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# Boswellic Acids in Chronic Inflammatory Diseases

Review

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## Abstract

Oleogum resins from *Boswellia* species are used in traditional medicine in India and African countries for the treatment of a variety of diseases. Animal experiments showed anti-inflammatory activity of the extract. The mechanism of this action is due to some boswellic acids. It is different from that of NSAID and is related to components of the immune system. The most evident action is the inhibition of 5-lipoxygenase. However, other factors such as cytokines (interleukins and TNF-$\alpha$) and the complement system are also candidates. Moreover, leukocyte elastase and oxygen radicals are targets. Clinical studies, so far with pilot character, suggest efficacy in some autoimmune diseases including rheumatoid arthritis, Crohn's disease, ulcerative colitis and bronchial asthma. Side effects are not severe when compared to modern drugs used for the treatment of these diseases.



## Key words

Boswellic acids · anti-inflammatory actions · immune system · leukotrienes · chronic bowel diseases · arthritis · bronchial asthma


## Abbreviations

AKBA: Acetyl-11-keto-$\beta$-boswellic acid
BA: Boswellic acid
BE: Extract from oleogum resin of *Boswellia serrata*
BS: *Boswellia serrata*
BSA: Bovine serum albumin
$[Ca^{2+}]$: Cytosolic calcium concentration
CHE: Cholinesterase
Con A: Concanavalin A
COX1: Cyclooxygenase 1
COX2: Cyclooxygenase 2
cPLA: Phospholipase A
CRP: C-reactive protein
$EC_{50}$: Effective concentration 50
ESR: Erythrocyte sedimentation rate
$FEV_1$: Forced expiratory volume in 1 sec (liters)
FLAP: 5-Lipoxygenase activating protein
fMLP: *n*-Formyl-methionyl-leucyl-phenylalanin
FVC: Forced vital capacity (liters)
HAB: Homöopathisches Arzneibuch (German homeopathic pharmacopoeia)
5-HETE: 5-Hydroxyeicosatetraenoic acid
12-HETE: 12-Hydroxyeicosatetraenoic acid
12-HHT: 12-Hydroxyheptadecatrienoic acid
HLE: Human leucocyte elastase
$IC_{50}$: Inhibitory concentration 50
5-LO: 5-Lipoxygenase
IKK: I$\kappa$B$\alpha$ kinase
IL-1: Interleukin-1
IL-2: Interleukin-2
KBA: 11-Keto-$\beta$-boswellic acid
LPS: Lipopolysaccharide
$LTB_4$: Leukotriene $B_4$
$LTC_4$: Leukotriene $C_4$
$LTD_4$: Leukotriene $D_4$
$LTE_4$: Leukotriene $E_4$


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**Dedication**
In memory of Prof. E. Reinhard


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MAPK: Mitogen activated protein kinase
MCH: Mean corpuscular haemoglobin
MCHC: Mean corpuscular haemoglobin concentration
MCV: Mean corpuscular volume
MEK: Mitogen activated protein kinase kinase
NSAID: Non-steroidal anti-inflammatory drug
P38: p38 kinase
P42: p42 kinase
PEFR: Peak expiratory flowrate
PGF 1$\alpha$: Prostaglandin F 1$\alpha$
PHA: Phytohaemagglutinin
PI3-K: Phosphatidylinositol 3-kinase
PMNs: Polymorphonuclear neutrophils
RAS: 21 kDA-GTP-binding protein
TA: Tirucallic acid
TH1: T-Helper cells 1
TH2: T-Helper cells 2
TNF$\alpha$: Tumor necrosis factor $\alpha$



## Introduction

In the last decade preparations from the oleogum resin of *Boswellia serrata* (BS) and other *Boswellia* species, also called frankincense or olibanum, have become more and more popular in some European countries for the treatment of a variety of chronic inflammatory diseases including rheumatoid arthritis, chronic bowel diseases, bronchial asthma, peritumoural brain oedema and others. This review summarises the present evidence of pharmacological actions and clinical outcome of boswellic preparations with special reference to the actions of boswellic acids.

## Historical Background

Incense was known to all the ancient civilisations and used in rituals and prayers to the gods. Frank ("pure") incense and myrrh were the finest and most scarce, produced only in a small area of the Arabian peninsula, Somalia and Ethiopia. Because of their rarity and great cost, the gifts of the Magi were a sign of wealth and sacrifice. Beyond that, there is medical evidence that gold, frankincense and myrrh were important for wound healing, used by many cultures and societies for thousands of years. The Babylonians, Hindus, Buddhists, Chinese, Shintoists, Greeks and Romans incorporated the use of incense in their ritualistic ceremonies.

The oldest written document, which mentions frankincense as a drug is the papyrus Ebers. In 1873, the Professor of Egyptology, Moritz Fritz Ebers received a more than 20 m long papyrus from an Arab businessman. It had been found eleven years before between the legs of a mummy in Luxor. It contained practical information for medical doctors regarding diagnosis and treatment of internal diseases with about 900 prescription formulae. It was probably written about 1500 BC at the time of Pharaoh Amenophis I [1].

Remedies containing preparations from frankincense (here *Boswellia carterii* Birdw.) were used by Hippocrates, Celcus, Galenus and Dioskurides [1]. Main external uses of *Boswellia carterii* Birdw. preparations were treatment of tumours, carcinomas and oedemas. Moreover, inflammatory diseases including diarrhoea and diseases of the respiratory tract were treated.

The use of the oleogum resin of *Boswellia serrata* (BS) salai guggal is described in Ayurvedic text books (Charaka Samhita, 1$^{st}$ - 2$^{nd}$ century AD and in Astangahrdaya Samhita, 7$^{th}$ century AD). Medical preparations containing the bark or the oleogum resin were used to treat a variety of diseases. These included diseases of the respiratory tract like cough, other respiratory problems, as well as diarrhoea, constipation, flatulence, central nervous diseases and others (Table **1**).

Olibanum was still a remedy in the beginning of the 20$^{th}$ century in Europe. Thus, olibanum is mentioned in the supplement to the 6$^{th}$ edition of the German Pharmacopoeia, which appeared in 1926. Thereafter, olibanum disappeared from medical treatments due to the lack of scientific evidence be it pharmacological or clinical. Scientists of the Regional Research Laboratory in Jammu (India) were the first to describe anti-inflammatory properties of an extract of the oleogum resin of BS in animal models in the years up to 1986. After the detection of the inhibitory effects of the extract on leukotriene synthesis in 1991, the subject received large interest in the scientific world.

Table **1** Therapeutic uses of Salai guggal in the traditional Indian Ayurvedic medicine (from [2])

| *Organs and functional systems* | *Effects* |
|---|---|
| Nervous system | Analgesic<br>Mental tonic<br>Stimulation<br>Eye tonic |
| Cardiovascular system | Cardiotonic |
| Gastrointestinal tract | Regulating colour of stool<br>Carminative, stomachic<br>Improving digestion, antidiarrhoeic<br>Improving taste<br>Anthelmintic |
| Urogenital system | Diuretic<br>Aphrodisiac<br>Improving menstruation |
| Fever | Antipyretic |
| Skin | Increases perspiration<br>Wound cleaning |
| Whole organism | Anti-inflammatory<br>Antiseptic<br>Reducing fat<br>Haemostypic<br>Connecting tissue<br>Decreasing Kapha diseases<br>(in Ayurvedic nomenclature) |



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## Botanical Aspects

Incense is the oleogum resin produced in the bark of different *Boswellia* species belonging to the family of Burseraceae. Some of them are listed in Table **2**.

The author is aware that the chapters dealing with the historical and botanical aspects are rather incomplete and, for details, the study of special historical, botanical and phytochemical literature [1] is suggested.

## Composition of Oleogum Resins

More than 200 different compounds were identified in the oleogum resin of different *Boswellia* species. Main components are volatile oil, pure resin and mucus. The content of these differs from species to species, between different harvestings and different locations. An approximate composition of some oleogum resins is listed in Table **3**. The resins of *Boswellia* species contain pentacyclic and tetracyclic triterpenes. Among the pentacyclic triterpenes, some boswellic acids (BA) are mainly responsible for many of the pharmacological effects. Further compounds are tetracyclic triterpenic acids among which tirucallic acids were also shown to be biologically active. For the detailed chemical composition of the resin, the reader should refer to [1].

Among the **pentacyclic triterpenes**, a variety of BAs and other compounds were identified. Some of them are closely related to the pharmacological effects of BS. Fig. **1** and Table **4** show their chemical structure and the content in the oleogum resins of BS and African species. In 2003, Büchele et al. [3] identified 12 different pentacyclic triterpenes in different samples of *Boswellia*, i.e., frankincense from India and Africa using an extract. The authors reported marked differences in different species. A striking difference was observed in the content of the main active boswellic acids AKBA and KBA. It is evident that the Indian sample contained quite similar amounts of AKBA and KBA whereas the African samples contained less KBA than AKBA. Interestingly, as discussed later, in pharmacokinetic studies with extracts from *Boswellia serrata* oleogum resin only little AKBA was found in the plasma if compared to KBA. A new pentacyclic triterpene from BS, i.e., 3$\alpha$-acetyl-20(29)-lupene-24-oic acid was recently identified by Beisner et al. [4]. Employing a commercial extract from BS (H 15 Ayurmedica™), ~2.6 mg/100 mg KBA and ~2.8 mg/100 mg AKBA were detected on average in 11 different lots [5]. Ganzera et al. [6] studied 4 different commercial products containing the oleogum resin of BS together with up to 10 other plant extracts. Considering the manufacturer's dosing recommendations, the daily intake of total boswellic acids varies up to 6-fold (18.49 to 109.62 mg per day). Hamm et al. [7] tested volatile and semi-volatile terpenes from 6 different olibanum samples, i.e., *B. carterii*, *B. sacra*, *B. serrata*, *B. papyfera* and *B. frereana*. The chemical composition was different in all species and allowed identification of the taxonomic origin of frankincense samples purchased from various markets.

Among the **tetracyclic triterpenes**, three tirucallic acids were identified, i.e., 3-oxotirucallic acid, 3-hydroxytirucallic acid and 3-acetoxytirucallic acid (Fig. **2**), which were also shown to interact with the 5-LO-system [8].

Table **2** Some species of *Boswellia* trees producing incense (from [1])

| *Species* | *Growing* | *Product* |
|---|---|---|
| *B. carteri* Birdw. | Somalia, | Olibanum |
| *B. sacra* Flueck | Nubia, South Arabia | Olibanum |
| *B. frereana* Birdw. | Somalia | Olibanum |
| *B. bhau-dajiana* Birdw. | North Somalia | Olibanum |
| *B. papyrifera* Hochst. | Ethiopia | Olibanum |
| *B. neglecta* S. Moore | Somalia | Olibanum |
| *B. odorata* Hutch. | Tropical Africa | Olibanum |
| *B. dalzielli* Hutch. | Tropical Africa | Olibanum |
| *B. serrata* Roxb. | India | Salai guggal |

Table **3** Composition of oleogum resin of two different *Boswellia* species (from [2])

| | ***Boswellia carteri* Birdw.** | ***Boswellia serrata* Roxb.** |
|---|---|---|
| Volatile oil | 5 – 9% | 7.5 – 9% to 15% |
| Pure resin | ≈ 66% | 55 – 57% |
| Mucus | ≈ 12 – 20% | ≈ 23% |

## Pharmacological Effects

In experimental animals, the use of carrageenan and dextran to produce oedemas in the paws are common models for studying anti-inflammatory actions of drugs. Singh and Atal [9] observed that oral administration of an *alcoholic* extract of the oleogum resin of BS caused inhibition of the carrageenan-induced oedema in rats and mice and dextran-induced oedema in rats, suggesting antiphlogistic action. Since such an effect could also be observed in adrenalectomised rats, it indicates that the effect was not due to the liberation of glucocorticoids.

On the other hand, an *aqueous* extract from *Boswellia carterii* Birdw. showed no inhibitory activity in carrageenan-induced rat paw oedema [10]. This is in contrast to the observations of Fan et al. [11] who used an *acetone* extract from the same species in complete Freund's adjuvant-induced oedema. Probably poor water solubility of the active principles could be the reason for this discrepancy.

Introducing a new model, i.e., latex of papaya as an inflammagen causing rat paw inflammation, Gupta et al. [12] tested a variety of antirheumatic agents and BAs and compared the effects to rat paw inflammation produced by carrageenan. The latter is thought to respond to inhibitors of prostaglandin synthesis. In the carrageenan model, the effects of indomethacin, piroxicam, ibuprofen and acetylsalicylic acid were compared with the actions of prednisolone and BAs. It turned out that BAs were much more effective in the latex of papaya model than in carrageenan-induced inflammation whereas the action of prednisolone was almost similar in both models. This suggests that the anti-in-



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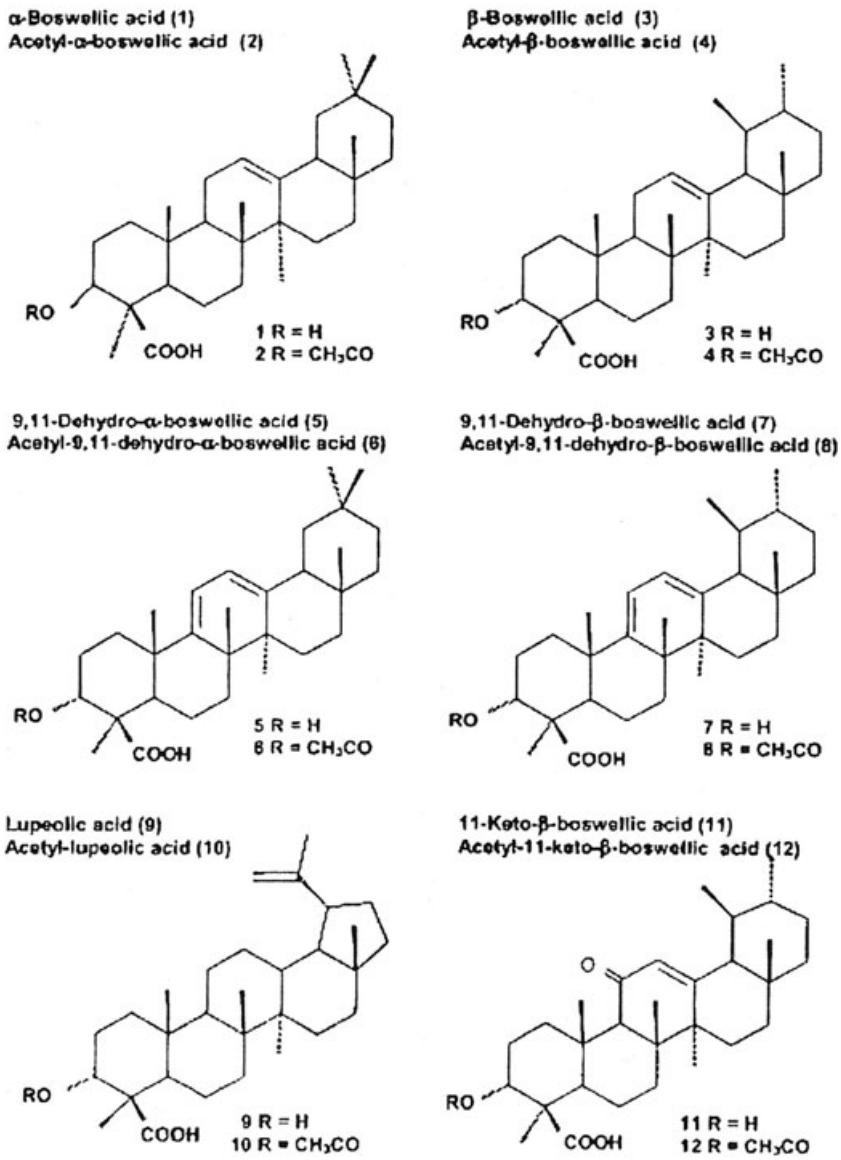


Fig. **1** Structures of different triterpenic acids present in frankincense (from [3]).

Table **4** Contents of pentacyclic triterpenic acids in the frankincense extract used as depicted in Figure **1** (from [40])

| *Compound* | *Content (mg/g extract)* | *Compound* | *Content (mg/g extract)* |
|---|---|---|---|
| **1** | 137.8 | 7 | 8.3 |
| **2** | 33.7 | 8 | 5.2 |
| **3** | 192.2 | 9 | 26.1 |
| **4** | 100.4 | 10 | 11.0 |
| **5** | 1.8 | 11 | 66.6 |
| **6** | 0.6 | 12 | 38.1 |

Total: 621.8 mg/g extract.

flammatory mechanism of BAs is different from so-called "aspirin-like" drugs and prednisolone. The latter inhibits prostaglandin and leukotriene synthesis. In fact, as discussed later, BAs did not inhibit prostaglandin synthesis but were effective in inhibition of leukotriene formation [13].

A delayed hypersensitivity was observed in mice immunised with *s.c.* administration of sheep erythrocytes into the right hind pad or intradermally after use of a mixture of BAs or azathioprine. In this experiment, the thickness of the left hind foot pad was measured. In a 4-day schedule, oral administration of a mixture containing BAs at doses of 50, 100 and 200 mg/kg on the day of sensitisation reduced foot swelling at 24 h at a dose of 200 mg/kg. The effect was comparable to the one observed following administration of an equivalent dose of azathioprine [14]. These data suggest that at least part of the anti-inflammatory action of *Boswellia* preparations could be linked to the immune system. The effects of BEs on other experimental inflammatory models such as ileitis and arthritis are discussed below.

Using the hot wire and mechanical pressure methods, Kar and Menon [15] and Menon and Kar [16] observed a significant analgesic effect of the non-phenolic fraction obtained from the oleogum resin of BS. In this study, a sedative action as evidenced by a reduction of motor activity was also described. On the other hand, Singh and Atal [9] failed to demonstrate analgesic or antipyretic properties after administration of an alcoholic extract of



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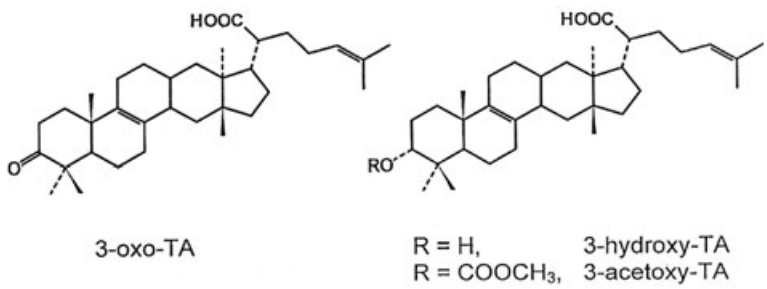


Fig. **2** Chemical structures of genuine tirucallic acids from *Boswellia serrata* oleogum resin: 3-oxo-TA (3-oxotirucallic-8,24-dien-21-oic acid), 3-acetoxy-TA (3-acetoxytirucallic-8,24-dien-21-oic acid) and 3-hydroxy-TA (3-hydroxytirucallic-8,24-dien-21oic acid).

the oleogum resin of BS. It is possible that the analgesic action is rather due to a reduction of inflammation and not to a direct effect.

In general, inflammation is the response of the body to damage of tissue either by injuries or by disorders of the immune system (auto-immune diseases). Inflammation is orchestrated by various mediators produced in mast cells, monocytes, granulocytes, macrophages and red blood cells. Inflammation is also related to the complement system in the blood where the C3 complement is converted to C3a and C3b for exerting a key function in the stimulation of inflammation and lysis by acting on different cells related to inflammation. The oleogum resin from BS and various boswellic acids were studied for their effects on different steps/factors in the cascades of events leading to inflammation. Different models have been used to study the action of oleogum resins of *Boswellia* species and BAs on the immune system.

## Effects on the Immune System

### Non-Specific Immune System

Inhibition of the guinea pig complement system by $\alpha$-boswellic acid and $\beta$-boswellic acid in a concentration range between 5 and 100 $\mu$M was reported by Wagner et al. [17]. Anticomplementary activities of a mixture of BAs were also described by Kapil and Moza [18]. They inhibited the *in vitro* immunohaemolysis of antibody-coated sheep erythrocytes by pooled guinea-pig serum. The reduced immunohaemolysis was found to be due to inhibition of C3-convertase of the classical complement pathway. The threshold concentration for inhibiting C3-convertase was 100 $\mu$g per 0.1 mL dilutent buffer added to the assay. BAs also weakly inhibited individual components of the complement system. Thus, at least *in vitro*, BAs can suppress the conversion of C3 into C3a and C3b with proinflammatory/lytic actions. Using the technique of complement fixing, a method for analysing antigen and antibody titres, oral administration of a mixture containing BAs (25, 50, 100 mg/kg) for 5 days around the time of immunisation resulted in a significant decrease in primary and secondary complement fixing antibody titres at 100 mg/kg [14]. Preincubation of peritoneal macrophages with different concentrations (1.95 – 125 $\mu$g/mL) of an undefined mixture of BAs was able to enhance the **phagocytotic function** of adherent macrophages with a maximal effect occurring at 62.25 $\mu$g/mL [14].

### Specific Humoral Defence

Humoral antibody synthesis was tested in serum from mice treated with sheep erythrocytes [14] by determining the haemagglutinating antibody titres. It was found that a single oral dose of BAs (50 – 200 mg/kg) on the day of sensitisation produced a dose-related reduction (10.4 – 32.8 %) in primary haemagglutinating antibody titres on day 4. A significant reduction in antibody production was obtained with 100 and 200 mg/kg doses. The secondary antibody titres were significantly enhanced at lower doses, the effect being most prominent at 50 mg/kg. Azathioprine (200 mg/kg *p.o.*) administered following the same schedule resulted in only 10.4 % inhibition of primary antibody synthesis and had no effect on the secondary antibody production. A marked (15.38 – 26.92 %) increase in antibody production on day + 7 was observed when a BA mixture (25 – 100 mg/kg) was given orally for 5 days around immunisation. The effect was more pronounced at a dose of 25 mg/kg than at 50 or 100 mg/kg. The secondary antibody titres were only marginally increased. Azathioprine treatment (100 mg/kg) had no significant effects on primary as well as on secondary antibody titres. In mice in which treatment was initiated 7 days prior to immunisation, BAs (25 – 100 mg/kg) elicited a dose related (37.93 – 63.79 %) increase in the primary humoral response without significantly affecting the expression of the secondary response. Levamisole (2.5 mg/kg, *p.o.*), an immunopotentiating agent, displayed only a 25 % increase in primary and a 6.66 % increase in secondary antibody titres. The different actions of BEs or BAs on leukotriene synthesis will be further discussed below.

### Specific Cellular Defence

Two studies investigated the effect of an extract of *Boswellia carterii* Birdw. and of BAs in the lymphocyte proliferation assay. This *in vitro* test utilises sensibilised lymphocytes, especially T-lymphocytes, and is used to establish immunomodulatory activity.

In 1996 Sharma et al. [14] reported that, if spleen cells from non-immunised mice were used, a mixture of various BAs in the range of 1.95 – 125.0 $\mu$g/mL showed no spontaneous mitogenic activity and the cell viability was comparable to controls. When the test was performed in the presence of mitogen stimulating lipopolysaccharide (LPS), phytohaemagglutinin (PHA), concanavalin A (ConA) and alloantigen, a concentration-dependent inhibition of lymphocyte proliferation was observed.

These data are in contrast to the observations of Badria et al. [19] who used this assay with isolated lymphocytes from venous human blood. In this study, a methylene chloride extract from the oleogum resin of *Boswellia carterii* Birdw. at 1 mg/mL stimulated lymphocyte transformation by 90 % ($EC_{50}$ = 0.55 mg/mL) in the presence of PHA or Con A. The different BAs and TAs tested, including acetyl-$\beta$-boswellic acid, acetyl-$\alpha$-boswellic acid, 3-oxo-TA, acetyl-11-keto-$\beta$-boswellic acid, $\beta$-boswellic acid, 3-hydro-

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xy-TA, and 11-keto-$\beta$-boswellic acid, showed a similar activity with $EC_{50}$ values from 0.001 to 0.005 $\mu$M. This is by far less active than the extract. Various compounds of the essential oil were active. The oil as such also exhibited 90% lymphocyte transformation. It can be concluded that this test is affected by a variety of compounds present in the extract.

### Immune Suppression

The question whether or not the overall actions of BAs will result in general immune suppression was also addressed by Sharma et al. [14]. They studied immunotoxicity in rats immunised with sheep erythrocytes and treated with undefined BAs at 25–100 mg/kg/day for 21 consecutive days. This treatment markedly increased the body weight, total leukocyte counts and primary and secondary antibody titres in rats. Beyond 50 mg/kg/day, a reduction of PMN and an increase in lymphocyte population was observed. In mice, 25–100 mg/kg on 21 days also elicited a dose-related increase in leukocyte counts without significantly affecting body weight and spleen weight, spleen cell population and cell viability. The results of this study show that the anti-inflammatory properties of boswellic acids are not associated with generalised immune suppression [14].

### Rejection of Transplants

Rejection of transplants by the immune system is still a matter of concern and is currently dealt with by treatment with immunosuppressive drugs including glucocorticoids and others. Their problem is the occurrence of severe side effects. Dahmen et al. [20] used a homeopathic preparation of olibanum (D1 trit. according to HAB 1, V6) in male mice undergoing heterotopic heart transplantation at a dose of 0.3 mg and 0.6 mg/kg body weight until sacrifice. In this study, the untreated control animals rejected their cardiac graft in 8.4 ± 1.5 days. Daily treatment with the boswellic preparation increased mean graft survival time to 14.5 days (claimed at 0.3 mg/kg/day), ranging between 8 and 59 days (and 16.7 days claimed at 0.6 mg/kg/day).

In conclusion, the studies on immunomodulatory actions discussed so far suggest an effect of BAs or at least of the oleogum resin of BS on immunological parameters. Whether or not these data are relevant in human autoimmune diseases remains to be established. At least some of these diseases, as discussed later, respond to extracts of BS.

## Mediators of Inflammation

Mediators of inflammation are produced and released by mast cells, granulocytes, macrophages, thrombocytes, red blood cells, endothelial cells and fibroblasts. They transport the information to related tissues and produce the inflammatory symptoms. Studies with BEs and/or BAs have focused so far on histamine prostaglandins, leukotrienes, IL-1, TNF-$\alpha$ and oxygen radicals.

**Histamine** causes vasodilatation, construction of bronchial smooth muscle, secretion of gastric acid and interacts with nociceptors. It binds to $H_1$ and $H_2$ receptors. Its effects in allergic reactions type I are very well known. Histamine is released from mast cells. In addition, mast cell activation results in release of leukotrienes and platelet-activating factors. In 2003, Pungle et al. [21] evaluated an extract of the oleogum resin of BS consisting of AKBA along with other constituents such as KBA and acetyl-$\beta$-boswellic acid for antianaphylactic and mast cell stabilising activity. Passive paw anaphylaxis and compound 48/80 as inducer of mast cell degranulation were used as model. The extract inhibited passive paw anaphylaxis in rats in a dose-dependent manner (20, 40 and 80 mg/kg, *p.o.*). However, dexamethasone (0.27 mg/kg, *p.o.*) serving as positive control for the extract proved to be superior. A significant, dose-dependent inhibition (20, 40 and 80 mg/kg, *p.o.*) in compound 48/80-induced degranulation of mast cells was also observed, thus showing a mast cell stabilising activity. The positive control disodium cromoglycate (50 mg/kg, *i.p.*) afforded maximum protection against degranulation as compared to the extract containing 60% AKBA. The results suggest promising antianaphylactic and mast cell stabilising activity of the extract.

**Prostaglandins** are produced via the arachidonic acid cascade either by action of the constitutive cyclooxygenase 1 (COX-1) or the inducible cyclooxygenase 2 (COX-2) enzymes. Prostaglandin production appears to depend mainly on the COX-2 products, which are responsible for inflammatory symptoms, including vasodilatation, permeability and sensitisation of nociceptors. Two types of drugs are used to treat pains/inflammation. One type (acetylsalicylic acid) does not distinguish between COX-1 and COX-2 whereas compounds such as celecoxib preferentially inhibit COX-2. In polymorphous nuclear leukocytes (PMN) stimulated with the calcium ionophore A23187, an alcoholic extract from BS inhibited 6-keto-$PGF_{1\alpha}$ formation, which was substantial at 100 $\mu$g/mL being two to three times higher than the level needed for inhibition of leukotriene synthesis [22].

Acetylboswellic acids were tested in human platelets which contain COX-1 but no 5-LO. In concentrations up to 400 $\mu$M they showed no effect on 12-HHT-COX formation [13], [23]. This is in line with data of Gupta et al. [12] who observed only little effect of BAs in the carrageenan (aspirin) model compared to the latex papaya model in which prednisolone and levamisole were effective and in which "aspirin" compounds were not active. Thus, it appears that inhibition of prostaglandin synthesis may play a minor role in the anti-inflammatory action of BEs.

**Leukotrienes** are inflammatory mediators of the immune system. They are produced by neutrophils and eosinophils, macrophages and mast cells. Their functions include: chemotaxis, plasma exudation (oedema), stimulation of oxygen radical formation and phagocytosis (partially mediated by $LTB_4$) as well as bronchoconstriction, mucus secretion and vasoconstriction (coronary arteries) (partially mediated by $LTC_4$, $LTD_4$ and $LTE_4$). These actions are different from the actions of prostaglandins. It is therefore not surprising, as discussed above, that there are differences in the anti-inflammatory actions between BAs and "aspirin"-like drugs.

Based on the observations of Singh and Atal [9], Ammon et al. [22] studied the effect of an ethanolic extract of the oleogum resin of BS on leukotriene $B_4$ formation in rat PMN. After stimulation of the leukotriene synthesis in PMN with the calcium ionophore A 231876 the extract inhibited $LTB_4$ and 5-HETE (a metabolite of the 5-LO cascade) formation in the range between 10 to 80 $\mu$g/mL in a concentration-dependent manner (Fig. **3**). In this



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assay, prednisolone was without any effect suggesting that the pharmacodynamic target is not phospholipase $A_2$.

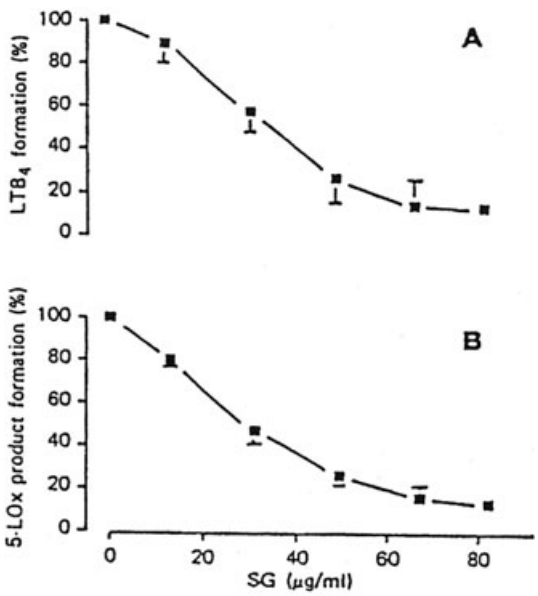


Fig. **3** Concentration-dependent inhibition of $LTB_4$-formation by an ethanolic extract of the oleogum resin of *Boswellia serrata* in stimulated rat peritoneal PMN (**A**), and the decrease in the formation of the sum of 5-lipoxygenase products (**B**), i.e., $LTB_4$, two 5-all-*trans* isomers of 5,12-diHETE and 5-HETE (mean ± S.D.; n = 3–4) (from [22]).

Table **5** Effect of boswellic acids on leukotriene formation/5-lipoxygenase activity (from [32])

| | $R^1$ | $R^2$ | $IC_{50}$ (µM) |
|---|---|---|---|
| AKBA | AcO | O | 2.7[1] (1.5) |
| 3-Acetyl-11-OH-BA | AcO | OH/H | not tested |
| 3-Acetyl-11-MeO-BA | AcO | MeO/H | partial inhibition[4] |
| KBA | OH | O | 3.0 |
| β-BA | OH | 2H | partial inhibition |
| 3-Acetyl-β-BA | AcO | 2H | partial inhibition |
| Acetyl-9,11-dehydro-BA | AcO | – | 0.75 |
| 9,11-Dehydro-BA | OH | – | partial inhibition |

In 1992, BAs were reported to be specific, non-redox inhibitors of 5-LO [13]. In this study, isomers (α-and β-) of BAs, i.e., 11-keto-β-boswellic acid and their acetyl derivatives were isolated from the oleogum resin of BS. BAs and derivatives decreased the formation of $LTB_4$ in calcium-stimulated PMN in a concentration-dependent manner. Acetyl-11-keto-β-boswellic acid (AKBA) was most effective with an $IC_{50}$ value of 1.5 µM [13], [23]. To find out whether or not the inhibitory action of BAs depends on specific chemical structures, Sailer et al. [24] studied the effect of a variety of derivatives of BAs on leukotriene synthesis in Ca ionophore-stimulated PMN. From the $IC_{50}$ value, it is obvious that not all of the compounds tested inhibited leukotriene synthesis and that some exhibited only a partial effect. The findings revealed that a hydrophilic function at C-4 in combination with an 11-keto group is essential for the inhibition of leukotriene synthesis by BAs (Table **5**).

As discussed, there is a cascade of events starting with stimulation of leukocytes and resulting in the production of leukotrienes by **5-lipoxygenase (5-LO)**. In a cell-free system from PMN, where such cascades are interrupted, the effect of various derivatives of BAs on 5-LO activity was tested in the presence of exogenous arachidonic acid (Table **6**). It was found that in this system the effects of different BAs were qualitatively similar to those in intact PMN. However, the $IC_{50}$ was higher which may be due to the different environment of 5-LO in a cell-free system. On the other hand the structure activity-relationships were the same indicating that effective BAs inhibit leukotriene synthesis by interaction with 5-LO.

Further studies addressing the mechanism of direct 5-LO inhibition used the supernatant of PMN, a cell-free system. In this study, pentacyclic triterpenes lacking the 11-keto function and/or carboxyl function on ring A (e.g., amyrin and ursolic acid) did not inhibit 5-LO. These compounds even caused a concentration-dependent reversal of 5-LO inhibition by AKBA whereas the inhibitory actions of 5-LO inhibitors from different chemical classes were not modified. Thus, it can be concluded that AKBA acts directly on the 5-LO enzyme at a site selective for pentacyclic triterpenes which is different from the arachidonate substrate binding site [25]. Using the technique of photoaffinity labeling it was studied whether or not azido-$^{125}$I-KBA (4-azido-5-$^{125}$iodo-salicy-

Table **6** Chemical structures of AKBA and analogues and their effects on 5-LO product formation (i.e. sum of 5,12-di-HETES plus 5-HETE) from endogenous arachidonic acid in ionophore-stimulated intact rat PMN (system A) and from 20 µM exogenous substrate in 105.000 g supernatants of rats PMN (system B) (from [24])

| Compound | $R^1$ | $R^2$ | $R^3$ | $IC_{50}$ (µM) system A | $IC_{50}$ (µM) system B |
|---|---|---|---|---|---|
| AKBA | COOH | α-OAc | O | 1.5 ± 0.2[a] | 7.0 ± 2.2[a] |
| KBA | COOH | α-OH | O | 2.8 ± 0.2[a] | 14.6 ± 7.6[a] |
| β-BA | COOH | α-OH | 2H | partial inhibition | partial inhibition |
| 11-Keto-diol BA | $CH_2OH$ | α-OH | O | 4.5 ± 1.2[a] | 45.3 ± 11[a] |
| 3α,24-Diol BA | $CH_2OH$ | α-OH | 2H | no effect[b] | no effect[a] |
| 11-Keto-β-BA methyl ester | $COOCH_3$ | α-OH | O | no effect[b] | no effect[b] |
| Amyrin | $CH_3$ | β-OH | 2H | no effect[b] | no effect[b] |
| Acetyl-11-keto-amyrin | $CH_3$ | β-OAc | O | no effect[c] | no effect[c] |

[a] $IC_{50}$ values of the biochemical effect were determined after logit-log transformation of the data of each experiment. Data of independent observations (n = 3) are shown as means ± S.D.
[b] No inhibition was observed for concentrations up to 50 µM.
[c] No inhibition was observed for concentrations up to 25 µM.

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loyl-β-alanyl-11-keto-β-boswellic acid), a photoaffinity analogue inhibiting 5-LO-activity as efficiently as a lead compound and specifically labeling human 5-LO protein, could be displaced from its binding site by AKBA. This was in fact the case. On the other hand, arachidonic acid had no such effect. These data also suggest that AKBA binds in the presence of calcium to a site distinct from the substrate binding site of 5-LO. The AKBA binding site is likely to be identical with a regulatory, second arachidonate binding site of the enzyme [26].

The physiological properties of **neutrophils** have already been described. In these cells, the transduction of external signals such as the toxin *N*-formyl-methionyl-leucylphenylalanine (fMLP) uses a variety of second messengers. Central factors are p38 kinase that activates mitogen activated protein kinase (MAPK) (Fig. **4**), a 21 kDA-GTP-binding protein (RAS) and phosphatidylinositol 3-kinase (PI3-K) which, in turn, causes translocation of cytosolic 5-LO and phospholipase $A_2$ ($cPLA_2$) to the nucleus via mitogen activated protein kinase kinase (MEK-1/2) and $[Ca^{2+}]i$. Here, they bind to 5-lipoxygenase activating protein (FLAP), and $Ca^{2+}$ activates leukotriene synthesis from arachidonic acid.

The fMLP-signalling is very complex. Thus, it activates phagocytic leukocytes resulting in chemotaxis, degranulation, generation of superoxide anions and activation of integrins; deletion of the fMLP receptor results in compromised host defence. In human neutrophils, it was observed that stimulation by fMLP induces MEK activation, 5-LO translocation and 5-LO product formation from endogenous substrate [27]. Recently, Altmann et al. [28], [29] demonstrated that 11-ketobo-swellic acids at 30 μM and more can activate PMN by mobilisation of $Ca^{2+}$, stimulation of p42 (MAPK) and p38 (MAPK). These data would suggest that BAs might even initiate leukotriene formation. This is, however, not the case since BAs directly inhibit 5-LO [25], [26] by binding to the enzyme in much lower concentrations (Tables **5** and **6**). The proposed cascade of events in stimulation of leukotriene synthesis and the possible effects of AKBA are shown in Fig. **4**.

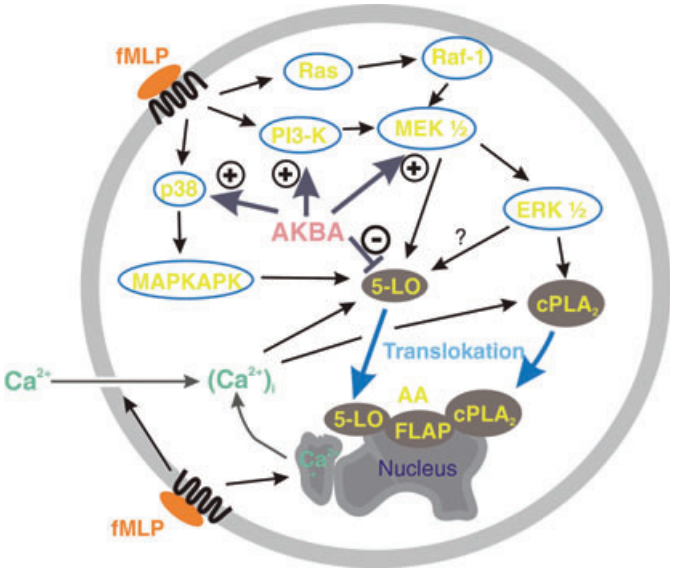


Fig. **4** Signal transduction cascade of the 5-LO-system: effects of AKBA.

In our first publication [22], we observed a concentration-dependent *in vitro* inhibition of leukotriene synthesis at a starting concentration of 10 μg/mL BE. Repeating these experiments with concentrations less than 10 μg/mL, an extract consisting of a mixture of resin exudates from *Boswellia carterii* Birdw. and *Boswellia bhaw-Dajiana* Birdw. as well as of BS [30] resulted in stimulation of leukotriene formation with a maximum up to 218 and 196% at 5 μg/mL. This effect was not due to the action of AKBA since this compound, even at lower concentrations, exhibited no such effect. Thus, other compounds in the extract must have caused this antagonistic action. One of these compounds has been identified by Boden et al. [8] to be **3-oxotirucallic acid (3-oxo-TA)**.

As discussed above [30], it was found that at low concentrations of a BE (5 μg/mL) there was a strong additional increase in leukotriene synthesis after stimulation of PMNs with $Ca^{2+}$ ionophore which was not due to the action of AKBA. In 2001, Boden et al. [8] reported that the tetracyclic triterpene 3-oxo-TA stimulated $Ca^{2+}$ ionophore-induced 5-LO product formation in intact PMNs by additional 54%. The maximum effect occurred at 10 μM whereas 20 μM seemed to be inhibitory. 3-Acetoxy-TA, a minor constituent of BS resin, also increased ionophore-stimulated $LTB_4$ synthesis by 35% at 2.5 μM. In contrast, the tetracyclic triterpene 3-hydroxy-TA, also a minor constituent, was inhibitory with an $IC_{50}$ value of about 5 μM, which is in the range of some of the BAs. In the absence of ionophore, 3-oxo-TA and 3-hydroxy-TA both initiated 5-LO product formation in substantial amounts. When the effect of 3-oxo-TA was tested in a cell-free system, its action was solely inhibitory ($IC_{50}$ ~3 μM) demonstrating the pivotal role of an intact cell structure for its activating property [8].

In non-primed resting PMNs, 3-oxo-TA initiated MEK-1/2 phosphorylation and 5-LO translocation as the early and crucial step of 5-LO activation which, in turn, consistently resulted in a substantial 5-LO product synthesis from endogenous substrate. A further effect of 3-oxo-TA consists in moderate mobilisation of intracellular calcium. This effect was inhibited by thapsigargin, an inhibitor of intracellular calcium release. Interestingly, in 3-oxo-TA-sensitive PMNs, the subsequent fMLP addition had no effect on $[Ca^{2+}]i$. In the absence of the ionophore, 3-oxo-TA and 3-acetoxy-TA at 5 and 10 μM both initiated 5-LO product formation in substantial amounts. This was associated with concomitant induction of 5-LO translocation. In the cell-free 5-LO assay, 3-oxo-TA, 3-acetoxy-TA and 3-hydroxy-TA inhibited 5-LO product formation in the presence of exogenous arachidonic acid [8].

3-Oxo-TA and 3-acetoxy-TA as well as fMLP initiated MEK-1/2 phosphorylation. In contrast, the non-inhibitory and non-stimulatory ligand of the 5-LO allosteric site, amyrin, induced no MEK-1/2 stimulation. 3-Hydroxy-TA, which acts as a 5-LO inhibitor in both intact cell and cell-free assays, did not mediate substantial MEK-1/2 phosphorylation [8]. Very recently, it was reported that fMLP does not induce LT production as reported repeatedly in literature [31].

Even though some BAs were shown to inhibit leukotriene synthesis/5-LO, it must be considered that extracts from boswellic species may contain antagonistic compounds. Thus, pentacyclic





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and tetracyclic triterpenes interact differently with 5-LO. The final outcome on leukotriene synthesis depends on the content of different BAs in different species, harvesting localisation, of growth and methods of extraction. These data again indicate that extract formulations of BEs must be standardised not only for their content of active boswellic acids but also for their 5-LO inhibitory actions [32]. The use as food supplements is not justified since boswellic preparations are drugs and therefore must meet regulations of drug laws.

**Human leukocyte elastase (HLE)** is a serine protease produced and released by PMN and, because of its aggressive destructive properties, some investigators have suggested that HLE may play a role in several diseases, such as pulmonary emphysema, cystic fibrosis, chronic bronchitis, acute respiratory distress syndrome, glomerulonephritis and rheumatic arthritis [33]. In 1995, it was demonstrated that granulocyte-mediated hepatotoxicity after endotoxin stimulation depends on elastase release [34].

Using pure HLE, Safayhi et al. [35] screened several pentacyclic triterpenes for inhibitory actions on HLE. This is of therapeutic interest since leukotriene formation and HLE release are increased simultaneously during neutrophil stimulation in a variety of inflammatory and hypersensitivity based human diseases. Thus, decrease of chemotaxis together with inhibition of this enzyme would lower the destructive actions of HLE, especially at the locus of diseases. In the study of Safayhi et al. [35], AKBA decreased the activity of HLE *in vitro* with an $IC_{50}$ value of roughly 15 $\mu$M. Among the pentacyclic triterpenes tested in concentrations up to 20 $\mu$M, they also observed substantial inhibition by $\beta$-boswellic acid, amyrin and ursolic acid, but not by 18-$\beta$-glycyrrhetinic acid. The data show that the dual inhibition of 5-lipoxygenase and HLE is unique to BAs: other pentacyclic triterpenes with HLE inhibitory activities (e.g., ursolic acid and amyrin) do not inhibit 5-LO.

The question may arise whether or not the effects of the aforementioned pentacyclic triterpenes are of practical relevance since the $IC_{50}$ value is much higher than that of some BAs (around 1.5 – 5 $\mu$M in PMN). However, the $IC_{50}$ of AKBA in a cell-free system on 5-LO has also been shown to be higher than in that intact cells [24]. It could therefore be that the $IC_{50}$ values of pentacyclic triterpenes are lower in a natural environment than in the enzymatic test. Moreover, it is also possible that with extract of the resin of BS the different pentacyclic triterpenes may act in a synergistic way. In fact, when an extract was employed in the enzyme test (H15™), half maximal inhibition occurred at ~7.5 $\mu$g/mL (personal observation).

**Oxygen radicals** are also factors involved in tissue destruction in, i.e., rheumatoid arthritis. Heil et al. [36] studied the effects of AKBA and of BEs on SOD-quenchable $O_2^-$ radical formation in intact PMNs and in a cell-free system. AKBA ($IC_{50}$ ~10 $\mu$M) and extracts ($IC_{50}$ ~13 $\mu$g/mL) consistently inhibited PMA-stimulated NADPH oxidase activity in rat peritoneal PMNs and reduced fMLP and PMA-induced oxidative burst in stimulator-sensitive human blood PMN preparations, but failed to block the NADPH-oxidase activity in the membrane fraction of PMA prestimulated PMNs.

The **cytokines** studied so far in connection with the anti-inflammatory actions of BAs are TNF-$\alpha$ and IL-1 that are released from macrophages, for example, after activation by TH1 cytokines such as interferon-$\gamma$ or by bacterial endotoxin. TNF-$\alpha$ and IL-1 are involved in antibacterial and inflammatory reactions, TH-2 cytokines are activating B-lymphocytes.

Inhibition of TNF-$\alpha$ and its signalling has been recognised as a highly successful strategy for the treatment of chronic inflammatory diseases such as rheumatoid arthritis. Previously, it has been shown by Shrives et al. [37] that acetyl-$\alpha$-BA and AKBA inhibited the generation of TNF-$\alpha$ in concentrations between 1 and 10 $\mu$M in lipopolysaccharide-stimulated human monocytes. AKBA was found to be the most effective compound. The effect was mediated by a direct inhibitory action on I$\kappa$B$\alpha$ kinesis (IKK), conveyed inhibition of NF-$\kappa$B and subsequent down-regulation of TNF-$\alpha$ expression in human monocytes. In human monocytes, Borsches and Grim (personal communication 2000) observed a concentration-dependent inhibition of IL-1$\beta$ and TNF-$\alpha$ production in a concentration range of 5 to 20 $\mu$M.

Roy et al. [38] tested the genetic basis of the anti-inflammatory effects of a standardised BE in a system of TNF-$\alpha$-induced gene expression in human micro vascular endothelial cells. Acutely, TNF-$\alpha$ induced 522 genes and down-regulated 141. 113 genes were clearly sensitive to BE treatment. Such genes are directly related to inflammation, cell adhesion and proteolysis. DNA micro array analysis in connection with Remap, gene ontology data mining tool and others led to the recognition of primary BE-sensitive TNF-$\alpha$-inducible pathways. BE was found to prevent TNF-$\alpha$-induced expression of matrix metalloproteinases and mediators of apoptosis. In this context it is important to note that most TNF$\alpha$-induced genes are NF-$\kappa$B-dependent.

The effect of an extract from *Boswellia carterii* on the production of TH1 and TH2 cytokines by murine splenocytes was studied by Chevrier et al. [39]. In these *in vitro* experiments, application of the resin extract using ethanol as a solvent resulted in significant cellular toxicity not seen with ethanol alone. Interestingly, use of an extract with sesame oil as solvent resulted in a dose-dependent inhibition of TH1 cytokines (IL-2 and $\gamma$ interferon) and a dose-dependent potentiation of TH2 cytokines (IL-4 and IL-10).

As far as the anti-inflammatory actions of BEs/BAs are concerned, it appears that these may have different targets related to the actions of the immune system in chronic inflammatory diseases.

## Action in Diseases – Experimental and Clinical

There are several severe chronic diseases mostly related to autoimmune disorders. Among these are rheumatic diseases, inflammatory bowel diseases, bronchial asthma and others.

In fact, several clinical studies were performed. However, the results published so far must be regarded as having pilot character because some were performed in order to test whether or not the theoretical considerations could also be of clinical relevance. Therefore, further studies confirming these findings are necessary.

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## Pharmacokinetics

As discussed before, most of the active principles are BAs. AKBA and KBA are the most active ones while other BAs may contribute to the actions when an extract or crude product is employed for therapeutic purposes. The contents of these BAs vary from species to species and also between different procedures (extractions) in the production of the drug. Since the *in vitro* tests have shown that the $IC_{50}$ for AKBA ranges from 1.5 – 3.0 $\mu$M for inhibition of leukotriene synthesis to 15 $\mu$M for elastase inhibition, it is necessary to determine pharmacokinetic parameters for absorption, plasma half-life time and time to reach maximal concentration in the blood. In fact, BAs have already been detected in human plasma after oral administration of an extract.

In 2003, Büchele et al. [3] identified 12 different pentacyclic triterpenic acids in frankincense. Their structures and contents are shown in Fig. **1** and Table **4**. Using high performance liquid chromatography and photodiode array detection, Büchele and Simmet [40] could identify most of them in the plasma of a patient with a brain tumour after 10 days of treatment with 4 × 786 mg per day of an Indian frankincense oleogum resin extract (Table **7**). It is interesting to note that 2 hours after administration of the last dose the concentration of AKBA in human plasma was only 0.1 $\mu$M whereas that of KBA was 0.34 $\mu$M. This suggests that the most active compound *in vitro*, AKBA, is either only marginally absorbed or deacetylated in the liver and converted to KBA. These *in vivo* concentrations are low if compared to the $IC_{50}$ values determined in the *in vitro* tests.

In an open uncontrolled trial with 12 healthy male volunteers [41], a single oral dose of a commercial product of BS extract (WokVel™) containing 333 mg was given *after* a meal. The extract contained 6.44% KBA, 2% AKBA, 18.51% $\beta$-BA, 8.58% 3-*O*-acetyl-$\beta$-BA, 6.93% $\alpha$-BA and 1.85% 3-*O*-acetyl-$\alpha$-BA. In this study, only the concentration of KBA in the plasma was determined. Maximum concentration was measured after 4.5 h and was 2.72 $\mu$M, which is in the range of the $IC_{50}$ of KBA for the inhibition of leukotriene synthesis. The absorption half life ($t^1/_2$ a) was 2.35 hours, and elimination half life ($t^1/_2$ b) was 5.97 hours. The apparent volume of distribution was 142.97 litres, and the plasma clearance averaged 296 mL/min. No adverse events were seen in this study. The elimination half life in this study of nearly 6 hours suggests that BE needs to be given every six hours.

Table **7** Contents of pentacyclic triterpenic acids in plasma from a brain tumor patient (glioblastoma multiforme) treated with the frankincense extract for 10 days (from [40])

| *Compound* | *Content (mg/g extract)* | *Compound* | *Content (mg/g extract)* |
|---|---|---|---|
| **1** | 3.50 | 7 | 0.47 |
| **2** | 4.00 | 8 | 0.29 |
| **3** | 10.10 | 9 | n.d. |
| **4** | 2.40 | 10 | n.d. |
| **5** | 0.06 | 11 | 0.34 |
| **6** | 0.10 | 12 | 0.10 |

n.d.: not detectable.

According to this study, kinetics for KBA indicate that the steady state plasma concentration will be reached after approximately 30 hours [42]. Since a single dose of a BE shows a very high volume of distribution, the drug either penetrates specifically into "deep" tissues in peripheral compartments such as fat or is bound to distinct biological materials [42].

Sterk et al. [43] studied the effect of food intake on the bioavailability of BAs from a dry extract of BS in healthy volunteers. Twelve healthy subjects were fasted during ten hours before and until four hours after drug administration (group A). A second group (B) received a high-fat meal together with the drug. The volunteers swallowed 3 capsules with 282 mg (total of 786 mg) extract, containing 143.4 mg $\beta$-BA, 103.71 mg $\alpha$-BA, 82.71 mg acetyl-$\beta$-BA, 48.12 mg KBA, 28.71 AKBA and 26.25 acetyl-$\alpha$-BA. The time course of the plasma concentrations of the most active boswellic acids, i.e., KBA and AKBA, was dramatically different in fasted and high-fat meal volunteers. The calculated plasma kinetic parameters are shown in Table **8**. With this preparation of BE, $t_{max}$ was 3.5 h (A) and 4 h (B) for KBA and 2 h (A) and 3 h (B) for AKBA. $C_{max}$ was found to be 83.6 ng/mL (A) and 227 ng/mL (B) for KBA. In case of AKBA, $C_{max}$ was much lower (6 ng/mL for A and 28.8 ng/mL for B). This is an important finding that has to be considered in therapy in order to achieve high plasma levels.

Elimination half-life ranged from 10.5 to 69.3 hours. Therefore, a repeated dose may lead to accumulation. In addition, patients reported that the therapeutic action occurs with a certain delay and that disease symptoms may even aggravate at the beginning of treatment in some cases. The data of Sterk et al. [43] Table **8** were re-calculated from ng/mL to nM in order to be comparable to the *in vitro* studies, the following observations can be made:

| | | | |
|---|---|---|---|
| KBA | $C_{max}$ | fasted | 178.5 nM |
| | | high fat meal | 483.7 nM |
| AKBA | $C_{max}$ | fasted | 11.7 nM |
| | | high fat meal | 56.2 nM |

If the $IC_{50}$ values for inhibition of leukotriene synthesis (1.5 – 2.7 $\mu$M for AKBA and ~3.0 $\mu$M for KBA) are considered, the measured plasma concentrations are not sufficient to exert a therapeutic effect as seen in the clinical studies even if KBA and AKBA and some other BAs may act in a synergistic manner. However, there are two possibilities for explaining the findings: firstly, accumulation may occur after repeated dosages, which still has to be confirmed. A second possibility is that lipophilic tissues may accumulate BAs. In support of this hypothesis, Raising et al. [42] showed that 99 ng/g KBA and 95 ng/g AKBA were detected in the brain after oral administration a single dose of 240 mg/kg of BE to Wistar rats.

The following conclusions can be drawn from the available pharmacokinetic studies:

- The content of the most active BAs differs between various species of *Boswellia*.
- Work-up processes may influence BA contents in pharmaceuticals.



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Table **8** Pharmacokinetics of 11-keto-β-boswellic acid (KBA) and acetyl-11-keto-β-boswellic acid (AKBA) after administration of three capsules BE (786 mg dry extract of the oleogum resin from *Boswellia serrata*) as oral single dose under fasted conditions (treatment A) and under fed conditions (treatment B) (from [43])

| *Parameters* | *Values (geometric mean + range)* | |
|---|---|---|
| | *Treatment A (fasted conditions) (n = 12)* | *Treatment B (fed conditions) (n = 12)* |
| 11-Keto-β-boswellic acid | | |
| $AUC_{(0-\infty)inf}$ (ng h $mL^{-1}$) | 1660.72 (840.3 – 3778.1) | 3037.15 (1481.9 – 6583.1) |
| $AUC_{(0-tz)}$ (ng h $mL^{-1}$) | 658.4 (137.0 – 2747.3) | 2451.8 (1085.0 – 6125.4) |
| $C_{max}$ (ng $mL^{-1}$) | 83.8 (24.9 – 243.8) | 227.1 (101.0 – 418.1) |
| $T_{max}$ (h)[a] | 3.5 (2.0 – 4.0) | 4.0 (3.0 – 8.0) |
| K ($h^{-1}$) | 0.017 | 0.027 |
| $T_{1/2}$ (h) | 40.8 | 25.7 |
| Acetyl-11-keto-β-boswellic acid | | |
| $AUC_{(0-\infty)}$ (ng h $mL^{-1}$) | 153.6 (59.2 – 647.9) | 748.9 (271.4 – 5316.8) |
| $AUC_{(0-tz)}$ (ng h $mL^{-1}$) | 47.4 (8.0 – 232.0) | 243.7 (53.0 – 3528.0) |
| $C_{max}$ (ng $mL^{-1}$) | 6.0 (0.9 – 45.7) | 28.8 (13.0 – 264.5) |
| $T_{max}$ (h)[a] | 2.0 (0.0 – 24.0) | 3.0 (0.5 – 60.0) |
| K ($h^{-1}$) | 0.066 | 0.046 |
| $T_{1/2}$ (h) | 10.5 | 15.0 |

$AUC_{(0-\infty)}$ = area under the concentration time curve, extrapolated to infinity; $AUC_{(0-tz)}$ = area under the concentration time curve, 0 – last quantifiable sample; $C_{max}$ = maximum plasma concentration; $T_{max}$ = time to $C_{max}$; K = overall elimination rate constant by log linear regression; $T_{1/2}$ = terminal elimination half-life from K (from:[41]).
[a] Median (and range)

- Different drug formulations, raw materials, tablets and capsules may contain different amounts of active compounds.
- Different doses may cause positive or negative results.
- Time of intake may be important to achieve an effective concentration in the blood.

Therefore, it is absolutely necessary to have standardised products with known pharmacokinetics and to measure the pharmacokinetic parameters at steady state during long-term administration.

## Use in Chronic Diseases such as Rheumatoid Arthritis and Osteoarthritis

In traditional Indian Ayurvedic medicine, Salai guggal is used to treat **rheumatoid arthritis**. In order to support this traditional use scientifically, Singh and Atal [9] from the Regional Research Laboratory (RRL) in Jammu, India described for the first time anti-inflammatory properties of Salai guggal ex *Boswellia serrata* in experimental animals. Further studies with the rat adjuvant arthritis model showed protective effects of Salai guggal and BAs. Classical animal models for testing antiarthritic actions of drugs are formaldehyde- and adjuvant-produced arthritis and the cotton pellet-induced granuloma test. In the study of Singh and Atal [9], arthritis was induced by injecting 0.1 ml of formaldehyde (2% v/v in normal saline) in the subplantar region on the 1st and 3rd day of experiment. Paw volume was measured before formaldehyde injection and during drug treatment. Drugs were administered *p.o.* daily. In a dose range of 50 – 200 mg/kg orally, the alcoholic extract of the oleogum resin of BS resulted in marked inhibition of swelling in these rats.

Adjuvant arthritis was caused by injecting 0.05 ml of a (0.5% w/v) suspension of killed *Mycobacterium tuberculosis* homogenised in liquid paraffin into the left hind foot. Oral administration of the test drug was started on the day before the injection of *Mycobacterium* and continued until day 14. Paw volume was measured on alternate days, and percent inhibition was calculated on day 14. In this model, BE at 100 mg/kg caused a reduction in swelling by 45%.

In the adjuvant arthritis model, Kesava-Reddy et al. [45] studied the effect of an extract of oleogum resin of BS (prepared according to Singh and Atal [9]) on urinary excretion of connective tissue metabolites, including hydroxyproline, hexosamine and uronic acid. Compared to controls, the arthritic animals showed increased excretion of these metabolites in the urine. The elevated levels of urinary hydroxyproline (free, total, non-dialysable and dialysable), hexosamine and uronic acid in the arthritic animals were found to be slightly decreased in the acute phase and significantly decreased in the chronic phase of the disease following administration of the drug suggesting a beneficial action.

In another study, Sharma et al. [46] investigated the effect of a BE on bovine serum albumin (BSA)-induced arthritis in rabbits. Oral administration of the BE (25, 50 and 100 mg/kg/day) significantly reduced the population of leukocytes in a BSA-injected knee and changed the electrophoretic pattern of the synovial fluid proteins. The local injection of the extract (5, 10 and 20 mg) into the knee 15 min prior to BSA challenge also significantly reduced the infiltration of leukocytes into the knee joint, reduced the infiltration of leukocytes into the pleural cavity and inhibited the migration of PMN *in vitro*. The leukocyte-inhibitory activity of BAs was not due to a cytotoxic effect and could later be explained by inhibition of leukotriene synthesis and therefore by a failure of the chemotactic action. The antiarthritic action of an acetone extract of *Boswellia carterii* Birdw. in adjuvant-induced arthritis of Lewin rats has been recently confirmed by Fan et al. [11].



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Taken together, these studies suggest an anti-inflammatory action of BEs in experimental arthritis.

## Hepatoprotection

One of the classic models for testing drugs for hepatoprotective activity is the use of galactosamine/endotoxin-induced hepatitis in mice. Endotoxin activates TLR, which triggers the transcription factor NF-κB to induce TNF$\alpha$, which in turn induces NF-κB activation and subsequent cytokine induction. The pathophysiological consequences of endotoxin injection in mice can be blunted by TNF antibodies. The galactosamine/endotoxin-induced liver injury is TNF-dependent [47]. Moreover, leukotrienes are thought to play a role in chronic hepatic diseases, i.e., liver cirrhosis.

Using this model, the effect of an extract of the oleogum resin of BS was tested by Safayhi et al. [48]. In this study, intraperitoneal application of galactosamine and *Salmonella* endotoxin produced acute liver injury in male albino NMRI mice. Within 8 h, serum sorbitol dehydrogenase, aspartate aminotransferase and alanine aminotransferase activities increased from 10 to 1330, from 170 to 1700 and from 30 to 2520 units/L, respectively. When given orally 1 h before the intoxication with galactosamine/endotoxin, extracts from the oleogum resin potently and significantly reduced serum enzyme activities. Since it is known that cyclooxygenase pathway inhibitors are not effective in this animal model, the protection by BE/BAs is interpreted in terms of inhibition of the production of TNF$\alpha$ and of their ability to decrease the formation of leukotrienes.

Up to now there are, however, no clinical studies proving an effect of BE in hepatic diseases.

## Clinical Studies

As early as 1982, the Regional Research Laboratory (RRL) newsletter from Jammu reports that Gufic Private Limited, Bombay, sells Sallai guggal under the trade name Sallaki (later also H 15™) for treatment of rheumatoid arthritis, osteoarthritis, soft tissue rheumatism, low back pain, myositis and fibrosis.

**Rheumatoid arthritis** belongs to the autoimmune diseases. The rheumatoid lesion in the synovial membrane includes macrophages and lymphocytes that produce and/or stimulate cytokines such as interleukins (IL) and TNF-$\alpha$. Neutrophils present in the synovial fluid of the inflamed joints produce leukotrienes, oxygen radicals and elastase activity, which finally cause synovialitis and destruction of cartilage. As discussed in the pharmacological section of this review, extracts from the oleogum resin of BS as well as its active principles AKBA and KBA inhibit IL-1, TNF-$\alpha$, leukotriene, and oxygen radical formation as well as elastase activity in macrophages and granulocytes. Thus, the available scientific evidence regarding both the pathophysiological mechanisms involved in rheumatoid arthritis as well as the pharmacotherapeutic effects of BA complement each other. However, as discussed elsewhere, the $IC_{50}$ value of AKBA in different *in vitro* tests varies between 1.5 $\mu$M (5-LO) [25], ~10 $\mu$M (oxygen radicals) [36], ~25 $\mu$M (IL-1$\beta$) (Bertsche and Greim, personal communication 2000), ~15 $\mu$M (TNF-$\alpha$) (Bertsche and Greim, personal communication 2000) and 15 $\mu$M (elastase) [35]. It therefore remains to be elucidated whether or not effective concentrations of BAs can be reached in the blood after oral administration of salai guggal or preparations from other *Boswellia* species. Fig. **5** shows a hypothetical scheme for the pathogenesis of rheumatoid arthritis-mediated cartilage and bone destruction and possible targets of BAs.

In 1996, Etzel [50] summarised in an overview the results of 11 mostly unpublished studies using extracts from the oleogum resin of BS in patients with chronic polyarthritis. The criteria were pain, swelling, sensitivity and tolerance. In 5 studies, patients were intraindividually, in 2 studies placebo-controlled. In a meta-analysis of the above studies, about 50–60% of the patients responded to this treatment. Pain and swelling of joints were improved by H 15™ if compared to the placebo group ($p < 0.05$). Unfortunately, all this material cannot be re-examined

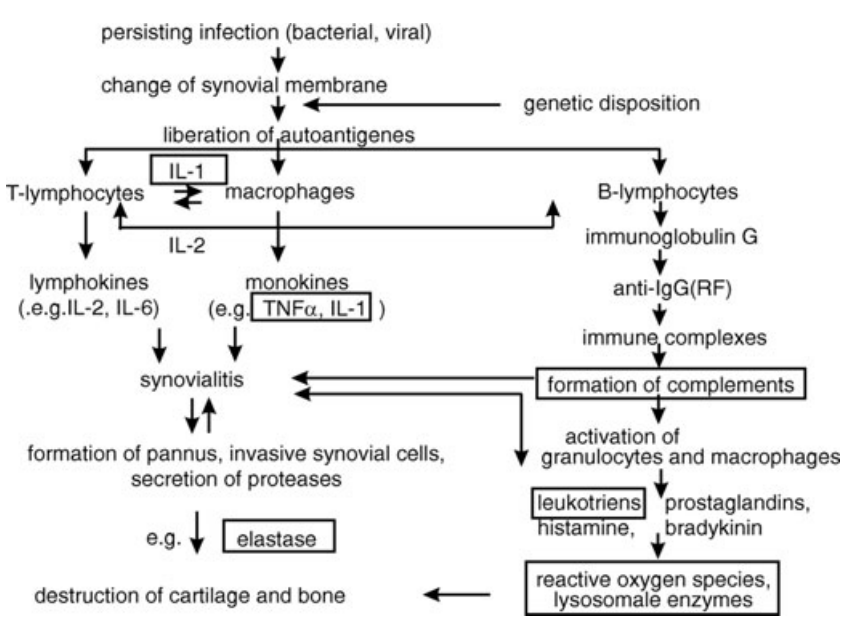


Fig. **5** Partial hypothetic pathogenesis of rheumatoid arthritis: possible targets of BAs (from [49]).



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since it was not published. It is therefore only of limited value. As a consequence, quality and the outcome of these studies were criticised by the German Society of Rheumatology in 1998. The arguments are mainly based on a study of Sander et al. [51]. In this multicentre controlled trial, the authors studied the effect of H 15™ versus placebo over a period of 12 weeks in 37 out-patients with rheumatoid arthritis and chronic polyarthritis under constant therapy with steroids and disease-modifying antirheumatic drugs. The patients received 9 tablets of H 15™ (3600 mg) or placebo daily in addition to their previous therapy. Doses of NSAIDs could be adjusted on demand. Efficacy parameters were the index for swelling and pain, ESR and CRP. Pain and NSAID doses were documented at the beginning and at 6 and 12 weeks after initiation. In this study, treatment with H15™ resulted in no measurable effect. However, this study suffers from the drawback that the effect of the BE alone in comparison to standard therapy was not tested. It can be assumed that administration of H15™ to patients already on steroids and basic therapy will not produce an additional effect. Moreover, only one study centre reported the data for 37 patients of the 78 originally recruited patients.

**Osteoarthritis** is a common chronic, progressive skeletal degenerative disorder, which often affects the knee joint and the shoulder. In a randomised, double-blind, placebo-controlled cross-over study, Kimmatkar et al. [52] studied efficacy, safety and tolerability of a BE (trade name WokVel™) in 30 patients with osteoarthritis in the knee, 15 of them receiving drug or placebo for eight weeks. Each capsule of WokVel™ contained a standardised extract of BS oleogum resin containing a minimum of 65% organic acids or a minimum of 40% total boswellic acids. The main components in boswellic acids are described in the pharmacokinetics section. The patients received three times a day one capsule with 333 mg of the extract and after a wash-out phase the alternate treatment. All patients reported decreased knee pain, increased knee flexion and an increase in the walking distance and in the ability to climb stairs. The symptoms returned after withdrawal of the treatment.

Despite justified criticisms, the clinical data are in favour of positive effects of a BE and/or certain boswellic acids in treatment of rheumatoid arthritis and osteoarthritis. However, further studies with standardised preparations and the determination of optimal doses are necessary.

### Chronic Inflammatory Bowel Diseases

In ancient Indian text books of Ayurveda (Charaka Samhita 1st–2nd century), the oleogum resin of BS was described to be an active component in decoctions for treatment of gastrointestinal symptoms including diarrhoea, flatulence, constipation and vomiting. The antiphlogistic effects of non-steroidal antirheumatics are due to inhibition of prostaglandin synthesis. However, these compounds are not effective in the treatment of inflammatory bowel diseases such as ulcerative colitis and Crohn's disease. It is known that the mucosa of patients with chronic inflammatory bowel diseases is synthesising considerable amounts of leukotrienes $LTB_4$, $LTD_4$ and $LTE_4$ that increase the production of mucus and stimulate contraction of the smooth muscle of the gastrointestinal tract. This effect can be inhibited by sulfasalazine and 5-aminosalicylic acid in a dose-dependent manner [53]. Moreover, IL-1 and TNF-$\alpha$ have also been shown to be of importance in intestinal inflammations [54]. Based on these findings, the effect of a preparation of the oleogum resin from BS was tested in animal experiments and in patients with chronic inflammatory bowel diseases.

Based on the data of Gupta et al. [55], Anthoni et al. [56] studied the effect of AKBA on experimental murine colitis induced by dextran sodium sulfate in comparison to the effects of corticosteroids. They used the adhesion of leukocytes and platelets in postcapillary venules of the inflamed colon which is mediated by P-selectin as parameter. Treatment with AKBA largely prevented the P-selectin up-regulation normally associated with dextran sodium sulfate colitis. All of the protective responses observed with AKBA were comparable to that found in mice treated with a corticosteroid. Whether or not this effect was due to inhibition of leukotriene synthesis or due to another mechanism remains to be elucidated. Other authors found BE to be ineffective in dextran sulfate sodium- or trinitrobenzenesulfonic acid-induced colitis in mice [57].

In a recent study [58], the effect of oleogum resin extract from BS (H 15™) and AKBA was investigated in an experimental model of **ileitis**. Ileitis was induced by two subcutaneous injections of indomethacin (7.5 mg/kg) 24 h apart in Sprague-Dawley rats. Rats also received oral treatment with the BE (H 15™) or AKBA at two different doses equivalent to recommendations in human diseases over 2 days. Controls received only the carriers $NaHCO_3$ (subcutaneously) and tylose (orally). Effectiveness of the treatment was assessed using intravital microscopy in ileal submucosal venules by analysing changes in the number of rolling and adherent leukocytes and by macroscopic and histological scoring. Increased leukocyte-endothelial cell adhesive interactions and severe tissue injury accompanied indomethacin-induced ileitis. Treatment with the BE or AKBA resulted in a dose-dependent decrease in rolling (up to 90%) and adherent leukocytes (up to 98%). High-dose BE as well as both low- and high-dose AKBA significantly attenuated tissue injury scores. Oral therapy with the BE or AKBA significantly reduced macroscopic and microcirculatory inflammatory features normally associated with indomethacin administration, indicating that the anti-inflammatory actions of the BE may be due at last in part to boswellic acids including AKBA.

**Ulcerative colitis** is a chronic inflammatory disease with remissions and exacerbations affecting primarily the rectal mucosa, the left colon, but in many instances also the entire colon. It is characterised by rectal bleeding and diarrhoea affecting mainly but not exclusively the youth and early middle age.

In 34 patients suffering from ulcerative colitis grades II and III, the effect of an alcoholic extract of BS oleogum resin according to Singh et al. [59] (350 mg thrice daily for 6 weeks) on stool properties, histopathology, scan microscopy of rectal biopsies and blood parameters including Hb, serum iron, calcium, phosphorus, proteins, total leukocytes and eosinophils was studied. Eight patients receiving sulfasalazine (1 g thrice daily) served as controls. All parameters tested improved after treatment with the extract. The results are similar to the ones for the controls:



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82% out of treated patients went into remission whereas the remission rate for sulfasalazine treatment was 75% [53]. Unfortunately, due to local reasons (cost factor), the number of control patients was smaller than that of patients receiving BE.

**Chronic colitis**: This disease was characterised by the authors [60] as vague lower abdominal pain, bleeding per rectum with diarrhoea and palpable tender descending and sigmoid colon. Its pathophysiology seems to be different from that of ulcerative colitis.

In this study, thirty patients, 17 males and 13 females aged between 18 and 48 years, were included. Twenty patients were given a preparation of the oleogum resin of BS containing KBA 0.63%, AKBA 0.7%, acetyl-$\beta$-boswellic acid and $\beta$-boswellic acid 1.5% (900 mg daily divided in three doses for 6 weeks) and ten patients receiving sulfasalazine (3 mg daily divided in three doses for 6 weeks) served as controls. Out of 20 patients treated with *Boswellia* oleogum resin, 18 patients showed an improvement in one or more of the following parameters: stool properties, histopathology as well as scanning electron microscopy, haemoglobin, serum iron, calcium, phosphorus, proteins, total leukocytes and eosinophils. Out of 20 patients treated with *Boswellia* oleogum resin, 14 went into remission; for the sulfasalazine-treated patients, the remission rate was 4 out of 10. In the control group, 6 out of 10 patients showed similar results with the same parameters. Therefore, an oleogum resin preparation from BS was effective in the treatment of chronic colitis [60].

A more rare chronic inflammatory bowel disease is **collagenous colitis**. It is characterised by aqueous diarrhoea, histological thickness of the mucosa, and subepithelial collagen band. In a randomised, placebo-controlled, double-blind study, quality of life and histology were studied in 25 patients receiving either 400 mg BE three times a day or placebo. After 6 weeks of treatment, significant improvements were reported for 58.3% of the *Boswellia* group patients and for 30.8% in the placebo group [61].

**Crohn's disease**: In a double-blind, verum-controlled parallel group comparison, 102 patients were randomised. The per protocol population included 44 patients treated with BE H15™ and 39 patients treated with mesalazine. As primary parameter, the change of the Crohn's disease activity index (CDAI) between the beginning and the end of the therapy was chosen. H15™ was tested for non-inferiority compared to standard treatment with mesalazine. In this study, the CDAI after treatment with H15™ was reduced by 90 and after therapy with mesalazine by 53 scores in the mean. A difference between both treatments could not be proven to be statistically significant. [62]. Thus, the data suggest that in treatment of Crohn's disease an extract from the oleogum resin is at least as effective as standard medication under the conditions of this study.

From the above studies, increasing evidence suggests that extracts of the oleogum resin of BS appear to be effective drugs in the treatment of chronic bowel diseases. It seems that this effect is comparable to conventional treatment and that the effect may at least in part be due to certain BAs. However, clinical trials with standardised preparations and establishment of appropriate dosages are necessary.

**Bronchial asthma**

Bronchial asthma is a chronic inflammatory condition characterised by bronchial hyper-responsiveness and reversible airways obstruction. Increased production of leukotrienes both during episodes of asthma and in patients with stable asthma was shown [63]. The finding that leukotrienes have proinflammatory biological properties relevant to the pathogenesis of asthma has stimulated the development of many potential therapeutic compounds for blocking these actions. A leukotriene receptor antagonist (Montelukast) is the first mediator antagonist shown to be effective in treating clinical asthma and as such represents one of the most interesting new classes of antiasthma drugs in development at present.

In Ayurvedic medicine, Salai guggal is used to treat respiratory disorders, i.e., cough, hoarseness, cold, dyspnoea and to produce mucolysis [from Ayurvedic texts: (Charaka Samhita 1st – 2nd century)]. BAs inhibiting leukotriene synthesis have been tested in a double-blind, placebo-controlled trial with asthma patients [64]. Forty patients, 23males and 17 females aged 18 – 75 years with a mean duration of bronchial asthma of 9.58 ± 6.07 years were treated with a preparation of oleogum resin of BS (S-Compound™, Rahul Pharma, Jammu Tawi, India) of 300 mg three times daily over a period of 6 weeks. The drug contained 0.63% KBA, 0.70% AKBA along with about 1.5% acetyl-$\beta$-boswellic acid and $\beta$-boswellic acid. In this study, 70% of the patients showed improvement of the disease scored by disappearance of physical symptoms and by signs such as dyspnoea, rhonchi, number of obstructive attacks, increase in $FEV_1$, FVC and PEFR as well as by a decrease in eosinophilic count and ESR. In the control group of 40 patients, 16males and 24 females aged 14 – 58 years with a mean duration of illness of 32.95 ± 12.68 were treated with lactose (300 mg thrice daily for 6 weeks). Only 27% of these patients showed improvements.

The data suggest a definite role of extracts of the oleogum resin of BS in the treatment of bronchial asthma.

**Autoimmune encephalomyelitis**

Multiple sclerosis is an autoimmune disease. The demyelination and perivascular mononuclear cell infiltration seen in the central nervous system seems to be a characteristic feature. Multiple sclerosis belongs to those diseases in which increased formation of leukotrienes is thought to play an important pathophysiological role.

Mixed acetyl-BAs, extracted from the oleogum resin of BS Roxb., significantly inhibited the ionophore-stimulated release of leukotrienes $B_4$ and $C_4$ from intact *human* PMN, with $IC_{50}$ values of 8.48 $\mu$g/mL and 8.43 $\mu$g/mL, respectively. AKBA was about three times more potent as inhibitor of the formation of both $LTB_4$ ($IC_{50}$ = 2.53 $\mu$g/mL) and $LTC_4$ ($IC_{50}$ = 2.26 $\mu$g/mL) from PMNs in the same assay [65]. For testing of drugs, autoimmune encephalomyelitis was used in guinea pigs as an animal model. After daily intraperitoneal dosage of mixed acetylboswellic acids (20 mg/kg) there was significant reduction of the clinical symptoms in guinea pigs between days 11 and 21. However, the inflammatory infiltrates in the brain and the spinal cord were not significantly





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less extensive in the treated animals than in the respective control group. In this animal model, however, the multiple intraperitoneal administration of boswellic acids did not inhibit the ionophore-challenged *ex vivo* release of leukotrienes $B_4$ and $C_4$ from PMNs separated from the blood of guinea pigs. It remains to be established whether or not these data are relevant for a possible effect of BEs. So far, no clinical studies in the human disease of multiple sclerosis are available.

### Other Diseases

There is a variety of other diseases where leukotrienes could contribute to their pathophysiology such as cystic fibrosis, adult respiratory distress syndrome, allergic rhinitis, lupus erythematosus, gout, Lyme arthritis, psoriasis, acute pancreatitis, liver cirrhosis, astrocytoma, multiple sclerosis, arteriosclerosis. It has not been studied so far whether or not boswellic acids may be of therapeutic benefit in these diseases.

### Side Effects

There is only little published material as far as unwanted effects are concerned. Taking into account the use of oleogum resin of different *Boswellia* species in ancient times and nowadays, especially in Eastern and Asian countries, side effects appear not to be a spectacular matter. In the clinical trials described above, two from 40 patients who received S-compound™ complained about epigastric pain, hyperacidity and nausea [62]. In the study dealing with ulcerative colitis [53], 6 out of 34 patients complained about retrosternal burning, nausea, fullness of abdomen, epigastric pain and anorexia. In a study reported by Böker et al. [66], some patients developed nausea and vomiting, in two patients skin irritations have also been observed. The side effects were reversible after omission of the treatment. In the study of Streffer et al. [67], the preparation H15™ was described to be well tolerated. Some gastrointestinal symptoms were observed.

Table **9** Laboratory parameters tested in patients receiving *Boswellia* extract H 15™ (from [69])

| *Clinical chemistry* | *Blood cells* |
|---|---|
| Sodium | Leukocytes |
| Potassium | Erythrocytes |
| Calcium | Haemoglobin |
| Iron | Haematocrit |
| Albumin | MCV |
| Glucose | MCH |
| Bilirubin | MCHC |
| Uric acid | Thrombocytes |
| Cholesterol total | |
| Triglycerides | Coagulation |
| Urea | Quick |
| Creatine | aPITT |
| Alkaline phosphatase | Fibrinogen |
| γ-Glutamyltransferase (γGT) | |
| Alanine aminotransferase (ALAT,GPT) | |
| Aspartate aminotransferase (ASAT, GOT) | |
| Cholinesterase (CHE) | |
| Lipase | |
| Ferritin | |
| C-Reactive protein | |
| ESR | |

Table **10** Grading of scientific evidence in the treatment of various diseases with boswellic preparations according to a review by the Natural Standard Research Collaboration [62]

| *Indication* | *Evidence Grade* | *Grading system link* |
|---|---|---|
| Asthma (chronic therapy) | B | A |
| Crohn's disease | C | B |
| Osteoarthritis | C | C |
| Rheumatoid arthritis | C | D |
| Ulcerative colitis | C | E |

In a retrospective analysis in 2000, the laboratory parameters before and after treatment of patients suffering from rheumatoid arthritis, ulcerative colitis, Crohn's disease, neurodermitis, lupus erythematosus, multiple sclerosis, astrocytoma, glioblastoma, bronchial asthma and psoriasis and receiving the *Boswellia* preparation H15™ over a period of 6 years before and after treatment were tested (Table **9**). No significant changes related to the therapy were observed [68].

Taken together, it appears that extracts from the oleogum resin of BS are relatively safe as far as side effects are concerned.

### Evidence-Based Evaluation

In an evidence-based systematic review including written and statistical analysis of scientific literature, expert opinion, folkloric precedents, historical pharmacology, kinetics/dynamics, interactions, adverse effects, toxicology, and dosing, Bash et al. [69] rated bronchial asthma (chronic therapy) with **B** and Cohn's disease, osteoarthritis, rheumatoid arthritis and ulcerative colitis all with *C* in a grading system from A to F (Table **10**).

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