene, menthol or eucalyptus oil. For each experiment, one group of rats only received the vehicle (without BAs). One group received 100 mg piroxicam gel (the standard group). All solutions were topically applied at a volume of 100 µl and 30 min prior to carrageenan injection. The volume of the paw was measured with a digital plethysmometer (Borj Sanat, Iran) immediately after carrageenan and also 4 h later. The difference in paw volume was considered as an index of inflammation and compared.

Statistical analysis

The data are presented as mean ± standard error of the mean one-way ANOVA followed by Duncan test was used for comparison of data and $P < 0.05$ was considered statistically significant. All statistical calculations were performed using SPSS version 13 (SPSS, Inc., Chicago, IL, USA) software.

Results

Topical anti-inflammatory activity of different concentrations of Boswellic acids in absolute ethanol

Three different concentrations (1%, 2%, and 4%) of BAs in absolute ethanol were applied topically and their anti-inflammatory activity was evaluated. These concentrations inhibited paw edema by 7.5%, 15%, and 21.5% respectively and the effect was significant ($P < 0.05$) for a concentration of 4% [Figure 1].

Comparative effect of ethanol 75% and 100% on topical anti-inflammatory activity of Boswellic acids

Ethanol 75% and 100% were used to dissolve BAs and their topical anti-inflammatory activity was assessed. BAs in ethanol 75% could not produce a significant effect while BAs in absolute alcohol reduced inflammation by 21.9% and this effect was significant ($P < 0.05$). Piroxicam

gel as the standard drug also exerted a significant effect ($P < 0.05$) [Figure 2].

Effect of propylene glycol vehicle on topical anti-inflammatory effect of Boswellic acids

A solution of 4% BAs in 2% propylene glycol could not produce a significant anti-inflammatory response but the same concentration of BAs in 5% propylene glycol reduced inflammation by 24% ($P < 0.05$ compared with control group). Piroxicam topical gel as the standard drug also significantly ($P < 0.05$) reduced the swelling and its percentage inhibition was 32% [Figure 3].

Effect of olive oil vehicle on topical anti-inflammatory effect of Boswellic acids

As shown in Figure 4, olive oil alone did not exert any significant anti-inflammatory activity. A 4% lotion of BAs in olive oil inhibited carrageenan-induced paw swelling by 35% and piroxicam gel as the standard drug caused a 32% reduction of inflammation ($P < 0.05$).

Effect of D-limonene on topical anti-inflammatory effect of Boswellic acids

The effect of D-limonene on topical anti-inflammatory activity of BAs is demonstrated in Figure 5. A 4% lotion of BAs in olive oil alone, D-limonene 0.5% and 1% when compared with control group produced 33%, 31%, and 27% decrease in inflammatory response, respectively. The percent inhibition of inflammation for piroxicam was 32%.

Effect of eucalyptus oil on topical anti-inflammatory effect of Boswellic acids

Compared with control group, BAs in olive oil, or olive oil with 0.5% and 1% eucalyptus oil inhibited inflammation by 33%, 36%, and 53%, respectively [Figure 6].

Effect of menthol on topical anti-inflammatory effect of Boswellic acids

As shown in Figure 7, BAs in olive oil alone, or olive oil containing 0.5% or 1% menthol showed significant ($P < 0.05$) anti-inflammatory effect so that they reduced inflammation by 33%, 35%, and 35%, respectively. The percent inhibition of inflammation for topical piroxicam was 32% ($P < 0.05$).

Discussion

Although systemic administration of NSAIDs and various herbal medicines is widely recommended to treat inflammatory diseases, in some cases, a topical formulation can provide more effective anti-inflammatory activity with minimal systemic adverse effects. Recently, it was reported that topically applied *B. serrata* showed anti-inflammatory activity.[13] The present study was aimed to enhance skin permeation of BAs as the main constituents of *B. serrata* using different enhancers.

In transdermal transport of drugs, *Stratum corneum* with multiple lipid structures plays a crucial role and therefore it is expected that lipid soluble agents can passively cross the skin.[20,21]

The low skin permeability for some drugs has encouraged the researchers to investigate a variety of penetration enhancers to overcome the skin flux barriers and to improve the topical delivery of these drugs.[17]

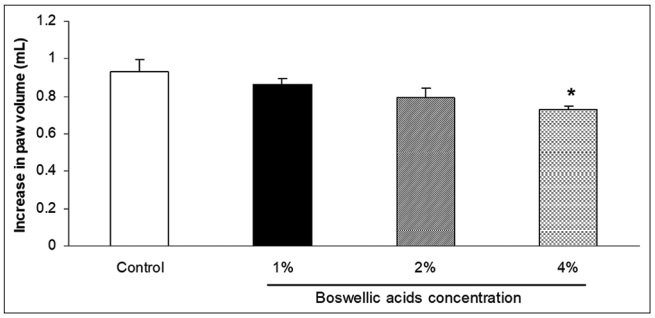

Figure 1: Topical anti-inflammatory effect of different concentrations of Boswellic acids in ethanol. One hundred microliter of 1%, 2%, and 4% Boswellic acids in absolute ethanol was applied on the right hind paw of rats and 30 min later carrageenan (100 µL) was injected. Data are mean ± standard error of the mean of paw edema in each group ($n = 6$). *$P < 0.05$ compared with control group

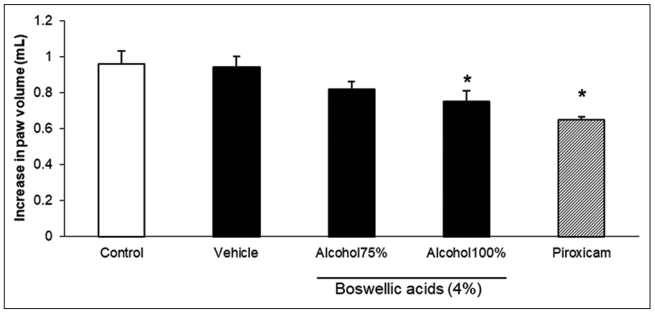

Figure 2: Effect of 75% and 100% ethanol on topical anti-inflammatory effect of Boswellic acids. Boswellic acids dissolved in 75% and 100% ethanol and 100 µL of the solutions was applied topically on right hind paw 30 min prior to carrageenan injection. Piroxicam gel (100 mg) was used as standard. Data are mean ± standard error of the mean of paw edema in each group ($n = 6$). *$P < 0.05$ compared with control group

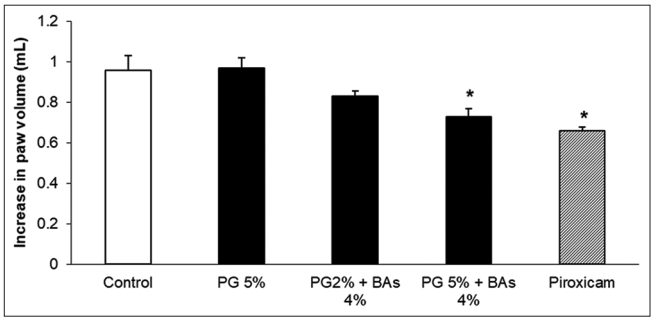

Figure 3: Topical anti-inflammatory effect of Boswellic acids 4% in propylene glycol 2%, 5%. A solution of 5% propylene glycol in ethanol (vehicle), Boswellic acids (4%) in propylene glycol 2%, Boswellic acids (4%) in propylene glycol 5% and Piroxicam 0.5% gel (100 mg) were topically applied 30 min prior to carrageenan injection. Data are mean ± standard error of the mean of paw edema in each group ($n = 6$). *$P < 0.05$ compared with control group

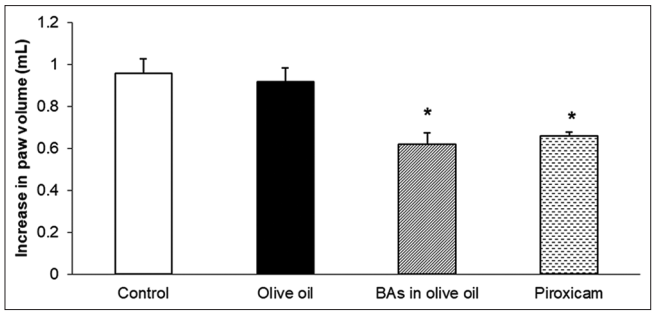

Figure 4: Effect of olive oil on topical anti-inflammatory effect of Boswellic acids. Treatments were performed 30 min prior to sub-plantar injection of carrageenan and the paw volume measured just after and 4 h after carrageenan. Olive oil was used as vehicle. Data are mean ± standard error of the mean of paw edema in each group (n = 6). *P < 0.05 compared with control group

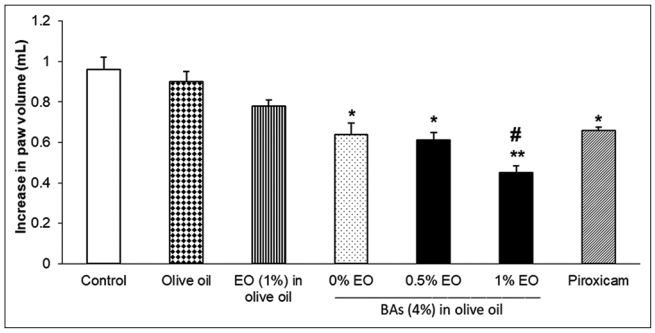

Figure 6: Effect of eucalyptus essential oil (EO) on topical anti-inflammatory effect of Boswellic acids. Olive oil (as vehicle), olive oil containing 1% eucalyptus oil, Boswellic acids 4% in olive oil, Boswellic acids (4%) in addition to 0.5 or 1% eucalyptus oil and piroxicam 0.5% gel (standard group) were topically applied on rats' paw and thirty minutes later all animals were injected with carrageenan 1% (100 µL). Data are mean ± standard error of the mean of paw edema in each group (n = 6). *P < 0.05 and **P < 0.01 in comparison with control group; #P < 0.05 compared with Boswellic acids in olive oil alone

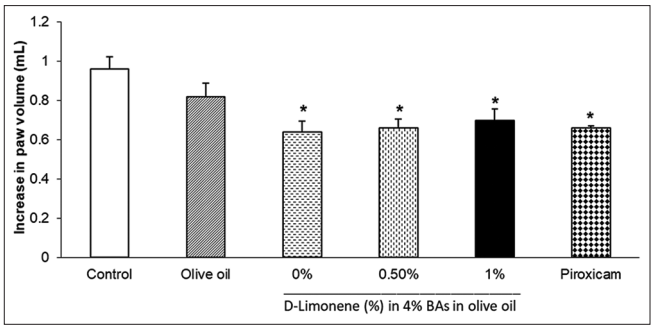

Figure 5: Effect of D-limonene on topical anti-inflammatory effect of Boswellic acids. Olive oil (as vehicle), Boswellic acids 4% in olive oil, Boswellic acids (4%) in addition to 0.5 or 1% D-limonene and piroxicam 0.5% gel (standard group) were topically applied on rats, paw and 30 min later all animals were injected with carrageenan 1% (100 µL). The paw volume measured just after and 4 h after carrageenan Data are mean ± standard error of the mean of paw edema in each group (n = 6). *P < 0.05 compared with control group

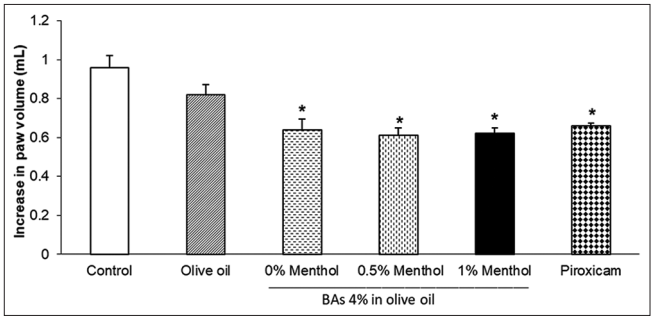

Figure 7: Effect of menthol on topical anti-inflammatory effect of Boswellic acids in carrageenan test. Olive oil (as vehicle), olive oil containing 4% Boswellic acids, Boswellic acids (4%) in addition to 0.5 or 1% menthol oil and piroxicam 0.5% gel (standard group) were topically applied on rats' paw and thirty minutes later all animals were injected with carrageenan 1% (100 µL). Data are mean ± standard error of the mean of paw edema in each group (n = 6). *P < 0.05 compared with control group

In the present study, different solvents and skin penetration enhancers were used for the assessment of topical anti-inflammatory effect of BAs in the well-known method of carrageenan rat paw edema. At first 1%, 2%, and 4% BAs in ethanol were prepared and results demonstrated that a 4% concentration of BAs had more effects than the other concentrations and selected for further studies. Furthermore, BAs in absolute ethanol showed a better anti-inflammatory activity compared with BAs in 75% ethanol (75/25 of ethanol/water). It has been reported that ethanol increases transdermal permeability of drugs by increasing their solubility in the membranes of the skin cells.[17,22]

When BAs dissolved in olive oil, it demonstrated considerable anti-inflammatory activity and our results are in agreement with previous studies.[17,23,24] Olive oil consists mostly of oleic acid (up to 83%) and both olive oil and oleic acid have been previously used as skin permeation enhancers. It has been reported that the application of oleic acid on the skin is associated with increased lipid fluidity in the stratum corneum and results in more permeability.[23]

In a recent study, the effect of several solvent systems and enhancers on skin penetration of thymoquinone was evaluated and azone, oleic acid, and transcutol were considered as suitable enhancers for topical delivery of thymoquinone.[24]

The other enhancer used in the present study was D-limonene. This substance is a non-polar hydrocarbon, and known as a potent enhancer for lipophilic drugs. The mechanism of permeability enhancement by limonene is lipid extraction causing improved drug partitioning.[17] Limonene was previously shown to be a potent skin permeation enhancer,[25] and its possible mechanism is disruption of lipid structures of the skin layers.[25,26] It has also been reported that tadalafil-loaded nanostructured lipid carriers dispersed with ethanol and limonene as skin permeation enhancers exhibited the highest skin flux (~4.8-fold) compared to that observed with tadalafil solution alone.[27] In our study, D-limonene could not change the penetration of BAs into the inflamed paws and further studies are needed to find out the reason. Among the enhancers tested in this study a

1% solution of eucalyptus oil in olive oil was the best carrier for transdermal delivery of BAs with the highest anti-inflammatory activity. Consistent with our study, results of a previous study conducted by Akram *et al*. showed that eucalyptus oil was very effective for transdermal delivery of glimepiride.[28] Also in a study by Biruss *et al*., eucalyptus oil was suggested as skin enhancer for selected steroid hormones.[29] According to our findings, it is suggested to clinically evaluate topical lotions of BAs in 1% solution of eucalyptus oil in olive oil in inflammatory diseases.

Conclusion

In our study, several skin penetration enhancers were used for topical application of BAs. Among the tested enhancers, 1% eucalyptus oil in olive oil considered as the best vehicle for topical BAs and this formulation can be a promising topical anti-inflammatory preparation.

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Conflicts of interest

There are no conflicts of interest.

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