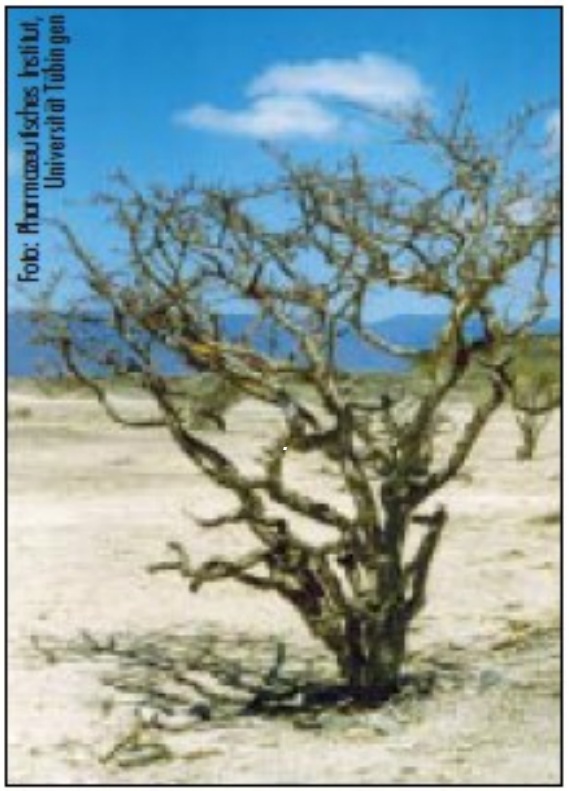
Weihrauch (Boswellia)



Die niedrig-wüchsige afrikanische Art des Weihrauchbaumes Boswellia serrata

Description:

- Boswellia is a genus-tree in the order Sapindales from the family Burseraceae and grows in moderate-sized flowering plants, including both trees and shrubs. Species include: *frankincense, B. carterii, B. sacra, B. serrata, B. papyfera and B. frereana.*
- The gummy exudate or the resin obtained by peeling away the bark is commonly known as *frankincense* or *olibanum*. The resin has traditionally been used as incense in religious and cultural ceremonies since the beginning of written history.
- It is native to Middle East, Ethiopia, Somalia and tropical regions of Africa and Asia. The distributions of the species are primarily associated with the tropics. The greatest diversity of species presently is in Africa and India.
- Boswellia acids are compounds of *Boswellia carterii Birdw.* which was included in German pharmaceutical registers **(Ergänzungsband DAB 6 und DAB 1)** as **Olibanum** and nowadays licensed for medical use against polyarthritis in India and Switzerland under brand names **Sallaki** or **H15**.

Tab. 1.1 A few representative *Boswellia* species and their geographical distribution. The trees of *Boswellia carterii* Birdw. and *Boswellia sacra* Flück. can be regarded as the same species, according to the chemotaxonomic and biological evaluations [11-13]. They are just differing because of their geographical origin.

Species	Geographical Distribution
Boswellia carterii Birdw.	Somalia, Nubia
Boswellia sacra Flück.	Oman, Yemen
Boswellia frereana Birdw.	Somalia
Boswellia papyrifera Hochst.	Ethiopia, Eritrea, Sudan
Boswellia serrata Roxb.	India
Boswellia neglecta S. Moore	Somalia
Boswellia odorata Hutch.	Tropical Africa
Boswellia dalzielli Hutch.	Tropical Africa
Boswellia ameero Balf. Fils.	Socotra
Boswellia elongata Balf. Fils.	Socotra
Boswellia socotrana Balf. Fils.	Socotra

Boswellia in medical history:

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.

Remedies containing preparations from frankincense (Boswellia carterii Birdw.) were used by Hippocrates, Celcus, Galenus and Dioskurides.

The use of the oleogum resin of Boswellia (salai guggal) is also described in Ayurvedic text books in 1st - 2nd century AD (Charaka Samhita) and in 7th century AD (Astangahrdaya Samhita).

Olibanum was still a remedy in the beginning of the 20th century in Europe. Thus, olibanum is mentioned in the supplement to the 6th 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, but is gaining interest in recent years due to new clinical evidence from pharmaceutical trials.

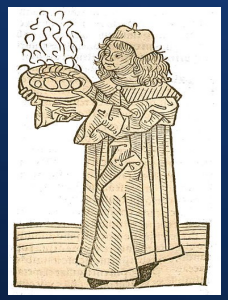
Organs and functional systems	Effects
Nervous system	Analgesic* Mental tonic Stimulation Eye tonic
Cardiovascular system	Cardiotonic
Gastrointestinal tract	Regulating colour of stool Carminative, stomachic Improving digestion, antidiarrhoeic Improving taste Anthelmintic
Urogenital system	Diuretic Aphrodisiac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

Table 1: Therapeutic uses in the traditional Indian Ayurvedic medicine:

*inaccurate according to clinical evidence

Tradition of medical use:

- Preparations from the gum resin of Boswellia serrata have been used as a traditional remedy in Ayurvedic medicine in India for the treatment of inflammatory diseases (arthritis, ulcerative colitis), snakebites, wound healing, coughs and bronchial asthma.



- 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.
- In clinical trials, promising results were observed in patients with bronchial asthma and ulcerative colitis.
- However, evidence is mixed for its benefits for osteoarthritis and collagenous colitis.
- Boswellia was also investigated for its role in maintenance of Crohn's disease remission, but it demonstrated no significant benefit.
- It may be considered as an alternative treatment to corticosteroids in reducing cerebral peritumoural oedema.
- The anti-inflammatory mechanism is different from that of NSAID (target: prostaglandins) and is related to components of the immune system (mainly leukotriens).
- Noticeably, unlike other non-steroidal anti-inflammatory drugs, boswellic acid failed to show analgesic or antipyretic effects.
- Essential oil of boswellia has antimicrobial activities.

Chemical composition:

- More than 200 different compounds were identified in the oleogum resin of different Boswellia species. 8-12% essential oils (amongst them mono-, di-, triterpenes) 45-60% polysaccharides, 25-35% higher terpenoids, phenolic compounds, diterpene alcohols (serratol, incensole, incensole acetate), sugars, compromises of proteins and inorganic compounds.
- The content differs from species to species, between different harvestings and different locations.
- Indian sample contained quite similar amounts of AKBA and KBA whereas the African samples contained less KBA than AKBA.
- Pentacyclic ring skeleton of boswellic acid is important for anti-topoimerase activity.

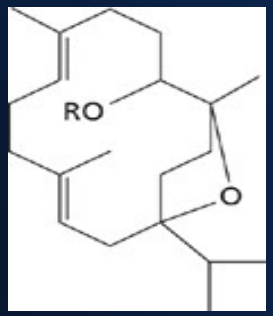


Fig. 3: Structures of incensole acetate (R = Ac) and incensole (R = H):

- Pentacyclic triterpenes: Boswellic acids amongst are mainlyactive AKBA and KBA, (biologically active); 3α-acetyl-20(29)-lupene-24-oic acid; roburic acids.

triterpenes. Higher terpenoids constitute the major fraction (25-35%) of the oleogum resin which are pentacyclic triterpenic acids known as boswellic acid such as β-boswellic acid (53.5-246.9 mg g^{-1}), 11-keto-boswellic acid (4.48-5.81 mg g^{-1}), 3-O-acetyl-β-boswellic acid (38.4-192.9 mg g^{-1}) and 3-O-acetyl-11-keto-β-boswellic acid (32.7-44.2 mg g^{-1}). The major

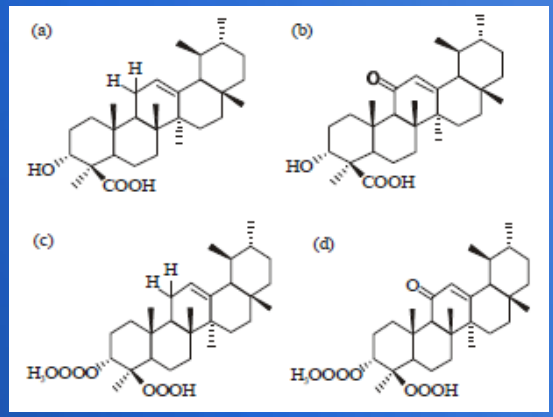


Fig. 1: Structure of boswellic acids:

- Tetracyclic triterpenic acids: Tirucallic acids (biologically active) e.g. 3-oxotirucallic acid, 3-hydroxytirucallic acid and 3-acetoxytirucallic acid.

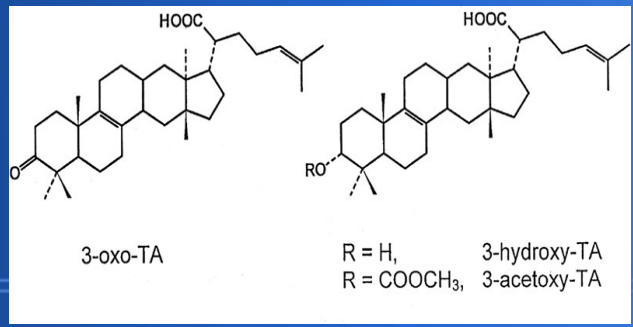


Fig. 2: Structures of tirucallic acids:

Anti-inflammatory activity

- Compounds from the gum with anti-inflammatory and pro-apoptotic effects are **pentacyclic triterpenes** of the *boswellic acid* type (e.g. AKBA and KBA).
- *In vitro* and animal studies show that *boswellic acid* shows anti-inflammatory activity by inhibition (non-competitive, non-redox) of 5-lipoxygenase and cyclooxygenase-1.
- As an effect, leukotriene biosynthesis declines in neutrophilic granulocytes.
- Boswellia also targets several cytokines (interleukins, TNF-α) and the complement system thus inhibiting the signaling pathways of transcription factor NF-KappaB in macrophages*, **markedly decreasing the production of the pro-inflammatory key cytokine tumor necrosis factor (TNF-α).**
- Effects on NF-KappaB may be mediated by diterpenes derivatives e.g. *incensole acetate*.

*as shown in a mouse model of psoriasis

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 LTB4) as well as **bronchoconstriction**, **mucus secretion and vasoconstriction** (coronary arteries).

Most symptoms of common diseases (**asthma, rheumatoid arthritis, oedemas, etc.**) that are positively responsive to Boswellia mediate their outburst through this pathway involving over-activation of leukotrienes within the immunitary response.

Anti-cancer activity: summary

- Extracts from Boswellia have been proposed to provide anti-neoplastic activity through their anti-proliferative, anti-angiogenesis and pro-apoptotic properties in numerous human and animal cancer cell lines including from **meningioma, leukemia, breast cancer, bladder cancer, hepatoma, melanoma, fibrosarcoma, colon cancer, and prostate cancer.**
- Anti-cancer efficacy was also established ***in vivo*** in animal models [1] [3]. More recently, it was reported that AKBA (20-200 mg/kg) inhibits the growth and metastasis of orthotopic tumors in mice with CRC, prostate cancer and pancreatic cancer without significant decrease in body weight.
- Boswellia acids **discriminate cancer cells against normal cells** and are less cytotoxic in normal cells.
- The effect of the extracts of Boswellia are **dose-dependent**.
- Anti-cancer activity of Boswellia can be **potentiated by incubation of cancer cell lines with LY294002** or **wortmannin**.
- **4-Amino analogues** prepared from β-boswellic acid and 11-keto-β-boswellic acid displayed improved cytotoxicity than the parent molecules [2]. The carboxyl group in ursane nucleus was replaced by an amino function via Curtius reaction.
- AKBA also sensitized the cells to apoptotic effects of gemcitabine [3].

References:

[1] Yadav VR, Prasad S, Sung B, Gelovani JG, Guha, S: Boswellic acid inhibits growth and metastasis of human colorectal cancer in orthotopic mouse model by down regulating inflammatory, proliferative, invasive and angiogenic biomarkers. Int. J. Cancer, 130; 2176-2184, 2012.

[2] Bhahwal A. Shah, Ajay Kumar, Pankaj Gupta, Madhunika Sharma, Vijay K. Sethi, Ajit K. Saxena, Jaswant Singh, Ghulam N. Qazi, Subhash C. Taneja: Cytotoxic and apoptotic activities of novel amino analogues of boswellic acids. Bioorganic & Medicinal Chemistry Letters. Volume 17, Issue 23, 1 December 2007, Pages 6411–6416.

[3] Park B, Prasad S, Yadav V, Sung B, Aggarwal B: Boswellic Acid Suppresses Growth and Metastasis of Human Pancreatic Tumors in an Orthotopic Nude Mouse Model through Modulation of Multiple Targets. Journal Plos One, Oct. 2011, Vol. 6, Issue 10.

Anticancer research studies (table):

Table 2: Anticancer research studies on cell lines and animal models

Boswellic acid	Cancer type	Cell lines	Analysis	Reference
Anticancer studies on human cancer cell lines				
AKBA	Colon cancer	HT-29, HCT-116	Flow cytometry, cell viability and DNA synthesis assay, western blot analysis, apoptosis assay	Liu and Duan (2009)
AKBA	Colon cancer	HT-29	ELISA, flow cytometry, Assay activities of caspase-3, caspase-8 and caspase-9, cell viability and DNA synthesis, western blot analysis	Liu *et al*. (2006)
AKBA	Leukemia	HL-60, CCRF-CEM	Flow cytometry, topoisomerase activity analysis, cDNA polymerase chain reaction approach	Hoernlein *et al*. (1999)
AKBA	Prostate cancer	LNCaP	Flow cytometry, MTT assay, Transient transfection assay, western blot analysis, Electrophoretic mobility shift assay	Yuan *et al*. (2008)
BSE	Leukemia, brain tumor	Five leukemia (HL-60, K 562, U937, MOLT-4, THP-1 and two brain tumor (LN-18, LN-229	WST-1 assay and flow cytometry	Hostanska *et al*. (2002)
BA	Breast cancer	MCF-7	Caspase activity assay, cytokine ELISA assay, superoxide dismutase activity, catalase activity, glutathione assay	Saraswati and Aggrawal (2012)
BAA	Leukemia	ML-1, HL-60, U937, K562	DNA fragmentation analysis, phase microscopy	Jing *et al*. (1999)
BAA	Leukemia	NB4, SKNO-1, K562, U937, ML-1 and HL-60	Clonogenic assay, mitochondria membrane potential assay, western blot analysis, northern blot analysis	Xia *et al*. (2005)
AKBA	Prostate cancer	PC-3, LNCaP	MTT proliferation assay, mitochondria membrane potential analysis, luciferase assay	Lu *et al*. (2008)
Anticancer research findings on both human cancer as well as on animal cell lines				
BAA	Metastatic Melanoma, fibrosarcoma	Mouse melanoma cells B16F10 and human fibrosarcoma cell line HT-1080	MTT proliferation assay, cell viability analysis, gelatin zymography, topoisomerase-II catalytic assay, flow cytometry and DNA fragmentation	Zhao *et al*. (2003)
BSE	Pancreatic and melanoma	Human MIA-PaCa, A375 and mouse fibroblast cell line L929	MTT proliferation assay, DNA fragmentation assay	Uthaman *et al*. (2012)
Anticancer research studies on both human cancer cell lines as well as on animal model				
AKBA	Pancreatic cancer	AsPC-1, BxPC-3, MIA PaCa-2, PANC-28, Orthotopic mouse model	MTT proliferation assay, western blot analysis and immunohistochemical analysis	Park *et al*. (2011)
AKBA	Colorectal cancer	HCT116, HT29, SW480 and SW620, Orthotopic mouse model	MTT proliferation assay, immunohistochemistry, colony formation assay, migration and invasion assay	Takahashi *et al*. (2012)
AKBA	Colorectal cancer	Orthotopic mouse model	Proliferative index, nuclear factor-κB (NF-κB) suppression	Yadav *et al*. (2012)

[a]AKBA: 3-acetyl-11-keto-β boswellic acid, [b]BSE: *Boswellia serrata* extract, [c]BA: Boswellic acid, [d]BAA: α- and β-Boswellic acid acetate, [e]ELISA: Enzyme Linked Immunosorbent Assay, [f]MTT: [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide], [g]WST: Water soluble tetrazolium salt

Source : Dureja N and H: Role of Boswellic Acids in Cancer Treatment. J. Med.Sci., 14(6-8) : 261-269. Aug.-Dec., 2014.

Impact on cell proliferation and growth (figures):

- **Histological findings:**

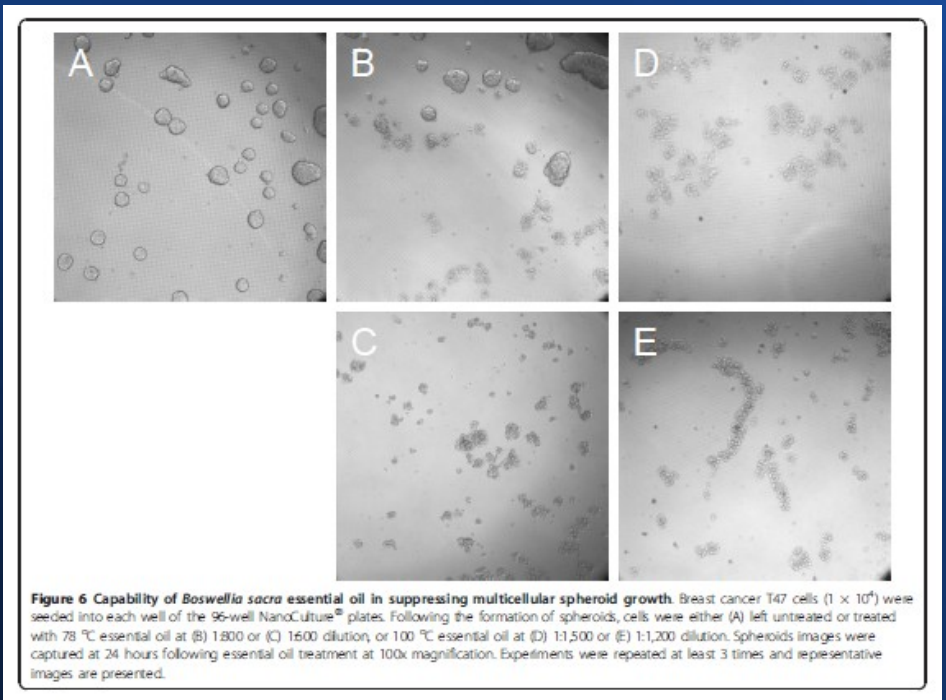
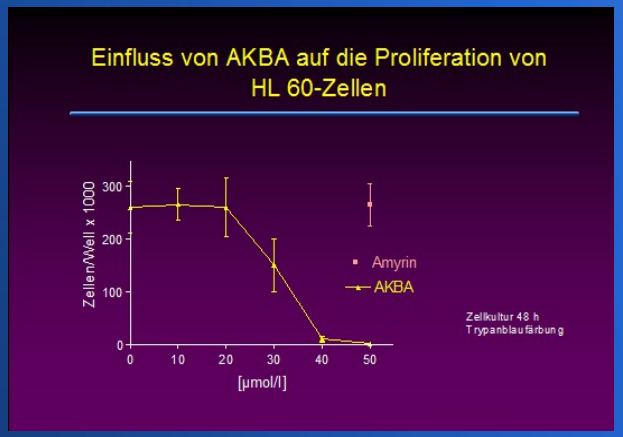


Figure 6 Capability of *Boswellia sacra* essential oil in suppressing multicellular spheroid growth. Breast cancer T47 cells (1×10^4) were seeded into each well of the 96-well NanoCulture® plates. Following the formation of spheroids, cells were either (A) left untreated or treated with 78 °C essential oil at (B) 1:800 or (C) 1:600 dilution, or 100 °C essential oil at (D) 1:1,500 or (E) 1:1,200 dilution. Spheroids images were captured at 24 hours following essential oil treatment at 100x magnification. Experiments were repeated at least 3 times and representative images are presented.



Source: [4] Mahmoud M Suhail,1 Weijuan Wu,2,3 Amy Cao,4 Fadee G Mondalek,2 Kar-Ming Fung,2,5 Pin-Tsen Shih,3,6 Yu-Ting Fang,3,6 Cole Woolley,7 Gary Young,7 and Hsueh-Kung Lin:Boswellia sacra essential oil induces tumor cell-specific apoptosis and suppresses tumor aggressiveness in cultured human breast cancer cells. BMC Complement Altern Med. 2011; 11: 129.

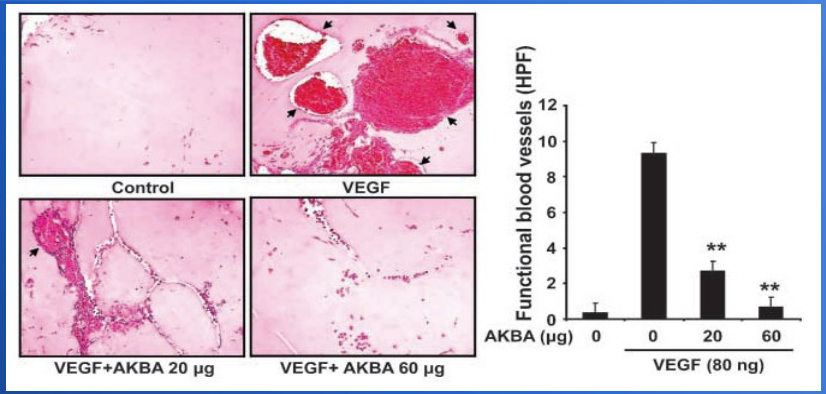
Inhibition of angiogenesis:

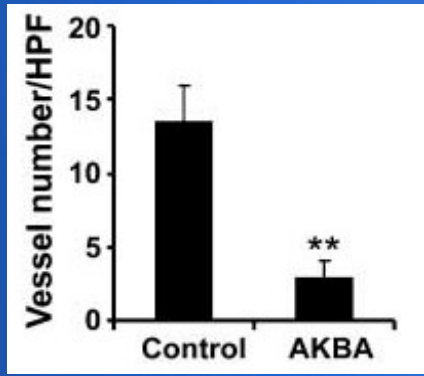
Boswellia inhibits angiogenesis and tumor cell proliferation via activation of signaling pathways:

- Acetyl-11-keto-beta-boswellic acid (AKBA) was found to inhibit human prostate tumor growth **via inhibition of angiogenesis** induced by **VEGFR2 signaling pathways** [5]. Furthermore, AKBA inhibited VEGF-induced cell proliferation, chemotactic motility, and the formation of capillary-like structures from primary cultured human umbilical vascular endothelial cells in a dose-dependent manner.

- Western blot analysis and *in vitro* kinase assay revealed that AKBA suppressed VEGF-induced phosphorylation of VEGF receptor 2 (VEGFR2) kinase (KDR/Flk-1) with IC50 of 1.68 Mmol/L. Specifically, AKBA suppressed the downstream protein kinases of VEGFR2, including Src family kinase, focal adhesion kinase, extracellular signal-related kinase, AKT, mammalian target ofrapamycin, and ribosomal protein S6 kinase.

- Acetyl-11-keto-beta-boswellic acid (AKBA) **inhibited the activation of signal transducers and activators of transcription-3** (STAT-3), which has been linked to survival, proliferation, chemoresistance, and angiogenesis of tumor cells.





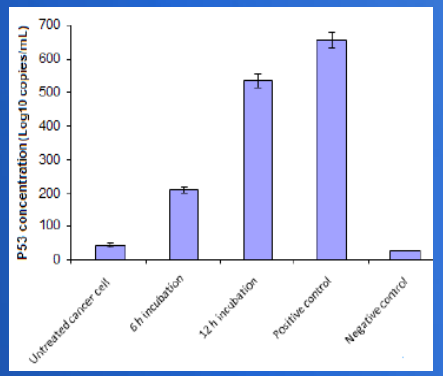
Reference:

[5] Pang X, Yi Z, Zhang X, et al. Acetyl-11-keto-beta-boswellic acid inhibits prostate tumor growth by suppressing vascular endothelial growth factor receptor 2-mediated angiogenesis. Cancer Res. 2009 Jul 15;69(14):5893-900.

Induction of apotosis:

Extracts of Boswellia increase transcription of tumor-suppressor genes:

- Research on the cytotoxic effects of boswellic acid indicates that it **induces p21 expression through a p53-independent pathway and causes apoptosis** in glioma [6] [7] and leukemia [8] cell lines.
- Another study [9] demonstrated that gum methanol extracts of *Boswellia th.* induced toxicity via **P53 gene transcription** in breast cancer cell lines. IC_{50} against cancer cells was 80 µg/mL. Effect was higher after 12 h treatment than it was after 6 h (see figure).
- AKBA significantly **up-regulated expression of the let-7 and miR-200 families** in various CRC cell lines. Both let-7 and miR-200 are putative tumor-suppressive miRNAs. AKBA modulated the expression of several downstream targets of the let-7 and miR-200 families, such as **CDK6, vimentin and E-cadherin** [10].
- Another apoptotic mechanism exhibited by Boswellia is via oxidative stress by **early generation of nitric oxide and reactive oxygen species that up regulate time-dependent expression of p53/p21/PUMA**. Boswellia extract induced apoptosis in a cervical cancer cell line by inducing endoplasmic reticulum (ER) stress [11].
- AKBA-induced apoptosis can enhanced up to 20-fold by pre-incubation of the cells with LY294002 (selective phosphatidylinositol 3-kinase inhibitor) or wortmannin (Liu and Duan, 2009).



Source: [7]

References :

[6] Glaser T, Winter S, Groscurth P, et al. Boswellic acids and malignant glioma: induction of apoptosis but no modulation of drug sensitivity. Br J Cancer. May 1999;80(5-6):756-765.

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[8] Jing Y, Nakajo S, Xia L, et al. Boswellic acid acetate induces differentiation and apoptosis in leukemia cell lines. Leuk Res. Jan 1999;23(1):43-50.

[9] Yazdanpanahi N, Behbahani M and Yektaeian A: Effect of *Boswellia Thurifera* Gum Methanol Extract on Cytotoxicity and *P53* Gene Expression in Human Breast Cancer Cell Line. Iranian Journal of Pharmaceutical Research (2014), 13 (2): 719-724.

[10] Takahashi M, Sung B, Shen Y, Hur K, Link A, Boland R, Aggarwal B and Goel A: Boswellic acid exerts antitumor effects in colorectal cancer cells by modulating expression of the let-7 and miR-200 microRNA family. Carcinogenesis vol.33 no.12 pp.2441–2449, 2012.

[11] Kim HR, Kim MS, Kwon DY, et al. Boswellia serrata-induced apoptosis is related with ER stress and calcium release. Genes Nutr. Feb 2008;2(4):371-374.

Induction of apoptosis (figures):

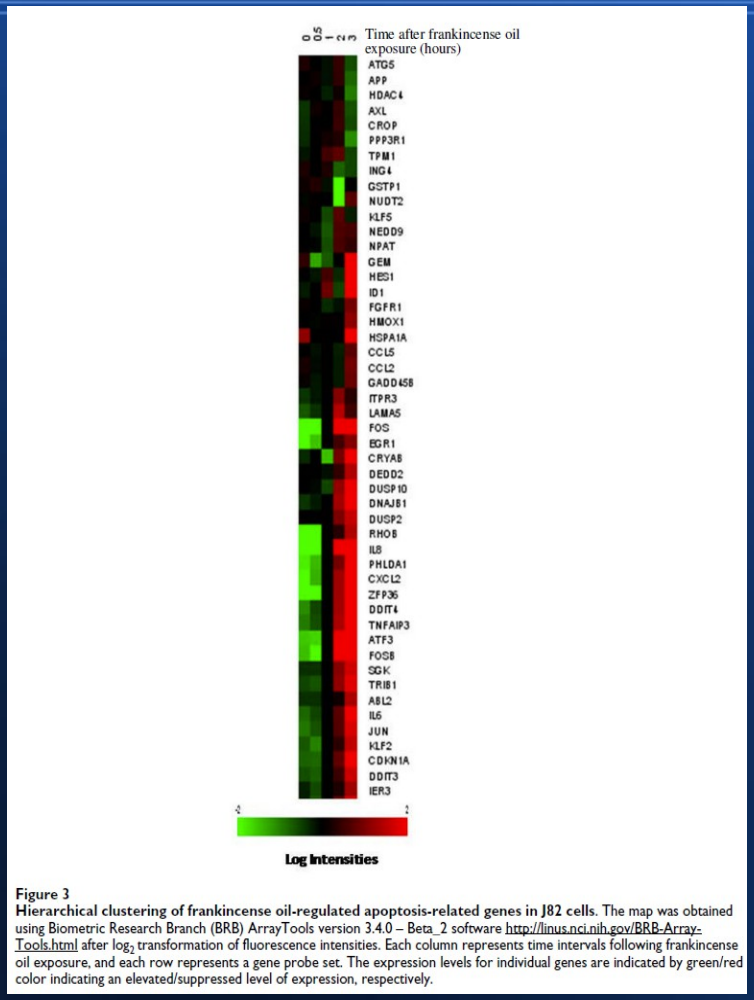


Figure 3
Hierarchical clustering of frankincense oil-regulated apoptosis-related genes in J82 cells. The map was obtained using Biometric Research Branch (BRB) ArrayTools version 3.4.0 – Beta_2 software http://linus.nci.nih.gov/BRB-ArrayTools.html after $\log_2$ transformation of fluorescence intensities. Each column represents time intervals following frankincense oil exposure, and each row represents a gene probe set. The expression levels for individual genes are indicated by green/red color indicating an elevated/suppressed level of expression, respectively.

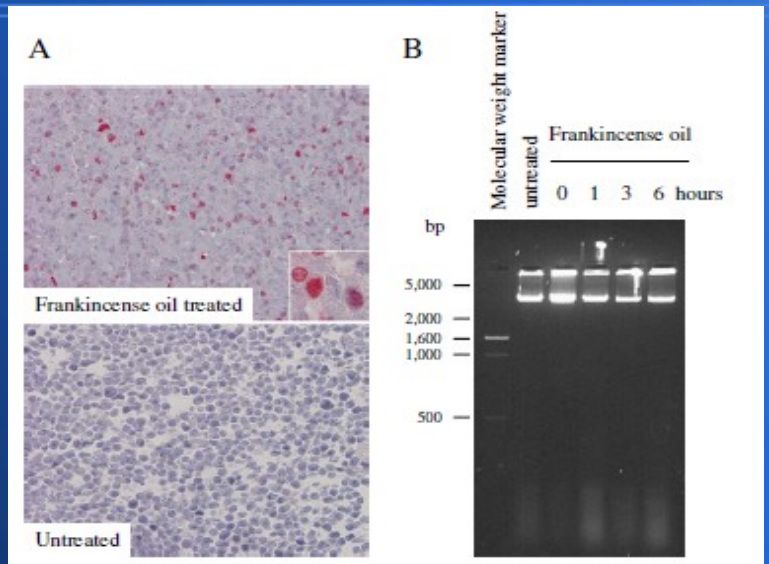


Figure 4
Frankincense oil-induced J82 cell death. To determine whether frankincense oil-induced apoptosis in bladder cancer cells, J82 cells were seeded in 60 mm tissue culture plates at the concentration of 2×10^5 cells per plate, cultured overnight for adherence, and either left untreated or treated with 1:1,000 dilution of frankincense oil. (A) TUNEL analysis was performed at 3 hours following treatment. Apoptotic cells with damaged DNA were stained positive with a bright red color (inserts). (B) DNA fragmentation was determined by separating genomic DNA on a 2% agaorse gel; and the gel image was captured using Gel Doc 100 system (Bio-Rad, Hercules, CA).

Source: [12] Mark Barton Frank, Qing Yang, Jeanette Osban, Joseph T Azzarello, Marcia R Saban, Ricardo Saban, Richard A Ashley, Jan C Welter, Kar-Ming Fung and Hsueh-Kung Lin: Frankincense oil derived from Boswellia carteri induces tumor cell specific cytotoxicity. The official journal of the International Society for Complementary Medicine Research (ISCMR)20099:6.

Induction of cell-cycle arrest:

Inhibition of DNA-replication:

- A semisynthetic analog of boswellic acid, 3-alpha-Butyryloxy-beta-boswellic acid, demonstrated significant growth inhibition in Ehrlich Ascitic Tumour (EAT), Ehrlich Ascitic Carcinoma (EAC) and Sarcoma- 180 tumour models, via **down-regulation of NF-KappaB** and by **induction of poly (ADP-ribose) polymerase (PARP) cleavage** [13].
- Acetyl-boswellic acids were shown to **inhibit topoisomerases** by competing with DNA for binding sites.

References :

[13] Qurishi Y, Hamid A, Sharma PR, et al.: NF-κB down-regulation and PARP cleavage by novel 3-α-butyryloxy-β-boswellic acid results in cancer cell specific apoptosis and in vivo tumor regression. Anticancer Agents Med Chem. 2013 Jun;13(5):777-90.

Classification of Boswellia-regulated genes and transcription factors (tables):

Table 3: Frankincense oil-regulated growth inhibitory genes in J82 cells

	Time after frankincense oil exposure(hours)				
Gene Symbol	0	<0.5	0.5–1	1–2	2–3
IL8	26.3	**32.6**	**146.2**	**693.6**	1241.4
CLK1	42.3	56.1	**57.9**	**121.2**	203.3
DLG1	48.3	41.2	**31.2**	**71.8**	37.2
H2AFX	105.6	103.3	**106.6**	**51.0**	96.1
ING4	73.7	63.0	**75.3**	**37.2**	43.6
KLF4	89.2	68.8	**123.2**	**326.0**	556.9
NEDD9	38.3	34.3	**23.8**	**59.7**	56.7
SSTR1	47.4	37.2	**28.4**	**70.3**	27.2
CDKN1A	56.3	54.5	95.7	**167.4**	**382.9**
DDIT3	346.7	316.0	523.2	**735.5**	**1647.7**
HDAC4	64.4	61.7	52.1	**68.0**	**31.5**
IL1A	63.4	44.5	72.1	**124.5**	**252.5**
IL6	190.5	235.4	337.2	**563.8**	**1326.4**
SNF1LK	40.6	38.8	48.9	**95.8**	**286.3**
IL8	26.3	32.6	146.2	**693.6**	**1241.4**
CLK1	42.3	56.1	57.9	**121.2**	**203.3**
DLG1	48.3	41.2	31.2	**71.8**	**37.2**

Normalized values of fluorescent intensities are presented. Bold font indicates a minimum two-fold change between adjacent time points.

Table 2: Frankincense oil-regulated transcription factors in J82 cells

	Time after frankincense oil stimulation (hours)			
	<0.5	0.5–1	1–2	2–3
Up-regulated	LOC126295 (NM_173480.1) * EGR1 (NM_001964.2)	ATF3 (NM_001040619.1) FOS (NM_005252.2) FOSB (NM_006732.1) KLF2 (NM_016270.2) ZNF234 (NM_006630.1)	KLF4 (NM_004235.3) KLF5 (NM_001730.3) ZBTB11 (NM_014415.1)	DDIT3 (NM_004083.4) DEDD2 (NM_133328.2) DENR (NM_003677.3) HES1 (NM_005524.2) ID1 (NM_181353.1) JUN (NM_002228.3) JUNB (NM_002229.2) SNAPC1 (NM_003082.2) TSC22D1 (NM_006022.2) UBTF (NM_014233.1) ZNF682 (NM_033196.1)
Down-regulated	POLR2K (NM_005034.3)	ING4 (NM_198287.1)		HDAC4 (NM_006037.2) RAI1 (NM_030665.3) TAF15 (NM_003487.2)

*GenBank accession number.

Table 1: Functional groups of frankincense oil-regulated genes in bladder cancer J82 cells

Function	Gene Symbol	Description
Cytokines		
	CCL2	chemokine (C-C motif) ligand 2
	CCL5	chemokine (C-C motif) ligand 5
	CMTM8	CKLF-like MARVEL transmembrane domain containing 8
	CXCL2	chemokine (C-X-C motif) ligand 2
	IL1A	interleukin 1, alpha
	IL6	interleukin 6 (interferon, beta 2)
	IL8	interleukin 8
Enzymes – kinases		
	ABL2	v-abl Abelson murine leukemia viral oncogene homolog 2 (arg, Abelson-related gene)
	AXL	AXL receptor tyrosine kinase
	CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)
	CLK1	CDC-like kinase 1
	DLG1	discs, large homolog 1 (Drosophila)
	FGFR1	fibroblast growth factor receptor 1 (fms-related tyrosine kinase 2, Pfeiffer syndrome)
	PSTK	phosphoseryl-tRNA kinase
	SGK1	serum/glucocorticoid regulated kinase 1
	SNF1LK	SNF1-like kinase
	TAOK1	TAO kinase 1
	TRIB1	tribbles homolog 1 (Drosophila)
Enzymes – peptidases		
	RCE1	RCE1 homolog, prenyl protein peptidase (S. cerevisiae)
Enzymes – phosphatases		
	DUSP10	dual specificity phosphatase 10
	DUSP2	dual specificity phosphatase 2
	DUSP5	dual specificity phosphatase 5
	MTMR6	myotubularin related protein 6
	NUDT2	nudix (nucleoside diphosphate linked moiety X)-type motif 2
	PPP3R1	protein phosphatase 3 (formerly 2B), regulatory subunit B, alpha isoform
	PTPN23	protein tyrosine phosphatase, non-receptor type 23
Membrane Receptors		
	PLAUR	plasminogen activator, urokinase receptor
	PLXNA1	plexin A1
	PLXNA3	plexin A3
	SSTR1	somatostatin receptor 1

Source: [12] Mark Barton Frank, Qing Yang, Jeanette Osban, Joseph T Azzarello, Marcia R Saban, Ricardo Saban, Richard A Ashley, Jan C Welter, Kar-Ming Fung and Hsueh-Kung Lin: Frankincense oil derived from Boswellia carteri induces tumor cell specific cytotoxicity. The official journal of the International Society for Complementary Medicine Research (ISCMR)20099:6.

Activation of signaling molecules and cell cycle-related protein expression (figure):

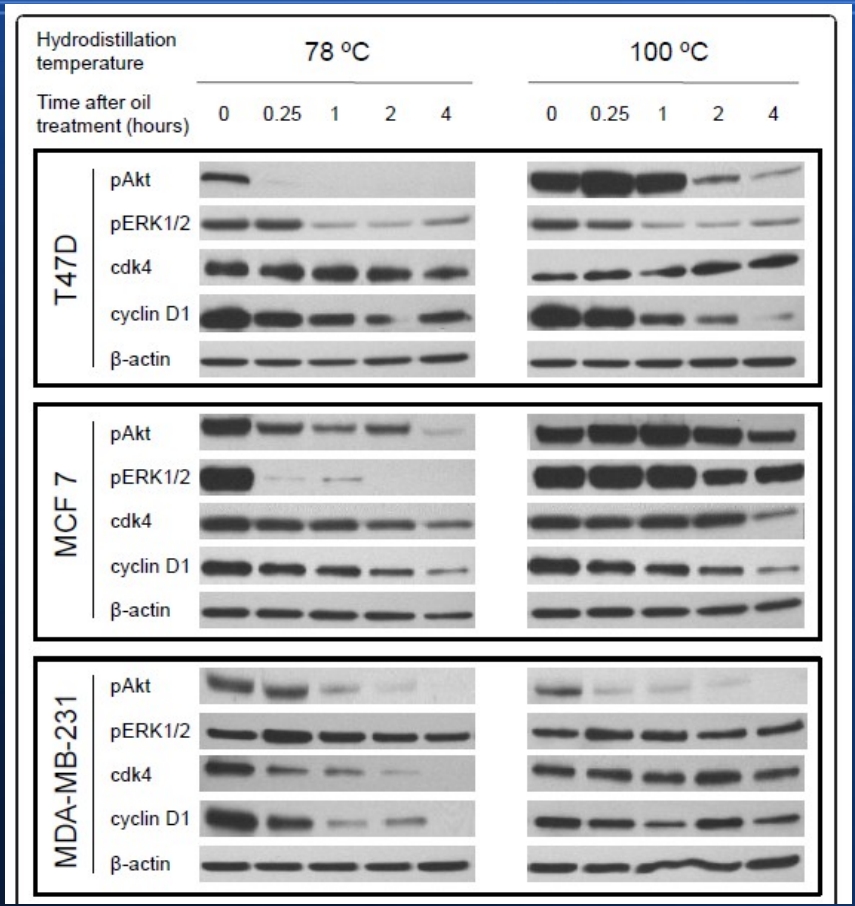


Figure 7 ***Boswellia sacra*** **essential oil-regulated signaling molecules activation and cell cycle-related proteins expression in human breast cancer cells.** Breast cancer cells were seeded at the concentration of 5×10^5 cells/60 mm tissue culture plate. After adherence, cells were treated with either 1:800 dilution of 78 °C or 1:1,200 dilution of 100 °C essential oil. Total cellular proteins were isolated between 0 (untreated control) and 4 hours following essential oils treatment. Western blot analysis was performed to determine levels of Akt and ERK1/2 phosphorylation as well as cyclin D1 and cdk4 proteins expression. Expression of β-actin was also determined in parallel and used as a protein loading control. Experiments were repeated at least twice for each cell line and representative results are presented.

Source: [4] Mahmoud M Suhail,1 Weijuan Wu,2,3 Amy Cao,4 Fadee G Mondalek,2 Kar-Ming Fung,2,5 Pin-Tsen Shih,3,6 Yu-Ting Fang,3,6 Cole Woolley,7 Gary Young,7 and Hsueh-Kung Lin: Boswellia sacra essential oil induces tumor cell-specific apoptosis and suppresses tumor aggressiveness in cultured human breast cancer cells. BMC Complement Altern Med. 2011; 11: 129.

Tumor-specific cytotoxicity:

- In all clinical trials with Boswellia it suppressed cell viability in cancer cells but not/less in normal cells.
- It appears to discriminate cancerous against normal cells.
- As shown in figure 1 (right), histology analysis show that cell morphology was not altered in normal cells after treatment with frankincense oil.

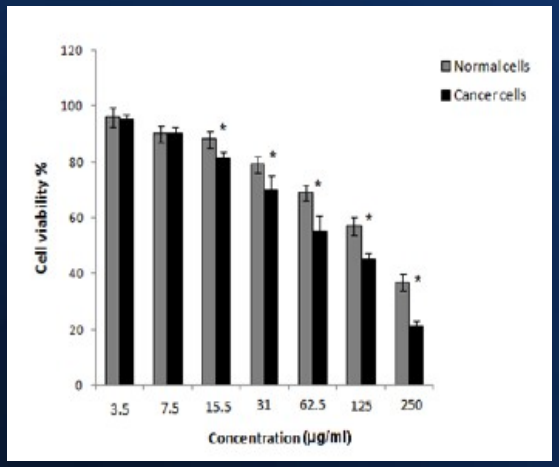


Figure 1. Cell viability assay of normal cells and MDA-MB-231 cancer cells 48 h after treatment with Boswellia thurifera gum methanol extract. The data was expressed as the mean±SD from 3 independent experiments.

Source: [9] Yazdanpanahi N, Behbahani M and Yektaeian A: Effect of *Boswellia Thurifera* Gum Methanol Extract on Cytotoxicity and *P53* Gene Expression in Human Breast Cancer Cell Line. Iranian Journal of Pharmaceutical Research (2014), 13 (2): 719-724

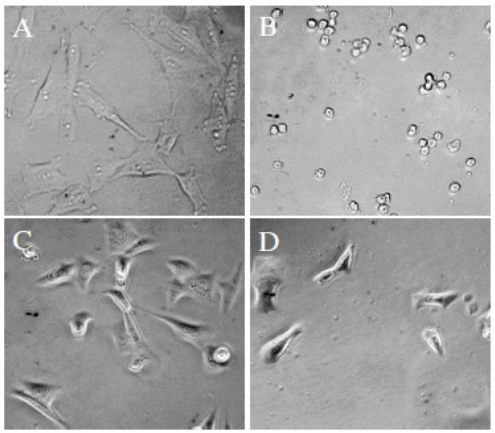


Figure 1
Morphological changes of bladder carcinoma J82 and bladder urothelial UROtsa cells following frankincense oil stimulation. Bladder J82 and UROtsa cells were seeded in 96-well tissue culture plates at the concentration of 1 × 10^4 cells/mm^2, cultured overnight for adherence, and either left untreated or subjected 1:1,000 dilution of frankincense oil stimulation. Images were taken at 24 hours following treatments for (A) untreated J82 cells, (B) J82 cells treated with frankincense oil, (C) untreated UROtsa cells, and (D) UROtsa cells treated with frankincense oil using Olympus IX51 inverted microscope. Notice cell shrinkage observed in J82 cells following frankincense oil treatment. In contrast, UROtsa cells did not experience noticeable morphological alteration following the same concentration of frankincense oil exposure.

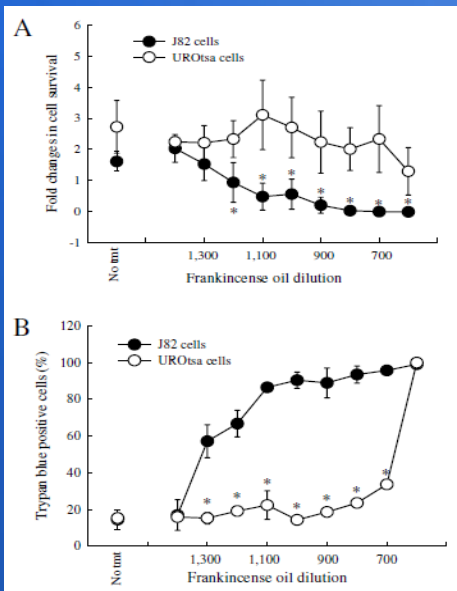


Figure 2
Bladder cell survival in response to frankincense oil exposure. Cell viability was determined using (A) a colometric XTT assay at 24 hours and (B) trypan blue exclusion at 3 hours after frankincense oil stimulation. All experiments were prepared in triplicate for XTT assay and duplicate for trypan blue exclusion. Data were presented as mean ± standard error of mean (SEM) from at least 3 independent experiments. * indicates statistical difference between frankincense oil-treated J82 cells and UROtsa cells ($P < 0.05$).

Source: [10] Mark Barton Frank, Qing Yang, Jeanette Osban, Joseph T Azzarello, Marcia R Saban, Ricardo Saban, Richard A Ashley, Jan C Welter, Kar-Ming Fung and Hsueh-Kung Lin: Frankincense oil derived from Boswellia carteri induces tumor cell specific cytotoxicity. The official journal of the International Society for Complementary Medicine Research (ISCMR)20099:6.

Enhancement of the effects of Gemcitabine:

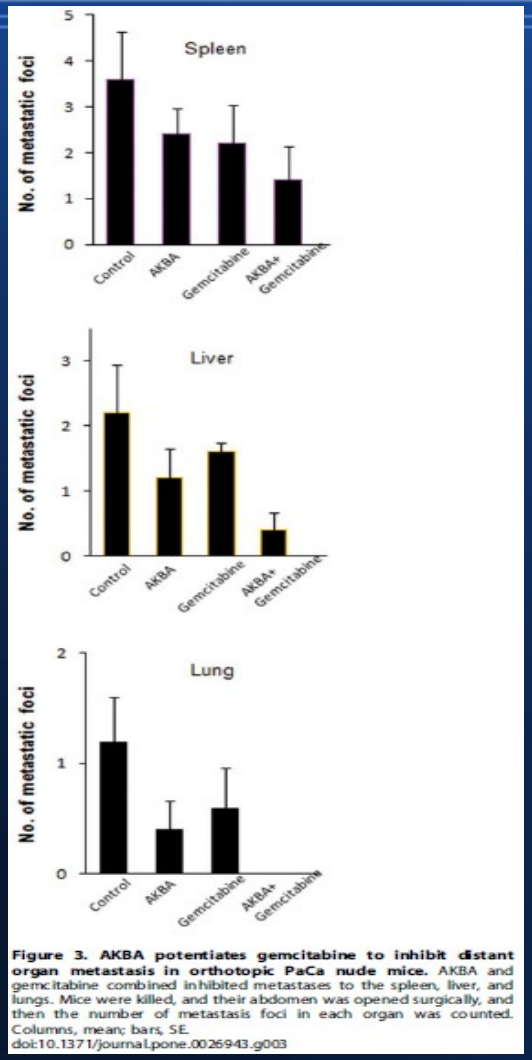


Figure 3. AKBA potentiates gemcitabine to inhibit distant organ metastasis in orthotopic PaCa nude mice. AKBA and gemcitabine combined inhibited metastases to the spleen, liver, and lungs. Mice were killed, and their abdomen was opened surgically, and then the number of metastasis foci in each organ was counted. Columns, mean; bars, SE.
doi:10.1371/journal.pone.0026943.g003

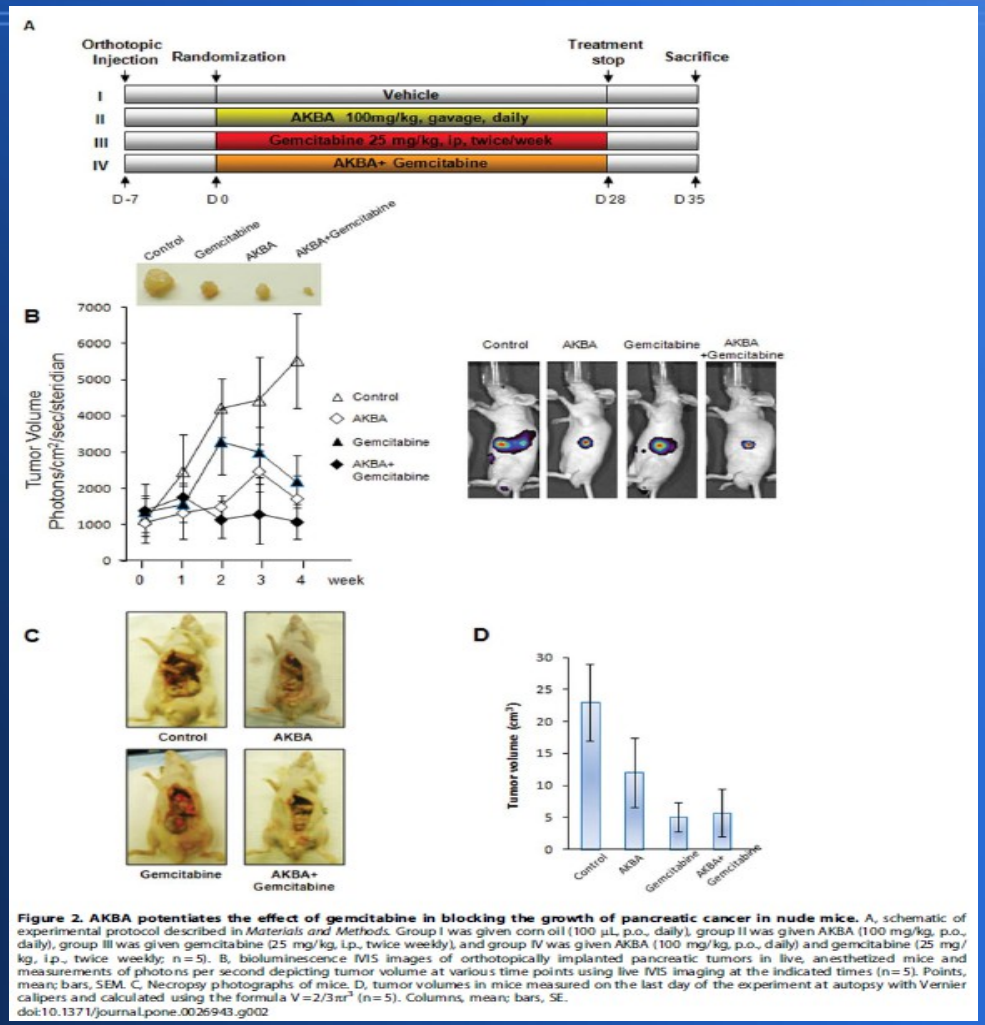


Figure 2. AKBA potentiates the effect of gemcitabine in blocking the growth of pancreatic cancer in nude mice. A, schematic of experimental protocol described in *Materials and Methods*. Group I was given corn oil (100 μL, p.o., daily), group II was given AKBA (100 mg/kg, p.o., daily), group III was given gemcitabine (25 mg/kg, i.p., twice weekly), and group IV was given AKBA (100 mg/kg, p.o., daily) and gemcitabine (25 mg/kg, i.p., twice weekly; n = 5). B, bioluminescence IVIS images of orthotopically implanted pancreatic tumors in live, anesthetized mice and measurements of photons per second depicting tumor volume at various time points using live IVIS imaging at the indicated times (n = 5). Points, mean; bars, SEM. C, Necropsy photographs of mice. D, tumor volumes in mice measured on the last day of the experiment at autopsy with Vernier calipers and calculated using the formula $V = 2/3\pi r^3$ (n = 5). Columns, mean; bars, SE.
doi:10.1371/journal.pone.0026943.g002

Source: [3] Park B, Prasad S, Yadav V, Sung B, Aggarwal B: Boswellic Acid Suppresses Growth and Metastasis of Human Pancreatic Tumors in an Orthotopic Nude Mouse Model through Modulation of Multiple Targets. Journal Plos One, Oct. 2011, Vol. 6, Issue 10.

Enhancement of the effects of Gemcitabine (2):

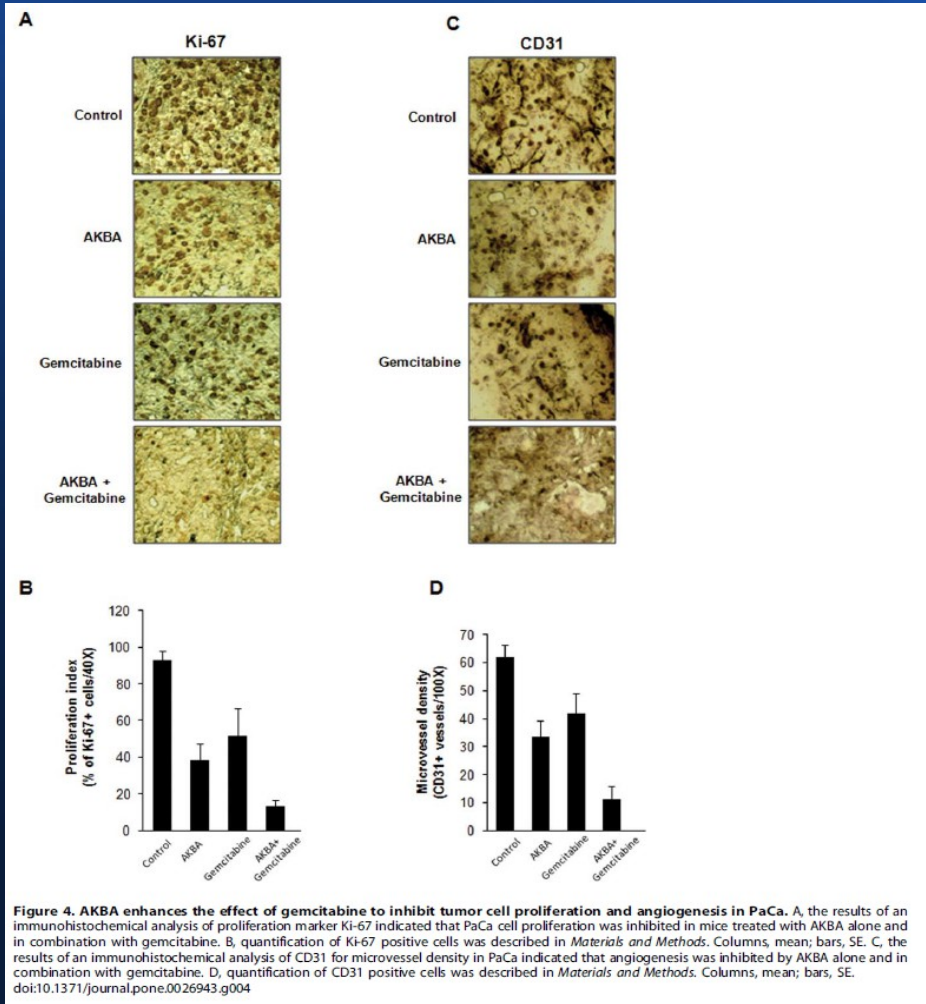


Figure 4. AKBA enhances the effect of gemcitabine to inhibit tumor cell proliferation and angiogenesis in PaCa. A, the results of an immunohistochemical analysis of proliferation marker Ki-67 indicated that PaCa cell proliferation was inhibited in mice treated with AKBA alone and in combination with gemcitabine. B, quantification of Ki-67 positive cells was described in *Materials and Methods*. Columns, mean; bars, SE. C, the results of an immunohistochemical analysis of CD31 for microvessel density in PaCa indicated that angiogenesis was inhibited by AKBA alone and in combination with gemcitabine. D, quantification of CD31 positive cells was described in *Materials and Methods*. Columns, mean; bars, SE.
doi:10.1371/journal.pone.0026943.g004

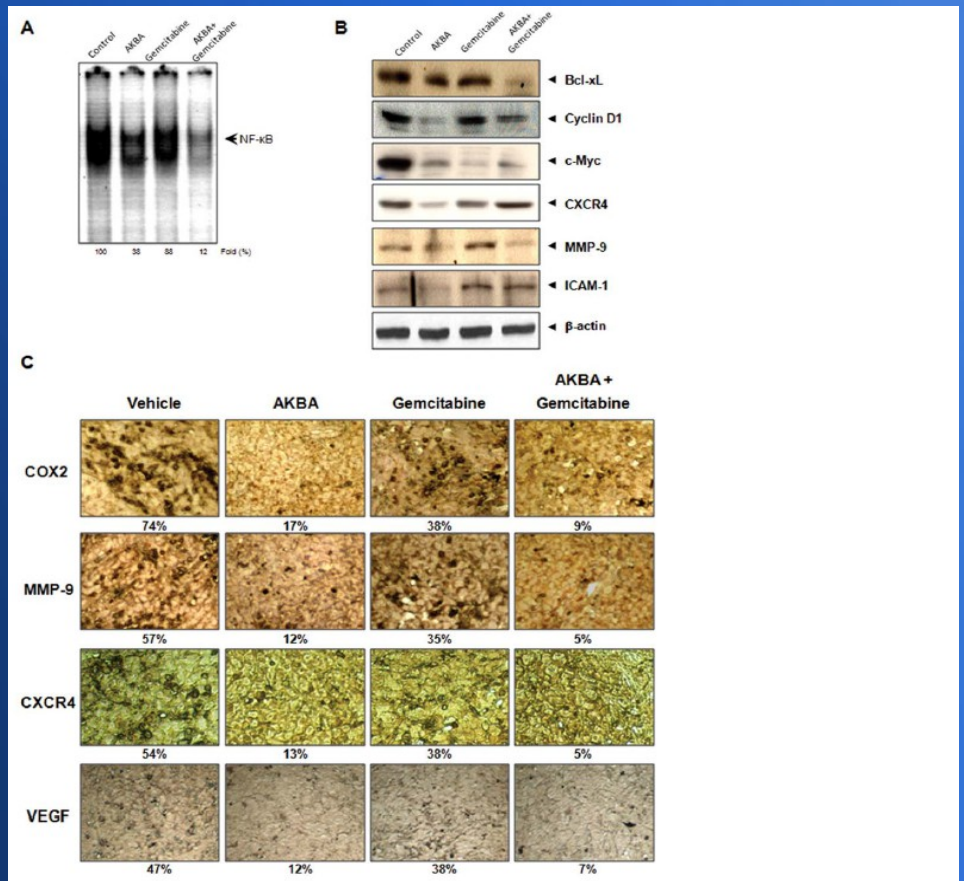


Figure 5. AKBA enhances the effect of gemcitabine against the expression of NF-κB and NF-κB–regulated gene products in pancreatic cancer tissues. A, detection of NF-κB by DNA binding in orthotopic tumor tissue samples showed the inhibition of NF-κB by AKBA. B, Western blot analysis showed that AKBA alone or combination with gemcitabine inhibited the expression of NF-κB–dependent genes. C, immunohistochemical analysis of NF-κB–regulated genes (COX-2, MMP-9, CXCR4 and VEGF) in pancreatic tumor tissues from mice. Percentages indicate positive staining for the given biomarker. Samples from three animals in each group were analyzed, and representative data are shown.
doi:10.1371/journal.pone.0026943.g005

Source: [3] Park B, Prasad S, Yadav V, Sung B, Aggarwal B: Boswellic Acid Suppresses Growth and Metastasis of Human Pancreatic Tumors in an Orthotopic Nude Mouse Model through Modulation of Multiple Targets. Journal Plos One, Oct. 2011, Vol. 6, Issue 10.

Risk-factor and radiotherapy side-effects reduction :

Source: [17]

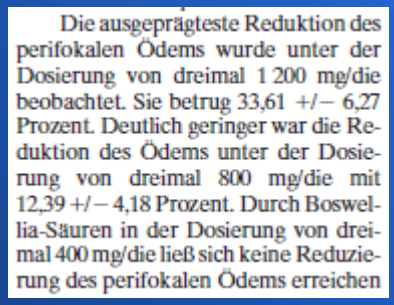
Protection against side-effects of radiotherapy:

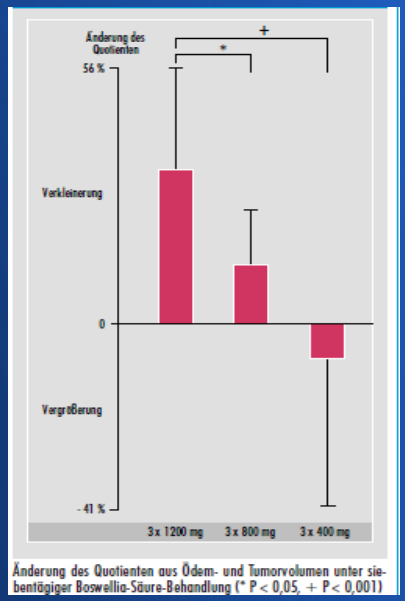
- Preliminary findings suggest effectiveness in **reducing cerebral edema in patients with brain tumors** following radiotherapy [14]. Compared with baseline and if measured immediately after the end of radiotherapy and BS/placebo treatment, a reduction of cerebral edema of >75% was found in 60% of patients receiving BS and in 26% of patients receiving placebo (P = .023).

- Also, a boswellia-based cream was found to be effective in preventing skin damage due to radiotherapy in breast cancer patients [15].

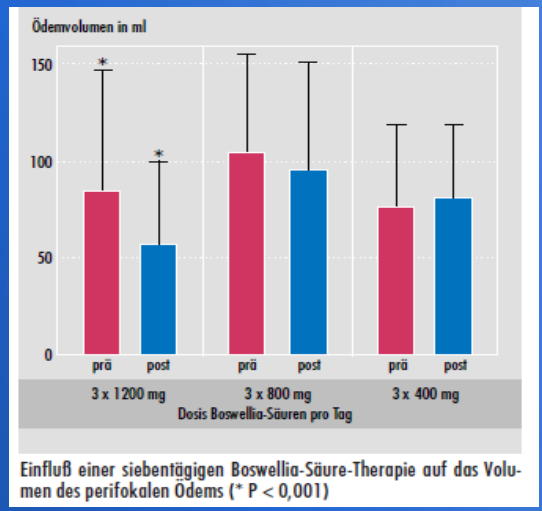
Risk-factor reduction:

- Other data suggest that a combination of boswellic acid, betaine, and myo-inositol treatment may help to reduce mammary density, a risk factor for breast cancer [16].

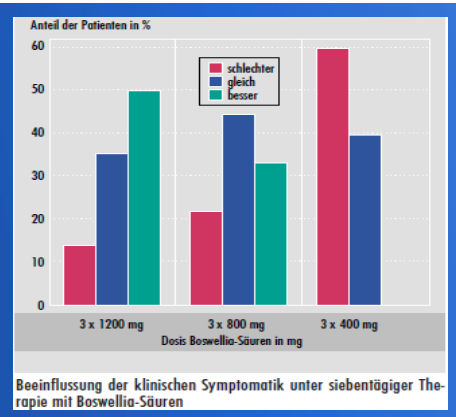

Die ausgeprägteste Reduktion des perifokalen Ödems wurde unter der Dosierung von dreimal 1 200 mg/die beobachtet. Sie betrug 33,61 +/– 6,27 Prozent. Deutlich geringer war die Reduktion des Ödems unter der Dosierung von dreimal 800 mg/die mit 12,39 +/– 4,18 Prozent. Durch Boswellia-Säuren in der Dosierung von dreimal 400 mg/die ließ sich keine Reduzierung des perifokalen Ödems erreichen



Änderung des Quotienten aus Ödem- und Tumorvolumen unter siebentägiger Boswellia-Säure-Behandlung (* P < 0,05, + P< 0,001)



Einfluß einer siebentägigen Boswellia-Säure-Therapie auf das Volumen des perifokalen Ödems (* P < 0,001)



Beeinflussung der klinischen Symptomatik unter siebentägiger Therapie mit Boswellia-Säuren

References:

[14] Kirste S, Treier M, Wehrle SJ, et al. Boswellia serrata acts on cerebral edema in patients irradiated for brain tumors: A prospective, randomized, placebo-controlled, double-blind pilot trial. Cancer. 2011;117(16):3788-95.

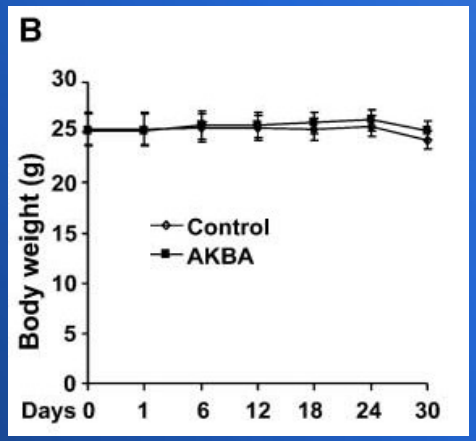
[15] Togni S, Maramaldi G, Bonetta A, Giacomelli L, Di Pierro F. Clinical evaluation of safety and efficacy of Boswellia-based cream for prevention of adjuvant radiotherapy skin damage in mammary carcinoma: a randomized placebo controlled trial. Eur Rev Med Pharmacol Sci. 2015 Apr;19(8):1338-44.

[16] Pasta V, Gullo G, Giuliani A, et al. An association of boswellia, betaine and myo-inositol (Eumastos(R)) in the treatment of mammographic breast density: a randomized, double-blind study. Eur Rev Med Pharmacol Sci. Nov 2015;19(22):4419-4426.

[17] Böker DK, Winking M: Die Rolle von Boswellia-Säuren in der Therapie maligner Gliome. Deutsches Ärzteblatt 94, Heft 18, 2. Mai 1997 (43).

Toxicity and adverse effects:

- No toxicity was established in animal models and no body weight losses were reported (see figure).
- Different evaluation studies including hematology, clinical chemistry, gross necropsy and histopathology showed no significant adverse changes on Newzealand white rabbits (Krishnaraju et al., 2010).



Source: [4]

- In some clinical trials epigastric pain, hyperacidity, anorexia, retrosternal burning, skin irritation or nausea have been reported in very few cases. The side effects were reversible after omission of the treatment.
- Allergic contact dermatitis was reported following use of a topical cream containing an extract of Boswellia serrata in combination with contact to latex.
- A 17-year-old girl with coeliac disease developed a gastric bezoar (accumulation of vegetable fiber, hair or other substances in the stomach or small intestine) after excessive intake of olibanum (frankincense). Surgical removal of the bezoar resolved symptoms of epigastric pain and vomiting.
- Elimination half-life ranged from 10.5 to 69.3 hours. Therefore, a repeated dose may lead to accumulation.

Reference:

[5] Pang X, Yi Z, Zhang X, et al. Acetyl-11-keto-beta-boswellic acid inhibits prostate tumor growth by suppressing vascular endothelial growth factor receptor 2-mediated angiogenesis. Cancer Res. 2009 Jul 15;69(14):5893-900.

Conclusions:

- Boswellic acids appear to be promising candidates for anticancer drug development in future.

- However, further *in vivo* studies are needed. Studies in combination with clinically used anticancer drugs also need to be carried out.