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Boswellia frereana (Frankincense) Suppresses Cytokine-Induced Matrix Metalloproteinase Expression and Production of Pro-Inflammatory Molecules in Articular Cartilage

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The aim of this study was to assess the anti-inflammatory efficacy of *Boswellia frereana* extracts in an *in vitro* model of cartilage degeneration and determine its potential as a therapy for treating osteoarthritis. Cartilage degradation was induced *in vitro* by treating explants with 5 ng/ml interleukin1α (IL-1α) and 10 ng/ml oncostatin M (OSM) over a 28-day period, in the presence or absence of 100 μg/ml *B. frereana*. Treatment of IL-1α/OSM stimulated cartilage explants with *B. frereana* inhibited the breakdown of the collagenous matrix. *B. frereana* reduced MMP9 and MMP13 mRNA levels, inhibited MMP9 expression and activation, and significantly reduced the production of nitrite (stable end product of nitric oxide), prostaglandin E2 and cycloxygenase-2. *Epi*-lupeol was identified as the principal constituent of *B. frereana.* This is the first report on the novel anti-inflammatory properties of *Boswellia frereana* in an *in vitro* model of cartilage degradation. We have demonstrated that *B. frereana* prevents collagen degradation, and inhibits the production of pro-inflammatory mediators and MMPs. Due to its efficacy we propose that *B. frereana* should be examined further as a potential therapeutic agent for treating inflammatory symptoms associated with arthritis. **Copyright © 2009 John Wiley & Sons, Ltd.**

Keywords: articular cartilage; *Boswellia frereana*; degradation; inflammation; MMPs; *in vitro* model.

INTRODUCTION

Articular cartilage degradation is a classic feature of both degenerative and inflammatory arthritides, e.g., osteoarthritis (OA) and rheumatoid arthritis, respectively. OA is characterized by a loss of the proteoglycans and collagen from the cartilage extracellular matrix (ECM). These catabolic events are primarily mediated by the matrix metalloproteinases (MMP) (Davidson *et al.*, 2006) and aggrecanases (ADAMTS) (Struglics *et al.*, 2006). The differential expression of these catabolic enzymes is related to the level of inflammatory mediators and cytokines in the OA joint. Cytokines such as interleukin-1 (IL-1) and tumour necrosis factor-α (TNF-α) are key mediators in driving ECM destruction. In addition to inducing the synthesis of MMPs and aggrecanases, IL-1 and TNF-α also stimulate production of nitric oxide, via inducible nitric oxide synthase (iNOS), and increase the synthesis of prostaglandin E_2 (PGE_2), by stimulating the expression of PGE_2 synthase and cyclo-oxygenase 2 (COX-2).

The incidence of OA is more prevalent in our ageing and increasingly obese population; therefore relieving pain and stiffness, improving physical function and delaying disease progression are important therapeutic goals for managing OA. Non-opioid analgesics, non-steroidal anti-inflammatory drugs (NSAIDs) and intra-articular therapies are currently prescribed; continued use of NSAIDs has been associated with an increased risk of other adverse side effects (Berenbaum, 2008) making them far from ideal for treating a chronic pathology. Therefore, identifying other potential pharmaceutical agents which may be used to alleviate the symptoms associated with OA, whilst remaining efficacious and non-toxic, is an ongoing pursuit.

There has been a growing interest in the use of compounds derived from herbs in alleviating OA symptoms, e.g., curcumin (Schulze-Tanzil *et al.*, 2004), resveratrol from red-skinned fruits (Shakibaei *et al.*, 2008) and catechins from green tea (Adcocks *et al.*, 2002). Oleogum resin from *Boswellia*, commonly known as frankincense, has been identified as a potent anti-inflammatory, anti-arthritic and anti-analgesic agent (Ernst, 2008). Traditionally, *Boswellia serrata* has been used extensively in treating conditions initiated by or maintained by inflammatory events, including both rheumatoid (Singh *et al.*, 2008) and osteoarthritis (Kimmatkar *et al.*, 2003; Sontakke *et al.*, 2007; Sengupta *et al.*, 2008). The main pharmacologically active ingredients of *B. serrata* are α- and β-boswellic acids (Pardhy and Bhattacharya, 1978). It is thought they act in both early and late phases of inflammation (Singh *et al.*, 2008) by inhibiting 5-lipoxygenase in a selective, enzyme-linked non-redox and non-competitive manner (Safayhi *et al.*, 1992),

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and by inhibiting COX-1 activity (Siemoneit *et al.*, 2008).

Recently, 5-Loxin®, a novel Indian *B. serrata* extract enriched with 30% 3-O-acetyl-11-keto-β-boswellic acid, significantly improved pain score, functional ability and reduced synovial MMP3 in human clinical trials for the treatment of knee OA (Sengupta *et al.*, 2008). Other identified members of the *Boswellia* family include *B. sacra* (native to Arabian Peninsula) and *B. carteri* and *frereana* (native to Africa).

There are no reported studies on the efficacy of *B. frereana* which is indigenous to Somalia. The aim of this study was to assess the efficacy of *B. frereana* to determine whether it could rescue cytokine-induced cartilage degradation *in vitro* by mediating anti-inflammatory effects, and therefore could be exploited as a therapy for treating inflammation associated with pathologies such as OA.

MATERIALS AND METHODS

All reagents were purchased from Sigma (Poole, UK) unless otherwise specified. Culture medium consisted of Dulbecco's modified Eagle's medium (DMEM glutamax I™/HAMS F12 media (1:1), Invitrogen, Paisley, UK) supplemented with 100Units/ml penicillin, 100 μg/ml streptomycin, 50 μg/ml L-ascorbate-2-phosphate and 1X Insulin-Transferrin-Sodium selenite (ITS).

Extraction of *Boswellia frereana*. Gum resin from *Boswellia frereana* was purchased from Hargeisa, North Somalia, and successively extracted with absolute ethanol for 7 days in a light-impermeable vessel. Insoluble gum resin was removed by filtration and the solvent evaporated at 60°C. The *B. frereana* extract was dissolved and stored in ethanol at 10 mg/ml. The constituents of the *B. frereana* extract were analyzed by gas chromatography-mass spectrometry (Agilent Technologies, Paisley, UK) and data acquired using the MSD Chemstation™ computer software. The identification of the individual peaks was conducted by comparing sample mass spectra to those stored in the NIST library database which contains over 54,000 spectra and by comparison of the sample mass spectra to published literature values.

Cell culture. Full-depth articular cartilage (6 mm diameter explants) was harvested from the *metacarpophalyngeal* joint of 7-day-old bovine calves using a biopsy punch. Immature cartilage was utilized as it results in reproducible responses to cytokine treatment with the well-characterized release of sGAGs within 7 days and collagen release after 22–28 days (Little *et al.*, 2005; Morgan *et al.*, 2006). Cartilage explants (approximately 35 mg wet weight) were stabilized for 3 days in culture medium (1 ml volume) prior to treatment. Cartilage degradation was initiated by treating cartilage explants with 5 ng/ml human recombinant IL-1α (Peprotech, U.K.) and 10 ng/ml human recombinant oncostatin M (OSM; Peprotech, London, UK) (Little *et al.*, 2005; Morgan *et al.*, 2006), in the presence or absence of 100 μg/ml *B. frereana* extract; untreated explants (containing 0.1% ethanol as the vehicle control) served as a control. A concentration of 100 μg/ml was selected as a result of performing a *B. frereana* dose response on isolated articular chondrocytes; the concentration was selected according to an observed maximal response on biochemical outputs with minimal cell death (data not shown). Cultures were maintained for 28 days, with replenishment of media/treatments every 3 days. Tissue and media were harvested after 3, 7, 14, 21 and 28 days of culture. Cartilage explants were freeze dried and all biochemical data normalized to dry weight of tissue (mg). For mRNA analysis, explants were cultured with treatments for 3 days to allow investigation of early gene changes.

Biochemical assays. Cell viability was assessed using the CytoTox 96® non-radioactive cytotoxicity assay (Promega) (Blain *et al.*, 2006). Tissue explants were digested with 300 μg/ml papain at 60°C for 1 h, or until the tissue had completely dissolved, and sulphated glycosaminoglycan (sGAG) levels in the tissue explants and released into the media determined using the Dimethylmethylene Blue assay (DMMB) (Blain *et al.*, 2006). The amount of collagen in and released from the cartilage was determined using the hydroxyproline assay (Blain *et al.*, 2006), and total collagen was calculated by multiplying the hydroxyproline values by a factor of 7.14 (Neuman and Logan, 1950). Quantification of MMP2 and MMP9 synthesis and activation were evaluated in experimental media samples by gelatin zymography (Blain *et al.*, 2001). Samples were loaded on an equivalent dry-weight value, taking into account differences in the volume of accumulated media over the 28-day culture. Absolute concentrations of nitrite (a stable end product of NO) and PGE_2 were determined in the culture media using the Griess Assay (Promega) and high sensitivity PGE_2 ELISA (Biosciences Cambridge, UK.) respectively.

RNA extraction, cDNA synthesis and quantitative PCR analysis. Cartilage explants were homogenized in 1 ml of Trizol™ Reagent (Invitrogen, Paisley, UK), in liquid N_2, using a dismembrator (Sartorius, Epsom, UK), total RNA extracted and cDNA generated as previously described (Blain *et al.*, 2006). Real-time PCR was carried out using an Mx3000® QPCR System (Stratagene, Stockport, UK). A real-time qPCR assay, based on SYBR Green® detection, was designed for the transcriptional profiling of iNOS, PGE_2 synthase, COX-2 (Chowdhury *et al.*, 2008), MMP9, MMP13, ADAMTS-4 and ADAMTS-5 in the cDNA samples, after normalization to the housekeeping gene 18S (Frye *et al.*, 2005). Primers were designed to span across different exons in the open reading frame of each gene using Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) (Table 1). All reactions were carried out at an annealing temperature of 60°C, except MMP13 (58°C). Relative quantification was calculated using the $2^{-\Delta\Delta C}{}_T$ method (Livak and Schmittgen, 2001), using the untreated controls as a reference group to quantify relative changes in target gene expression. Where there was no amplification of a gene product in the untreated cartilage cDNAs, gene expression changes were relative to the IL-1α/OSM treated cDNA samples.

Statistical analysis. Data were normalized to the dry weight of the cartilage explants and presented as mean

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Table 1. Primers used in quantitative PCR assays

Gene of Interest	Primer Sequence	Product size (bp)
iNOS	F 5′ – TGTTCAGCTGTGCCTTCAAC – 3′	232
	R 5′ – AAAGCGCAGAACTGAGGGTA – 3′	
PGE_2 synthase	F 5′ – GGACGCTCAGAGACATGGAG – 3′	206
	R 5′ – TATGCCACGGTGTGTACCATA – 3′	
MMP9	F 5′ – TAGCACGCACGACATCTTTC – 3′	121
	R 5′ – GAAGGTCACGTAGCCCACAT – 3′	
MMP13	F 5′ – CCCTTGATGCCATAACCAGT – 3′	202
	R 5′ – GCCCAAAATTTTCTGCCTCT – 3′	
ADAMTS-4	F 5′ – CTCCATGACAACTCGAAGCA – 3′	169
	R 5′ – CTAGGAGACAGTGCCCGAAG – 3′	
ADAMTS-5	F 5′ – CTCCCATGACGATTCCAAGT – 3′	155
	R 5′ – TACCGTGACCATCATCCAGA – 3′	

± SEM (n = 4 explants per treatment). Each experiment was repeated on three independent sources of articular cartilage and representative data are shown. Data were tested for normality (Anderson-Darling) and equal variances prior to parametric analyses (Minitab). Where data were not normal or of unequal variance, log transformation was performed and reanalyzed. Statistical analysis was performed using a 2-way ANOVA (and Tukey's post hoc test) and where indicated a Student's *t*-test was utilized (qPCR analysis). Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

B. frereana does not affect cartilage chondrocyte viability

Cartilage explants were treated with 5 ng/ml IL-1α and 10 ng/ml OSM (Barksby *et al.*, 2006) over a 28-day period, in the presence or absence of 100 μg/ml *B. frereana*. There was 25 ± 5.9% cell death in cartilage treated with IL-1α/OSM, 12.7 ± 2.3% after *B. frereana* treatment and 16.1 ± 5.0% when explants were cultured with both cytokine and extract, but these were not significantly different to the untreated control explants.

B. frereana does not affect IL-1α/OSM-induced sGAG release or regulate ADAMTS-4 and TS-5 transcription

Over the 28 days, a small amount of sGAG was released into the media (expressed as a percentage of total sGAG) from untreated explants or explants treated with *B. frereana* (Fig. 1A). IL-1α/OSM induced sGAG release which was maximal within the first 7 days of treatment ($p < 0.001$) as previously reported (Little *et al.*, 2005; Morgan *et al.*, 2006), and remained significantly elevated until day 14 ($p < 0.001$). *B. frereana* did not rescue cytokine-induced sGAG loss from the cartilage explants over the culture period (Fig. 1A). The primary mediators of proteoglycan degradation are the aggrecanases – ADAMTS-4 and TS-5 (Fosang and Little, 2008). To elucidate whether the inability of *B. frereana* to counteract IL-1α/OSM-mediated sGAG release was attributed to ADAMTS regulation, mRNA expression levels of these two genes were analysed (Fig. 1B). IL-1α/OSM significantly increased ADAMTS-4 mRNA expression ($p = 0.008$) which *B. frereana* was unable to reduce ($p = 0.173$). Hence, *B. frereana* appears to be ineffective at inhibiting ADAMTS-4 expression, at least at the gene level, which may partially explain its inability to prevent cytokine-induced sGAG loss. ADAMTS-5 mRNA levels did not significantly alter with any of the treatments including IL-1α/OSM (Fig. 1B).

B. frereana prevents IL-1α/OSM-mediated collagen release and reduces MMP9 and MMP13 transcription and translation

Over the 28-day period, minimal amounts of collagen were released (expressed as a percentage of total collagen) from untreated or *B. frereana*-treated explants (Fig. 2A), whereas IL-1α/OSM induced significant collagen release ($p < 0.01$; log transformation), as previously reported (Little *et al.*, 2005; Morgan *et al.*, 2006). Interestingly, *B. frereana* protected the cartilage from cytokine-mediated collagen release ($p = 0.012$; log transformation) returning levels to basal amounts. Key mediators of collagen breakdown include MMP9 and MMP13 (Milner *et al.*, 2006), levels of which are elevated in pathological articular cartilage (Davidson *et al.*, 2006). To ascertain whether *B. frereana* inhibited IL-1α/OSM–mediated collagen release by modulating MMP synthesis MMP9 and MMP13 mRNA expression levels were investigated (Fig. 2B). IL-1α/OSM induced MMP13 expression (2.3-fold; $p = 0.011$: log transformation of all data), which was reduced when explants were co-treated with *B. frereana* (4-fold; $p = 0.008$). *B. frereana* alone had no effect on MMP13 mRNA expression ($p = 0.216$). MMP9 mRNA was below the limit of detection in both untreated and *B. frereana*-treated cartilage explants (Fig. 2B). However, B. *frereana* reduced IL-1α/OSM-induced MMP9 transcription by almost 50%

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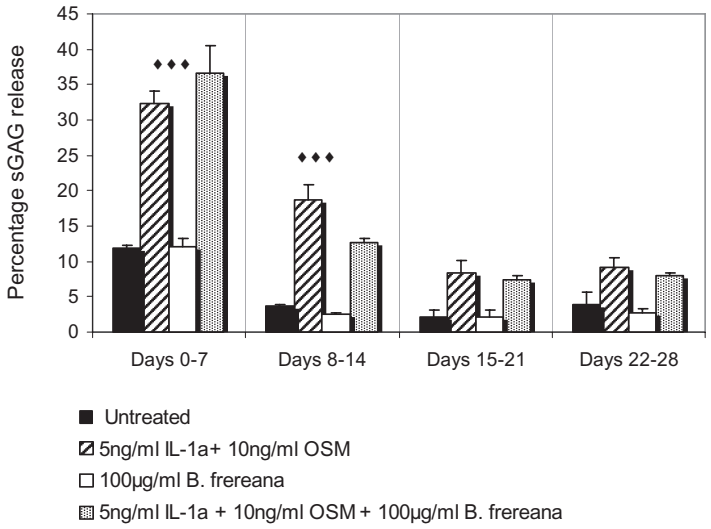
A.



B.

mRNA expression	untreated	5ng/ml IL-1a + 10ng/ml OSM	100µg/ml *B. frereana*	5ng/ml IL-1a + 10ng/ml OSM + 100µg/ml *B. frereana*
ADAMTS-4	1.77 ± 1.08	15.12 ± 5.29♦♦	0.35 ± 0.08	4.72 ± 0.80
ADAMTS-5	1.06 ± 0.21	1.44 ± 0.52	0.57 ± 0.07	0.75 ± 0.02

Figure 1. The effect of treating cartilage explants with *Boswellia frereana* (100 µg/ml) in the presence or absence of 5 ng/ml IL-1α and 10 ng/ml OSM over the 28-day period on (A) sGAG release (DMMB assay). Data are expressed as mean percentage sGAG release (of total sGAG) ± SEM (n = 4). (B) ADAMTS-4 and TS-5 mRNA expression levels after 3 days of treatment (qPCR). Data are presented as mean ADAMTS-4 and TS-5 expression (arbitrary units) ± SEM (n = 4) ^ $p < 0.05$, ^^ $p < 0.01$, ^^^ $p < 0.001$ for comparison between explants treated with IL-1α/OSM or left untreated; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ for comparison between explants treated with IL-1α/OSM +/– 100 µg/ml *B. frereana*.

($p = 0.079$; Student's *t*-test). To assess whether this regulation was evident at the protein level, the expression and activation status of pro-MMP9 was analyzed (Figs 2C and 2D). Only media removed from explants after 7 and 28 days have been included in the manuscript to maintain brevity but are representative of the trends observed over all of the time points. In untreated explants, there was no/minimal expression of pro-MMP9 at all time points. Pro-and active-MMP2 were observed in both the untreated and *B. frereana*-treated explants over the culture period. IL-1α/OSM-stimulated cartilage synthesized significantly higher levels of pro-MMP9 after 7 and 28 days ($p < 0.0001$; log transformation). From day 7 onwards, activation of pro-MMP9 increased substantially in cytokine-stimulated explants contributing to the significant release of collagen at days 21–28. Interestingly, *B. frereana* inhibited cytokine-induced pro-MMP9 synthesis ($p < 0.0001$; log transformation), and levels of active MMP9 were abolished. After 28 days, although *B. frereana* had no effect on IL-1α/OSM-mediated pro-MMP2 expression it did significantly counteract its activation ($p = 0.04$; ranked log transformation). This finding corroborates a study in which *B. carteri* inhibited MMP2 and MMP9 secretion from human fibrosarcoma HT-1080 cells (Zhao *et al.*, 2003), and also the recent clinical trial where 5-Loxin® (*B. serrata*) reduced synovial MMP3 levels (Sengupta *et al.*, 2008).

B. frereana reduces IL-1α/OSM-induced nitrite and PGE_2 production and suppresses iNOS, PGE_2 synthase and COX-2 mRNA expression

MMP9 synthesis is modulated by a variety of pro-inflammatory molecules including NO and COX-2 (Pelletier *et al.*, 1999), and both are involved in cartilage degradation (Lotz, 1999). The amount of nitrite (stable end product of NO) and PGE_2 released from the untreated explants and explants treated with *B. frereana* was low (≤5 µM nitrite/mg dry weight tissue and ≤25 pg PGE_2/mg dry weight tissue) and did not change appreciably over the 28-day culture period (Figs 3A and 3B). Cytokine treatment significantly increased production of nitrite ($p \leq 0.0033$; log transformation of data) and PGE_2 ($p \leq 0.012$; log transformation of data) at all time points. *B. frereana* significantly inhibited IL-1α/OSM-induced nitrite production from day 14 onwards ($p \leq 0.003$; log transformation of data) and PGE_2 production from day 7 onwards ($p \leq 0.03$; log transformation of data). To determine whether the inhibitory activity of *B. frereana* on nitrite and PGE_2 synthesis were regulated at the transcriptional level mRNA levels of iNOS, PGE_2 synthase and COX-2 – which regulates PGE_2 production, were quantified (Fig. 3C). PGE_2 synthase mRNA was not detected in untreated explants and all three transcripts were not detected in *B. frereana*-treated cartilage. IL-1α/OSM induced an approximate

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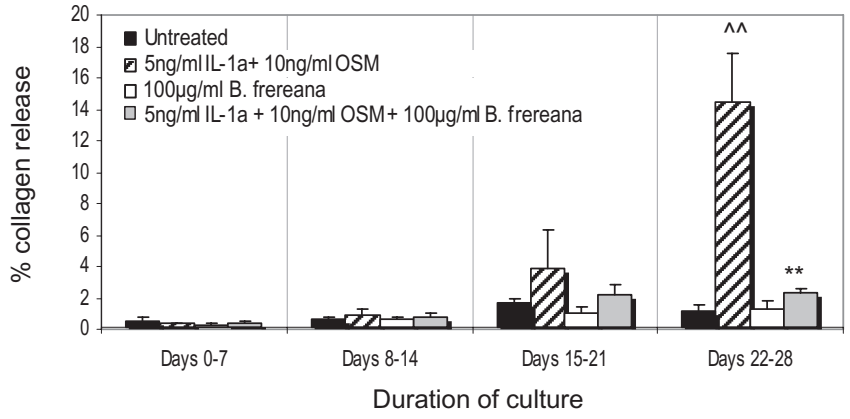
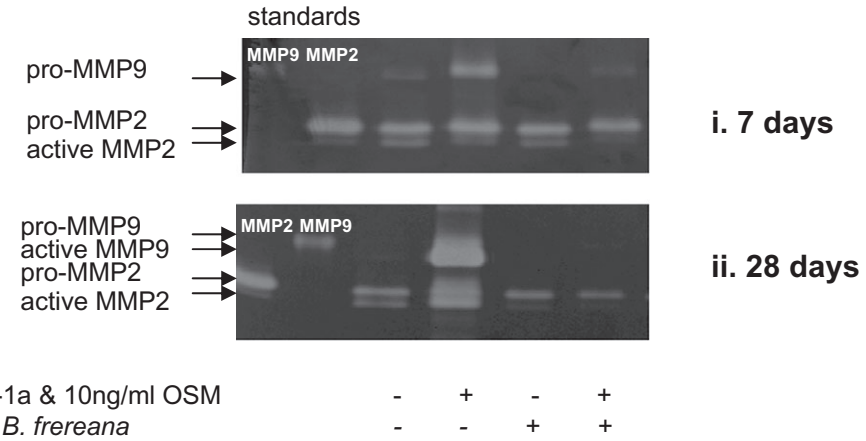
A.



B.

mRNA expression	untreated	5ng/ml IL-1a + 10ng/ml OSM	100µg/ml *B. frereana*	5ng/ml IL-1a + 10ng/ml OSM + 100µg/ml *B. frereana*
MMP13	1.04 ± 0.20	2.34 ± 0.32^^	0.24 ± 0.06	0.56 ± 0.09***
MMP9	N.D.	1.05 ± 0.18	N.D.	0.58 ± 0.13

C.



D.

		5ng/ml IL-1a & 10ng/ml OSM: -	100µg/ml *B. frereana*: -	+ -	+ +	- +	+ +
Day 7	**Pro-MMP9**	0.06 ± 0.02	0.59 ± 0.03♦♦♦	0	0.09 ± 0.01***
	Pro-MMP2	0.67 ± 0.03	0.76 ± 0.06	0.68 ± 0.02	0.60 ± 0.05
	Active MMP2	0.17 ± 0.05	0.11 ± 0.02	0.21 ± 0.02	0.10 ± 0.01
Day 28	**Pro-MMP9**	0	0.58 ± 0.04	0	0
	Active MMP9	0	0.98 ± 0.28	0	0
	Pro-MMP2	0.49 ± 0.02	0.58 ± 0.09	0.54 ± 0.13	0.27 ± 0.04
	Active MMP2	0.21 ± 0.04	0.38 ± 0.15	0.14 ± 0.05	0.06 ± 0.01*

Figure 2. The effect of treating cartilage explants with *Boswellia frereana* (100 µg/ml) in the presence or absence of 5 ng/ml IL-1α and 10 ng/ml OSM over the 28-day period on (A) collagen release (Hydroxyproline assay). Data are expressed as mean percentage collagen release (of total collagen) ± SEM (n = 4). (B) MMP9 and MMP13 mRNA expression levels after 3 days of treatment (qPCR). Data are presented as mean expression (arbitrary units) ± SEM (n = 4). (C) Representative gelatin zymograms from **i.** day 7 and **ii.** day 28. (D) densitometric analysis of MMP2 and MMP9 expression and activation. Data are expressed as mean pro- or active-MMP released (densitometric units/mg dry weight tissue) ± SEM (n = 4) (see Fig. 1 legend for p value significance).

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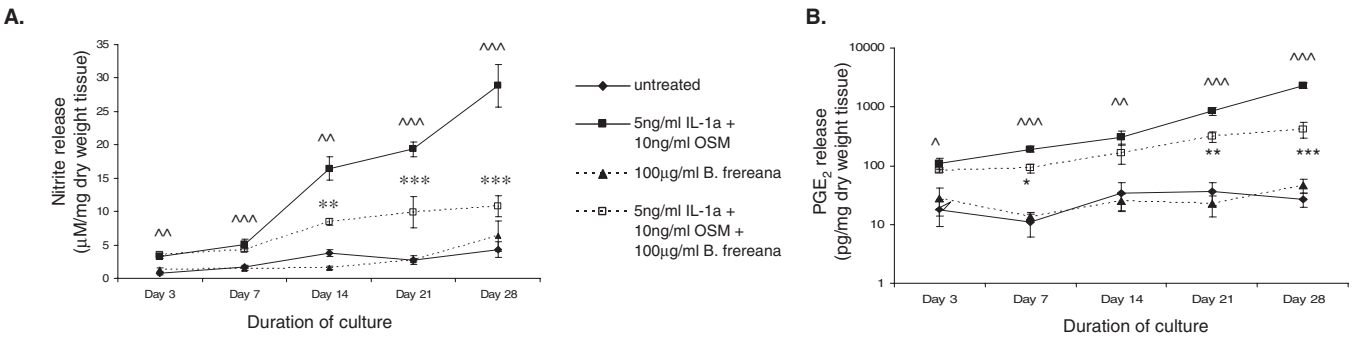

C.

mRNA expression	untreated	5ng/ml IL-1a + 10ng/ml OSM	100μg/ml *B. frereana*	5ng/ml IL-1a + 10ng/ml OSM + 100μg/ml *B. frereana*
iNOS	1.62 ± 0.86	81.1± 22.4^^^	N.D.	18.1 ± 6.3*
PGE_2 synthase	N.D.	1.02 ± 0.13	N.D.	0.42 ± 0.07**
COX-2	1.45 ± 0.59	17.11 ± 2.64^^^	N.D.	4.01 ± 0.78***

Figure 3. The effect of treating cartilage explants with *Boswellia frereana* (100 μg/ml) in the presence or absence of 5 ng/ml IL-1α and 10 ng/ml OSM over the 28-day period on (A) nitrite (Griess assay) and (B) PGE_2 (PGE_2 ELISA) release. Data are expressed as mean nitrite (μM) or PGE_2 (pg) per mg of dry weight tissue ± SEM (n = 4). (C) Expression levels of iNOS, PGE_2 synthase and COX-2 mRNAs after 3 days of treatment (qPCR). Data are presented as mean gene expression (arbitrary units) ± SEM (n = 4) (see Fig. 1 legend for p value significance).

50-fold increase in iNOS mRNA ($p < 0.0001$; log transformation) and a significant 12-fold increase in COX-2 mRNA levels ($p < 0.0001$). *B. frereana* reduced IL-1α/OSM-mediated transcription of iNOS (75% reduction, $p = 0.02$; log transformation), PGE_2 synthase (60% reduction, $p = 0.007$; Student's *t*-test) and COX-2 (75% reduction, $p = 0.0001$). Our data would suggest that the inhibitory action of *B. frereana* on nitrite and PGE_2 synthesis is controlled at the transcriptional level – reducing iNOS, PGE_2 synthase and COX-2 mRNA. We hypothesize that the vast increase in pro-MMP9 synthesis and activation (Fig. 2C) can, in part, be attributed to increased production of these pro-inflammatory molecules, and that this inhibitory action of *B. frereana* prevents downstream MMP9 synthesis.

Epi-lupeol is the major constituent of *B. frereana*

Although *B. frereana* has the geneology of the Boswellia species, little is known about the bioactive ingredients, except that it is devoid of the α- and β-boswellic acids that are characteristic of the other family members (Pardhy and Bhattacharya, 1978). Using gas chromatography in conjunction with mass spectrometry (Fig. 4A), approximately 81% of the components present in the ethanol extract of *B. frereana* were identified (Fig. 4B). The major component identified, and accounting for 59.3% of the *B. frereana* extract was *epi*-lupeol. Homologues of lupeol were also detected including lupeol acetate (3.10%) and a lupeol analogue (7.26%), as well as other minor components. *Epi*-lupeol is a pentacyclic triterpene; its epimer lupeol is a naturally occurring triterpene found in various fruit and vegetables including olives and strawberries. Lupeol has been shown to have anti-inflammatory and anti-arthritic activity *in vitro* and *in vivo* (Geetha and Varalakshmi, 1999; Fernandez *et al.*, 2001). In light of the evidence surrounding the anti-inflammatory properties of lupeol (Geetha and Varalakshmi, 1999; Fernandez *et al.*, 2001), we suggest that *epi*-lupeol is a potential candidate responsible for the effects observed in this study. We are currently isolating *epi*-lupeol from the ethanolic fraction of *B. frereana* to test this hypothesis.

CONCLUSION

This is the first report detailing the anti-inflammatory efficacy of *B. frereana* in articular cartilage. Our novel data demonstrates that *B. frereana* confers some protection against IL-1α/OSM-mediated cartilage degeneration by inhibiting breakdown of the collagenous matrix, through the inhibition of MMP9 and MMP13 transcription, inhibition of MMP9 synthesis, and reduced production of pro-inflammatory mediators including NO, PGE_2 and COX-2. We hypothesize that the active constituent of *B. frereana* may be *epi*-lupeol.

However, it remains to be determined whether an oral dosage or intra-articular administration would be more efficacious as an OA therapy. *B. frereana*, although efficacious in preventing collagen degradation, did not protect articular cartilage from cytokine-induced sGAG loss. Although a potential limitation as an OA therapy, the capacity of *B. frereana* to protect the tissue from collagen loss is important as it is well recognized that the proteoglycan component of articular cartilage is subject to self-renewal but that collagen loss is an irreversible process. A combined treatment of B. *frereana* with a nutraceutical such as glucosamine, which is known to protect against cytokine-induced sGAG loss (Fosang and Little, 2008), may offer the best protection as an OA treatment. *B. frereana*, which is non-toxic and efficacious, may offer an alternative therapy for treating inflammatory symptoms present in arthritis.

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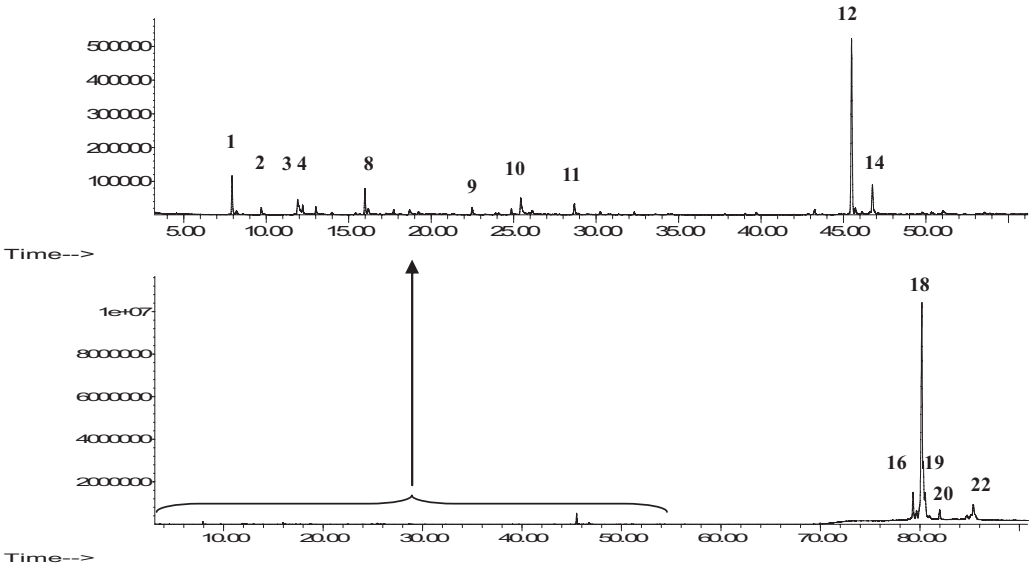

I.D.	Compound	% Peak Area
16	β-Amyrin	6.357
17	α-Amyrin	2.384
18	***epi*-Lupeol**	**59.328**
19	Lupeol analogue	7.266
20	Lupeol acetate	3.105
21	Unknown	2.093
22	Unknown	6.676
	% Compounds identified	80.85

Figure 4. *Epi-lupeol* is the predominant constituent of *Boswellia frereana.* Identification of the major chemical constituents of a *B. frereana* ethanolic extract using gas chromatography with mass spectrometry. (A) Total ion current generated gas chromatograms, where the numbers correspond to the constituents identified. (B) The largest peak (#18) was identified as *epi-lupeol* accounting for almost 60% of the *B. frereana* extract. Trace amounts of other compounds including lupeol derivatives were also identified. No isomers of Boswellic acid were detected.

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