Potential Treatment of Autism with Traditional Chinese Medicine

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Autism: The Facts

- **Fastest growing developmental disability**
- **Annual growth 10-17%**
- **1/10000 in 1960's, now 1/88 in USA**
- **More children was diagnosed with Autism this year than with Cancer, diabetes and AIDS combined**
- **Boy: girl 4:1**
- **Autism cost nation over $35 billion per year**
- **CDC has called autism a national public health crisis**

Autism: The Facts

How much do we know about the etiology of Autism?

Identified etiology is only 10%!

Autism and Genes
Brain Pathology-Selective area/Neurotransmitters
GI dysfunction
Immune dysfunction
Impaired detoxification system
Toxic exposure/increased burden
Inflammation and oxidative stress

Autism: New Research Frontier

- **Autism represents an immunological and inflammatory disorder with definable biomarkers, mainly targeting GI and Brain**

Immune dysfunction in Autism: A New Frontier for Autism Research

1. **Allergy /Autoimmnue dysfunction**

   Eczema as marker of Th2 shift
   Allergic rhinitis, seasonal exacerbation
   Asthma
   Food allergy
   Cerebral autoimmunity, antibodies detected, Myelin basic Ab, etc

   Immunological profile

   IL-1beta almost 100% with mutation in autism
   TH-1 cytokine—TNF-alpha, viral infection and cancer
   TH-2 cytokines--- IL-4,6,10,13, allergy/atopy

2. **Chronic low grade infections**
   Bacteria, strep. elevated urinary bacterial metabolites in 50% patients
   PANDAS (pediatric autoimmune neuropsychiatric disorder associated with streptococcus) OCD
   Fungal, dermatitis, candidiasis
   Virus, MMR, HSV, EBV,HPV warts
   Mycoplasma
   Lyme

3. **Chronic inflammation**
   Cytokines elevation

Immune dysfunction in Autism

There is potential that aberrant immune activity during vulnerable and critical period of neurodevelopment could participate in the generation of neurological dysfunction characteristic of ASD.

J. Leukas et al., Biol. 2006, 80 (1): 1-15.

Neuropathology of Autism Brain

1. **Enlarged brain size in autistic children**

   Autopsy data: 5-13 years old, fresh brain weight increase by 100-200g when compared with expected age and sex, 20% head circumferences over 97th percentile, mostly above average.

2. **Overgrowth and enlargement of white matter**

   Axon and myelination process (Herbert, MGH)

3. **Evidence of inflammation and oxidative stress in autistic brain tissue from childhood and middle age (Ann Neurol 57:67-81,2005)**

4. **impairment· in synaptic function** *[N A J Med Sci. 2011;4(3):112-115.]…..*

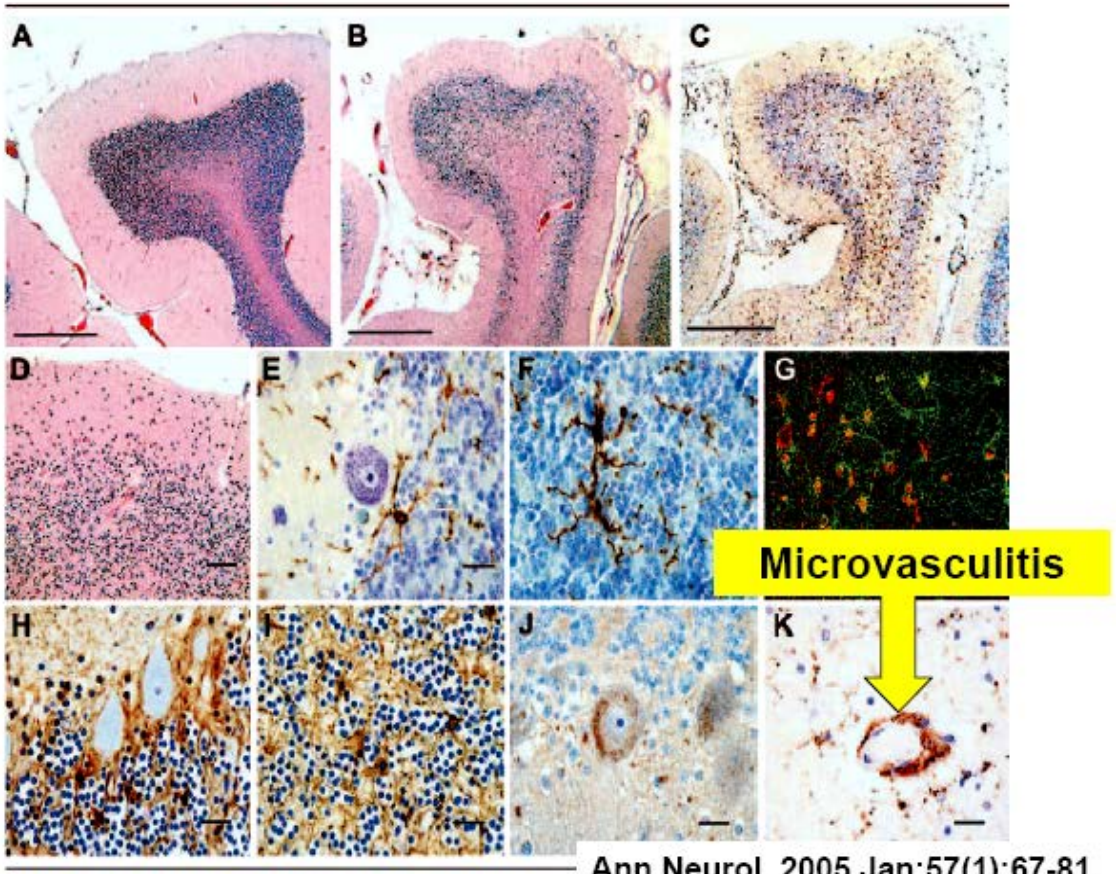
Inflammation and Immune-dysfunction in Autism Brain

Responses are primarily from Innate Immunity

Adaptive immunity markers like T cells or antibody reactions not found

Cytokines significantly elevated in brain and CSF of autistic patients

- **Pro-inflammatory: MCP-1, IL-6 and IFNγ**
- **Anti-inflammatory:TGFbeta**



Ann Neurol. 2005 Jan;57(1):67-81

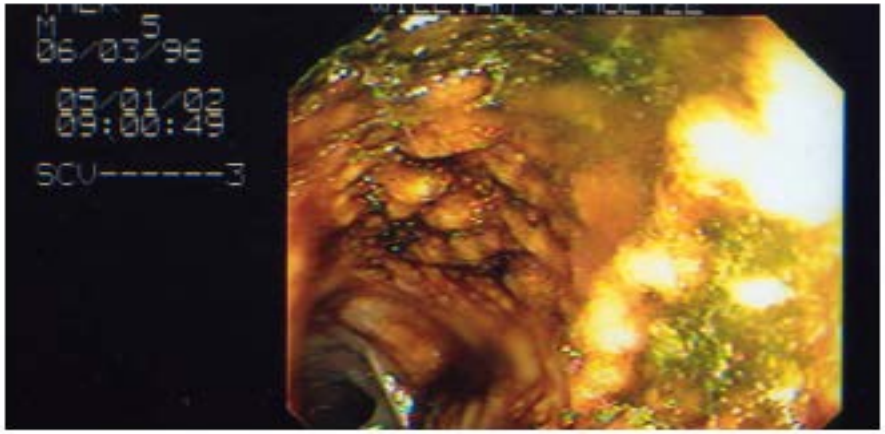
Gut dysfunction in Autism

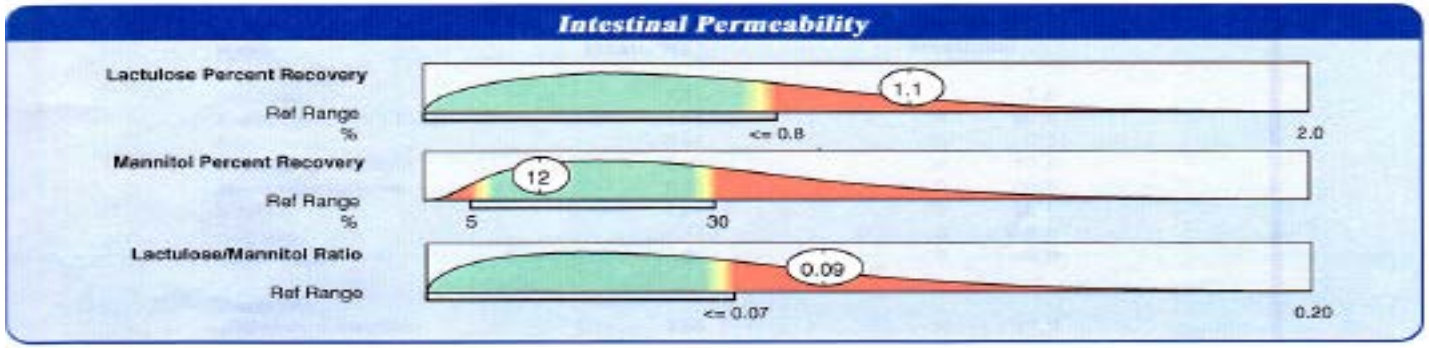
Chronic diarrhea
Food allergy/sensitivity
Endoscopy: inflammation
Infection: bacteria, yeast, virus

Stool analysis: Maldigestion and Malabsorption

Leak bowel syndrome
GI enzyme deficiency, Secretin, DDP-IV
Urinary peptides
Nutritional deficiency
Low B6 50%
Low Magnesium almost 100%
Low zinc almost 100%
Low selenium, vitamin A, biotin, B1, B3, B5, B12, Vitamin C,

Intestinal immune changes lead to *increased intestinal permeability*

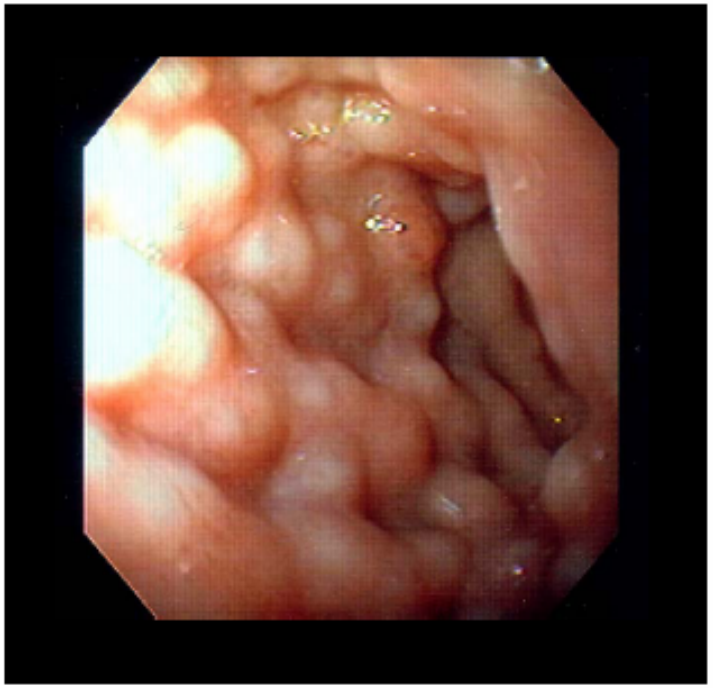


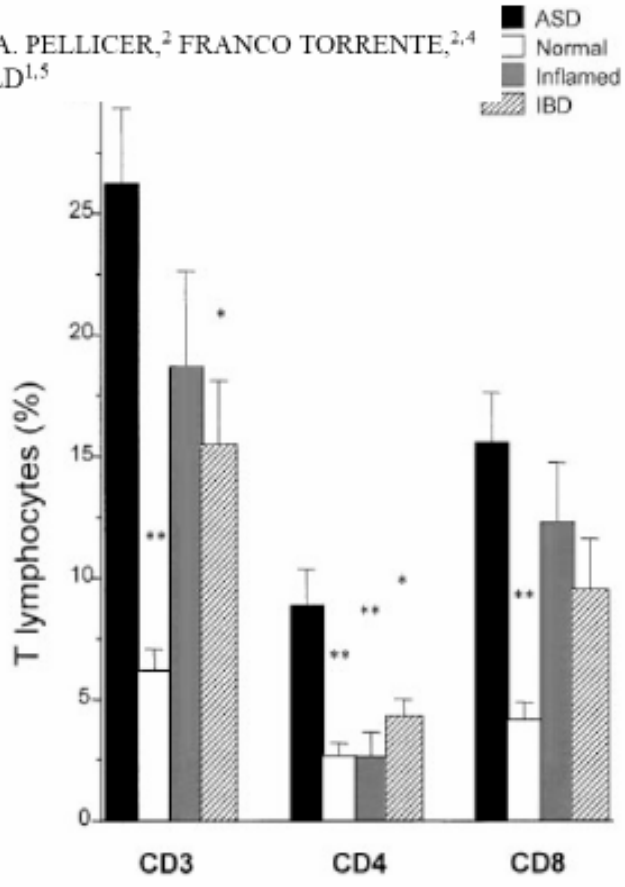


Intestinal Lymphocyte Populations in Children with Regressive Autism: Evidence for Extensive Mucosal Immunopathology

Journal of Clinical Immunology, Vol. 23, No. 6, November 2003 (© 2003)

PAUL ASHWOOD,[1,2,6] ANDREW ANTHONY,[1,3] ALICIA A. PELLICER,[2] FRANCO TORRENTE,[2,4] JOHN A. WALKER-SMITH,[2] and ANDREW J. WAKEFIELD[1,5]





Autism Treatments:

Traditional:
Behavioral and educational training
Medications

Biochemical and Alternative
Diet and nutritional supplement
Treatment for infection
Chelation
Immunomodulation: LDN, Actos
RNA therapy
TMS
Stem cell

Current Medications for Autism

There is no known cure for autism. Not everyone with Autism has the same symptoms, and not all symptoms can be treated with the same drugs.

Most often, the prescription is intended to address specific symptoms such as anxiety, depression, mood swings (bipolar disorder), obsessions, compulsions, inattention, and hyperactivity.

SSRIs
Antipsychotics
Anticonvulsants
Stimulants.

Because these medications give only symptomatic relief, and there is a large individual differences, therefore clinical improvement is quite limited.

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Modern Drug Development

Drug Candidate: Single Chemical Entity

↓

In Vitro Study

↓

In Vivo Study

↓

Clinical Study

↓

Modern Drug

Single component, clear mechanism of actions, good quality control, proven efficacy

Development of TCM

Man

↓

Foods = Herbs

↓

Foods =/= Herbs

↓

Herbs (up-mid-low)

↓

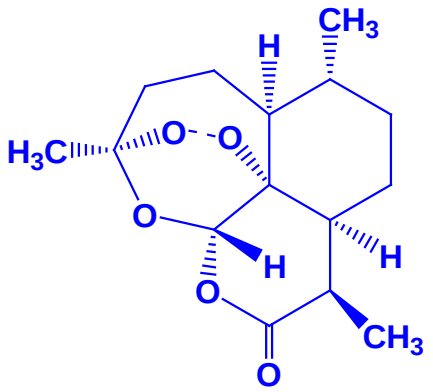
Herbs + Minerals

↓

TCM Herbal Remedies

Multi-components, bioactive components unknown, mechanism of actions unclear, quality control issues and questionable efficacy

New Anti-Malaria Drug



本草纲目 草部第十五卷 草之四 (1575)

【主治】疥瘙痂痒恶疮，杀虱，治留热在骨节间，明目（《本经》）。鬼气尸疰伏连，妇人血气，腹内满，及冷热久痢。秋冬用子，春夏用苗，并捣汁服。亦曝干为末，小便入酒和服（藏器）。补中益气，轻身补劳，驻颜色，长毛发，令黑不老，兼去蒜发，杀风毒。心痛热 黄，生捣汁服，并贴之（大明）。治疟疾寒热（时珍）。生捣敷金疮，止血止疼良（苏恭）。烧灰 隔纸淋汁，和锻石煎，治恶疮息肉 瘢（孟诜）。

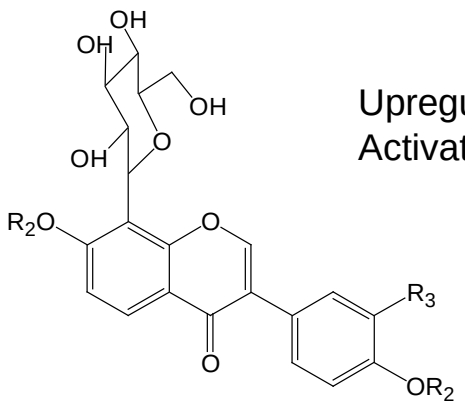
Fat: Fashion in Tang Dynasty (609-907)

Diabetes -Sugar Urine Disease

葛根 *

〔气味〕甘、辛〔三〕，平，无毒。〔别录曰〕生根汁：大寒。〔好古曰〕气平味甘，升也，阳也。阳明经行经的药也。

〔主治〕消渴 **身大热，呕吐，诸痹，起阴气，解诸毒。本经疗伤寒中风头痛，解肌发表出汗，开腠理，疗金疮，止〔四〕胁风痛。别录治天行上气呕逆，开胃下食，解酒毒。甄权治胸膈烦热发狂，止血痢，通小肠，排脓破血。傅蛇虫〔五〕咬，署毒箭伤。大明杀野葛、巴豆、百药毒。之才生者：堕胎。蒸食：消酒毒，可断谷不饥。作粉尤〔六〕妙。藏器作粉：止渴，利大小便，解酒，去烦热，压丹石，傅小儿热疮。捣汁饮，治小儿热痞。开宝猘狗伤，捣汁饮，并末傅之。苏恭散郁火。时珍〔发明〕〔弘景曰〕生葛捣汁饮，解温病发热。五月五日〔七〕中时，取根为屑，疗金疮断血为要药，亦疗疟及疮，至良。〔颂曰〕张仲景治伤寒有葛根汤，以其主大热，解肌、发〔八〕腠理故也。〔元素曰〕升阳生津。脾虚作渴者，非此不除。勿多用，



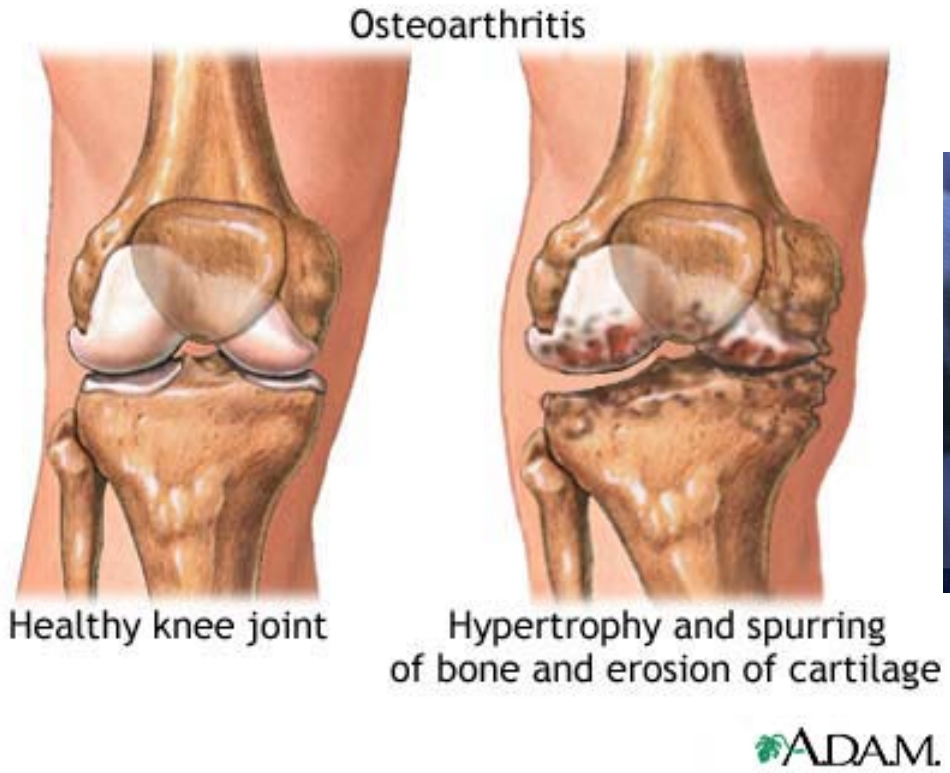
Upregulates peroxisome proliferator-Activated receptor **PPARα**

Osteoarthritis

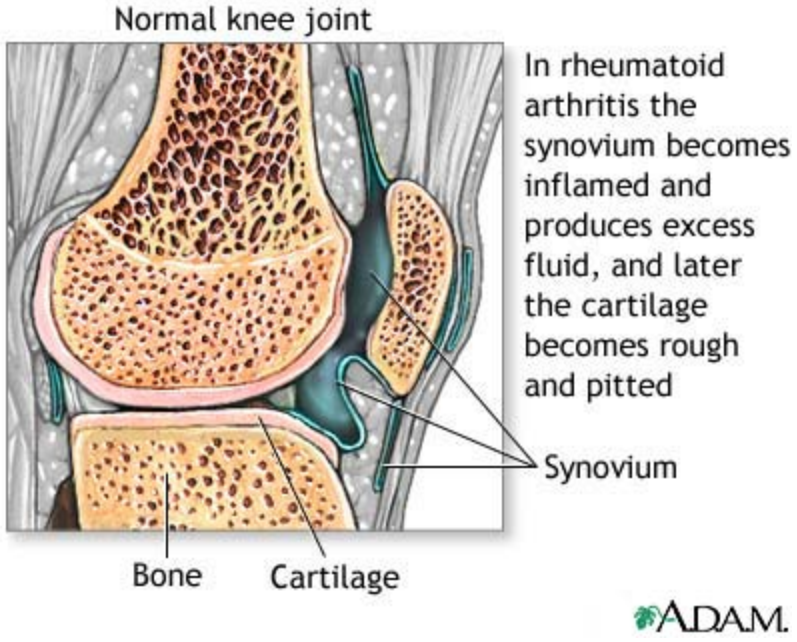
- It is estimated that Osteoarthritis (OA) alone will reach 7 billion and the total market size for arthritis drug will reach 20 billion in 2010.

- Modern COX2 type anti-inflammatory drug, such as (Vioxx) was recalled by FDA in 2004 and currently there is no effective prescription drug for OA.

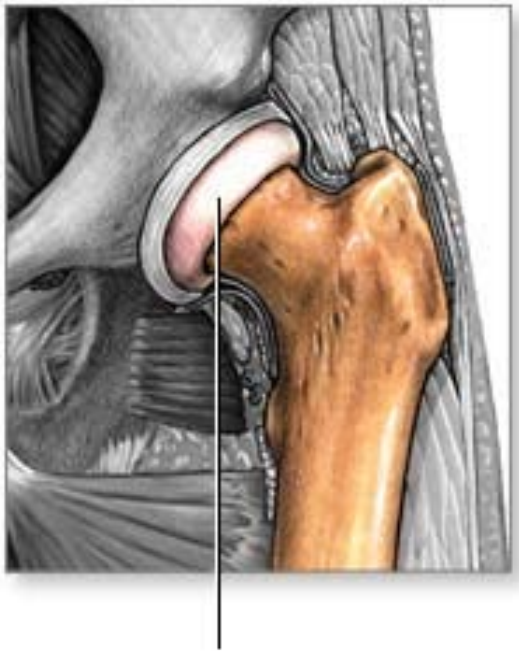
Osteoarthritis

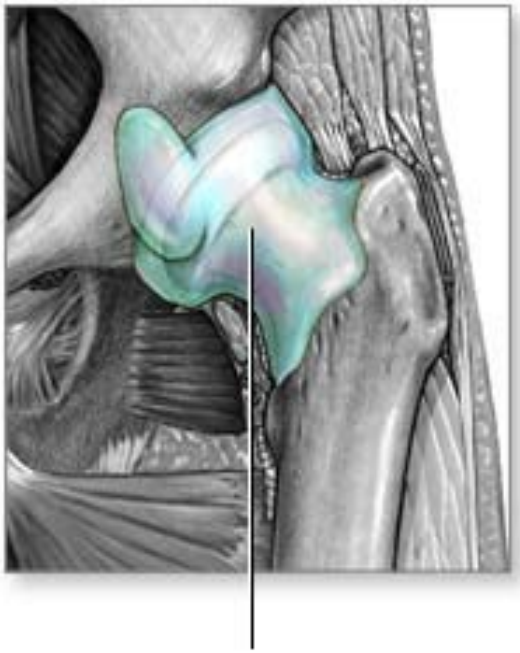


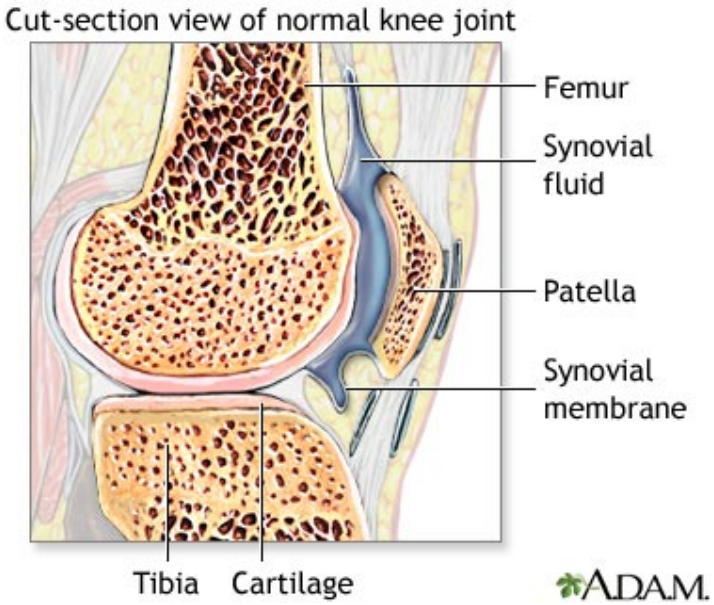
Rheumatoid Arthritis



Osteoarthritis vs Rheumatoid Arthritis







Prescribed Medications for Arthritis
Important Safety Information

Like all prescription NSAIDs, CELEBREX may increase the chance of a heart attack or stroke that can lead to death. It should not be used right before or after certain heart surgeries.

Serious skin reactions or stomach and intestine problems, such as bleeding and ulcers, can occur without warning and may cause death.
Patients taking aspirin and the elderly are at increased risk for stomach bleeding and ulcers.

People with aspirin-sensitive asthma or allergic reactions due to aspirin or other arthritis medicines or certain drugs called sulfonamides should not take

Prescription CELEBREX should be used exactly as prescribed at the lowest dose possible and for the shortest time needed.

Traditional Chinese Medicine for Arthritis

黄帝内经：素问：痺论篇

痺证 （**Arthritis**）：
风 **wind**
寒 **cold**
湿 **wet or damp**

凡痺之类， 逢寒则虫， 逢热则纵。
其病情昼轻夜重，痛如虎啮。

Traditional Chinese Medicine (HLXL) for Osteoarthritis

HLXL (Huo-Luo-Xiau-Lin Dan): A Traditional Chinese Remedy which consists of 11 herbs for treatment of arthritis

HLXL has been studied in the US for over 15 years and funded continuously by NIH (NCCAM-AT-P01-0026053)

HLXL is under Phase II Trial (IND#70324) in the US

Procurement of Botanical Materials in China

SPECIFIC AIMS:

- **To acquire authenticated quality individual crude plant drug materials used in the studies based on good sourcing practice (GSP), and to establish the chemical (chromatographic) profiles of the acquired materials for validation reference, and recollection**

THE INSTITUTE OF MEDICINAL PLANT DEVELOPMENT (IMPLAD)

CHINESE ACADEMY OF MEDICAL SCIENCES-PEKING UNION MEDICAL COLLEGE

IMPLAD is dedicated to protecting, developing and utilizing medicinal plant resources by means of modern scientific technology. IMPLAD has a staff of 680 individuals, including 150 professors and associate professors

- **The WHO Center for Collaboration on Traditional Medicine (1986)**
- **The Center for the Utilization and Conservation of Chinese Medicinal Herb Resources**
- **The National Medicinal Plant Seed Resource Bank (2005)**
- **The National Center for Chinese Medicinal Herb Reference Standards**
- **The Chinese Medicinal Herb Resource Conservation and Phytochemistry Laboratory**
- **US/NIH Project: Center for Chinese Herbal Therapy (CHT) for Asthma: Project 3 Chemical and Biological Characteristics of Botanicals (PI)**

Collection of 11 herbs of HLXL

Danshen

Salvia miltiorrhiza Bge.

Chuanxiong

Ligusticum chuanxiong Hort.

Yanhusuo

***Corydalis yanhusuo* W. T. Wang**

Danggui

Angelica sinensis (Oliv.) Diels.

Guizhi

Cinnamomum cassia Presl.

Duhuo

Angelica pubescens

Maxim.f.biserrata Shan et Yuan

Gancao

Glycyrrhiza uralensis Fisch.

Qinjiao

Gentiana macrophylla Pall.

Qianghuo

Notopterygium incisum Ting ex H. T. Chang

Chishao

Paeonia lactiflora Pall.

Ruxiang

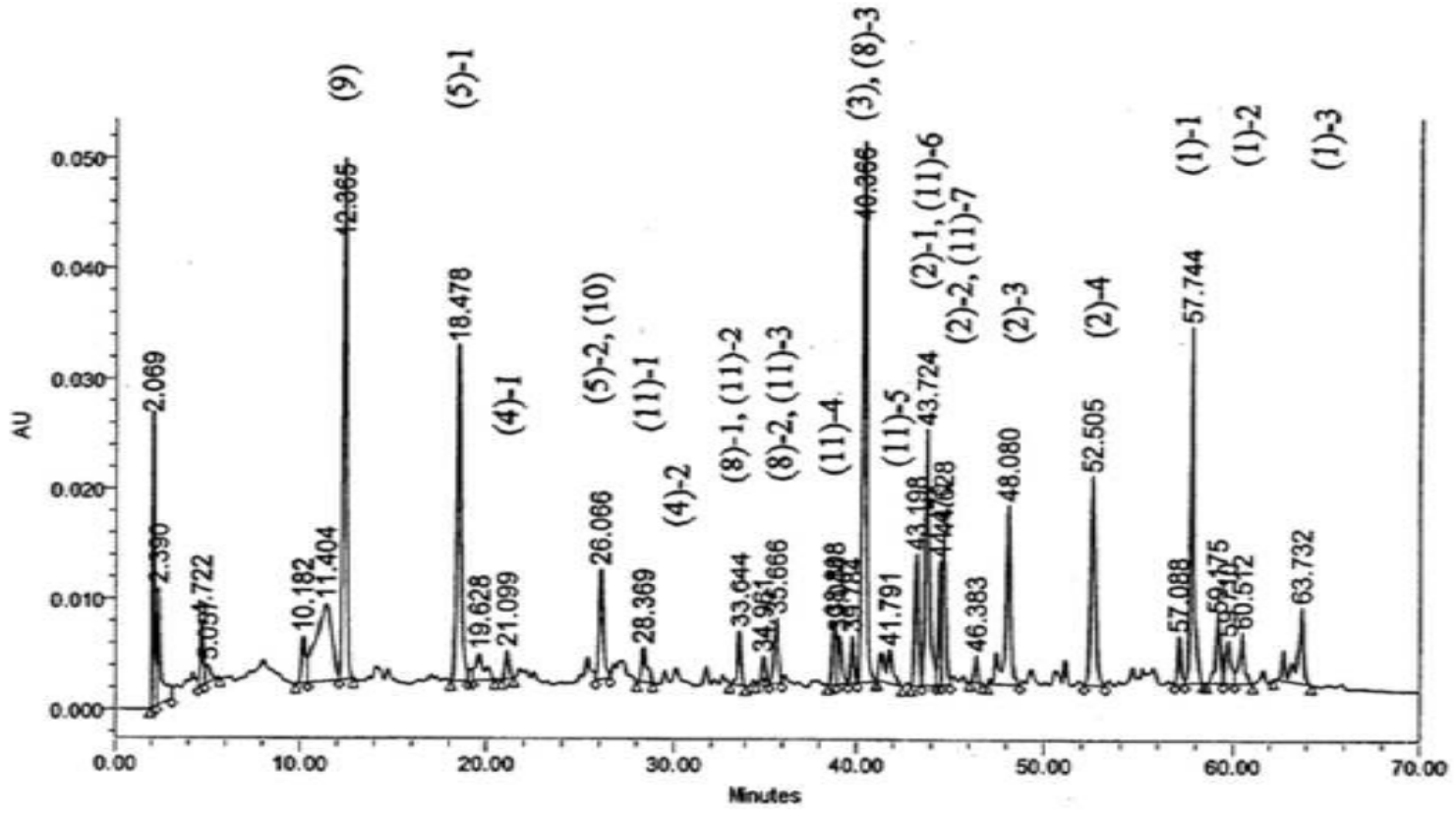
Boswellia carterii Birdw.

Radix Paeonia Rubra

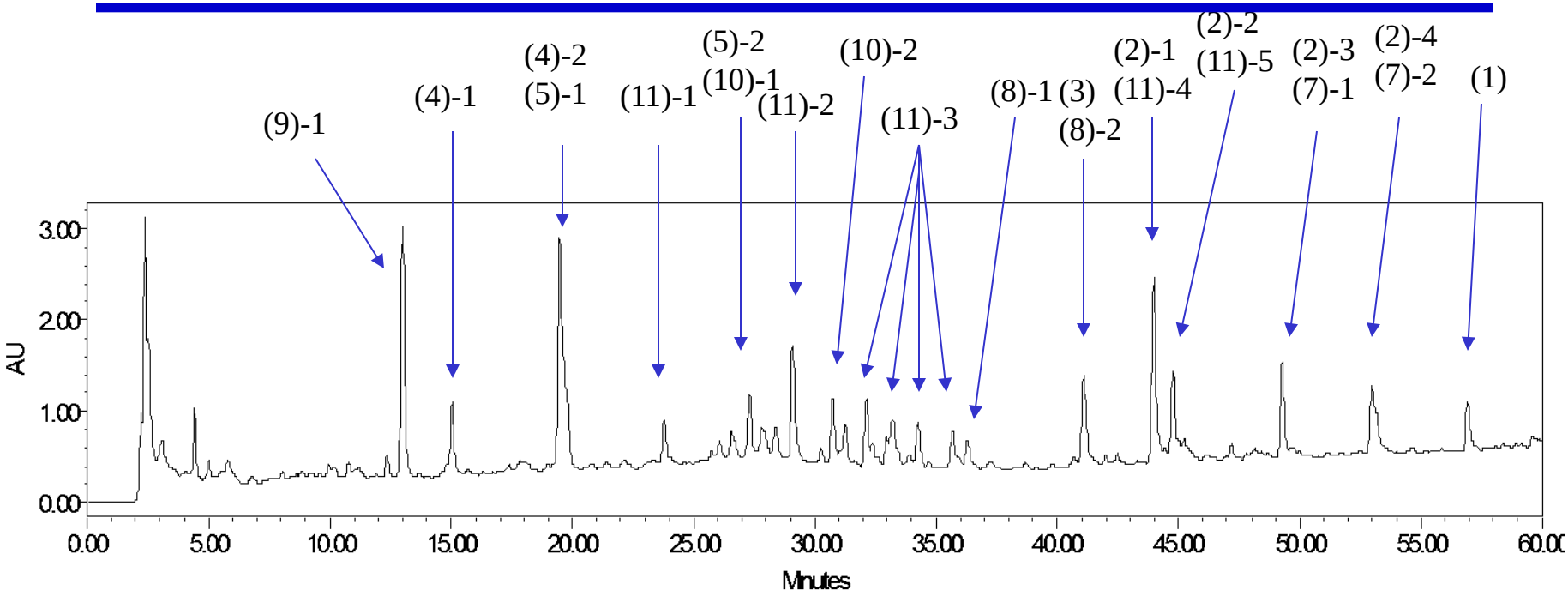
Collecting Number: Lin003
Collecting Site: Banshifangzi Town, Linxi District, Chifeng, Innermongolia
Harvesting Time: November, 2005
Medicinal Plant: *Paeonia lactiflora* Pall.
Processing: Dug up root, removed rhizome and rootlets, and dried.

Plant Discription:

HPLC Fingerprint - HLXL



HPLC Fingerprint

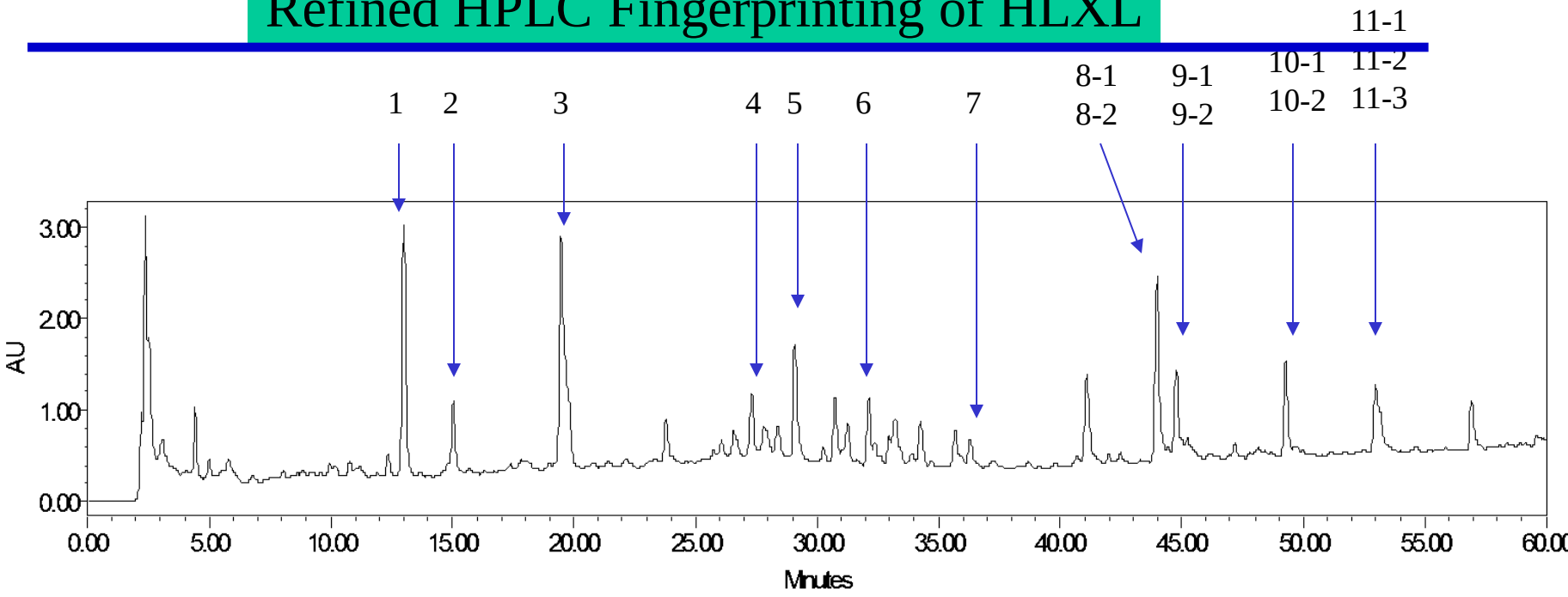


HPLC conditions

Waters Breeze HPLC systems — YMC ODS-A column (4.6 X 150 mm)
Flow Rate: 0.8 ml/min — Running time: 60 or 70 min
Wavelength: 210 nm — Mobile phase: 20-100% MeOH 0 - 60 min

(1). Boswellia carterii Birdw.
(2). Notopterygium incisum Ting ex HT Chang
(3). Angelica sinensis (Oliv.) Diels
(4). Paeonia Lactiflra Pall.
(5). Glycyrrhiza uralensis Fisch.
(6). Corydalis yanhusuo WT Wang
(7). Salvia miltiorrhiza Bge.
(8). Ligusticum chuanxiong Hort.
(10) Cinnamomum cassia Blume
(11). Angelica pubescens Maxim.f.biserrata Shan et Yuan

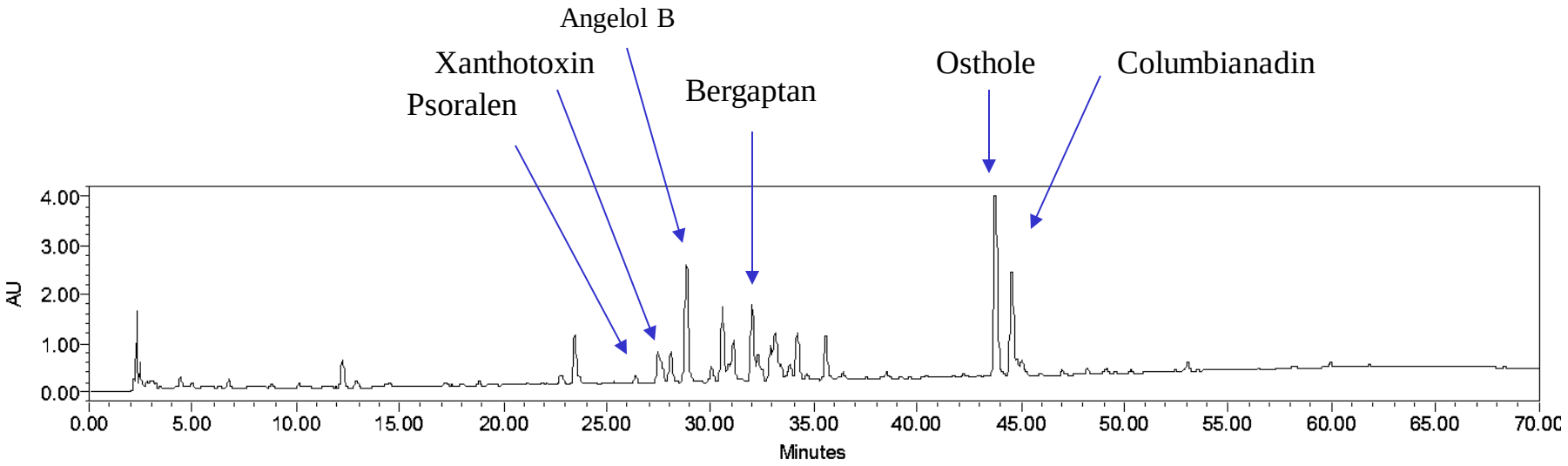
Refined HPLC Fingerprinting of HLXL

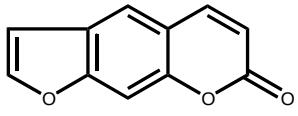


Marker compounds:

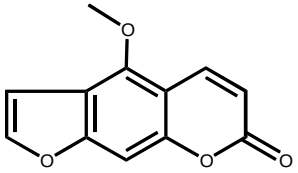
1. (9) Swertiamarin (秦艽 *Gentiana macrophylla*)
2. (4) Paeoniflorin (赤芍 *Paeonia Lactiflra*)
3. (5) Liquiritin (甘草 *Glycyrrhiza uralensis*)
4. (5) Liquiritigenin (甘草 *Glycyrrhiza uralensis*)
5. (11) Angelol B (独活 *Angelica pubescens*)
6. (11) Bergapten (独活 *Angelica pubescens*)
7. (8) Senkyunolide A (川芎 Ligusticum chuanxiong)
8-1. (2) Phenethyl *trans*-ferulate (羌活 *N. incisum*)
8-2. (11) Osthole (独活 *Angelica pubescens*)
9-1. (2) Isoimperotorin (羌活*Notopterygium incisum*)
9-2. (11) Columbianadin (独活 *Angelica pubescens*)
10-1.(2) Falcarindiol (羌活 *Notopterygium incisum*)
10-2. (7) Crytotanshinone (丹参 *Salvia miltiorrhiza*)
11-1. (7) Tanshinone IIA (丹参 *Salvia miltiorrhiza*)
11-2. (2) Ostruthin (羌活 *Notopterygium incisum*)
11-3. (2) Anhydronotoptol (羌活 *N. incisum*)

Angelica pubescens 独活

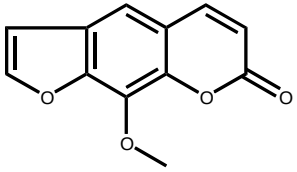




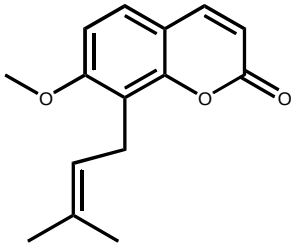
Psoralen



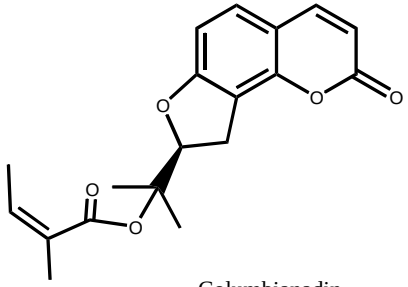
Bergaptan



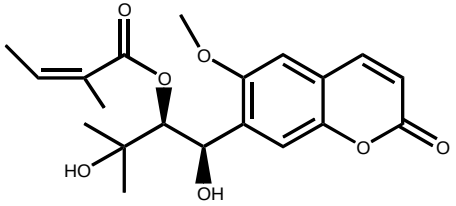
Xanthotoxin



Osthole

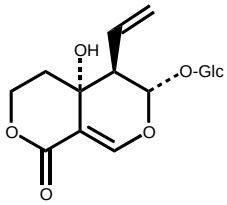


Columbianadin

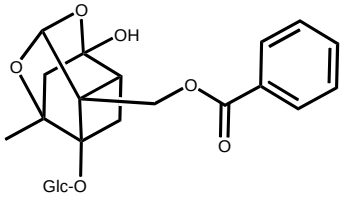


Angelol B

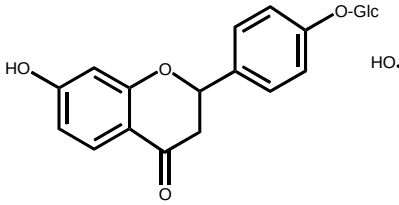
HPLC Marker Compounds



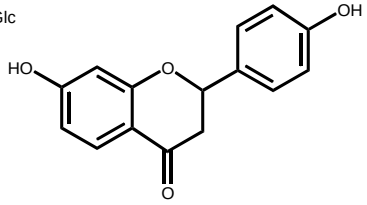
1. Swertiamarin



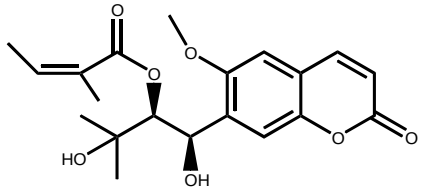
2. Paeoniflorin



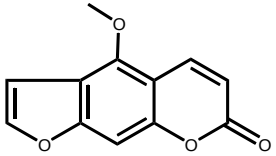
3. Liquiritin



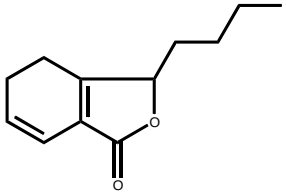
4. Liquiritigenin



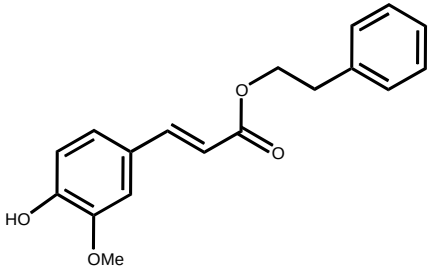
5. Angelol B



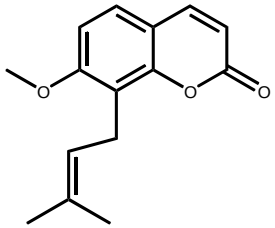
6. Bergaptan



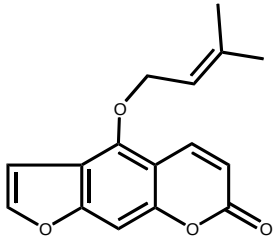
7. Senkyunolide A



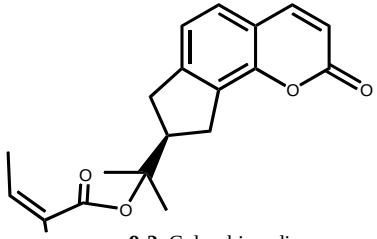
8-1. Phenethyl trans-ferulate



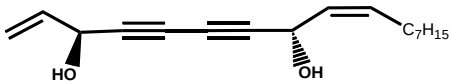
8-2. Osthole



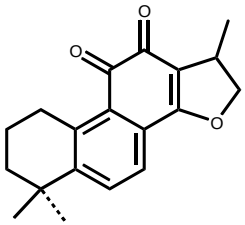
9-1. Isoimperatorin



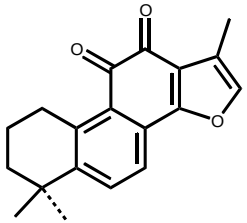
9-2. Columbianadin



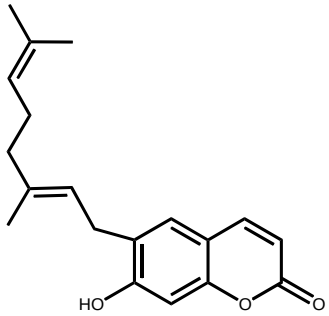
10-1. Falcarindiol



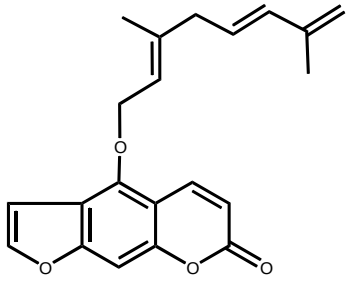
10-2. Cryptotanshinone



11-1. Tanshinone IIA



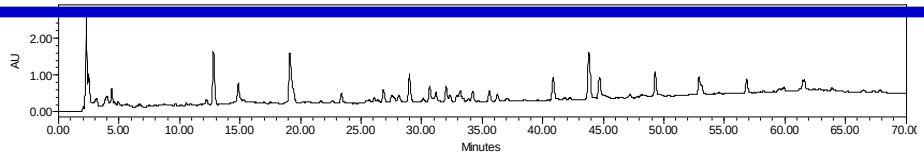
11-2. Ostruthin



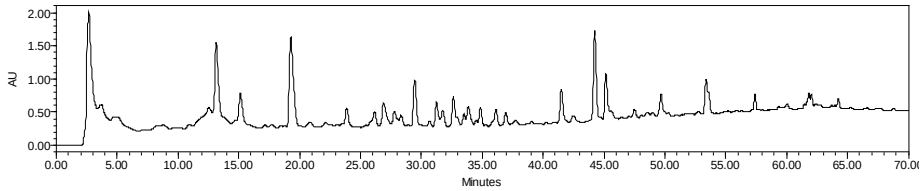
11-3. Anhydronotoptol

Stability Study of HLXL

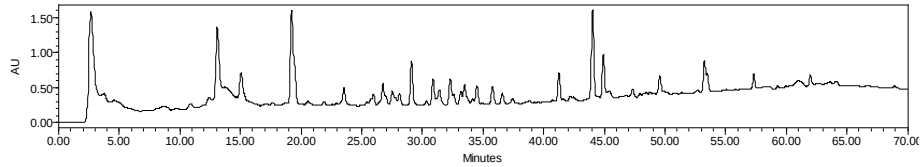
No detectable changes of peak shapes, numbers, intensities and retention times after two years of stability study.



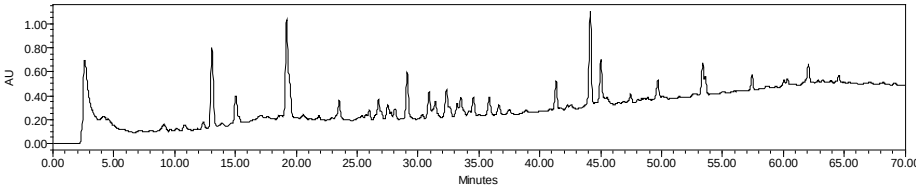
Standard HLXL Dan (210 nm)
01/17/2007



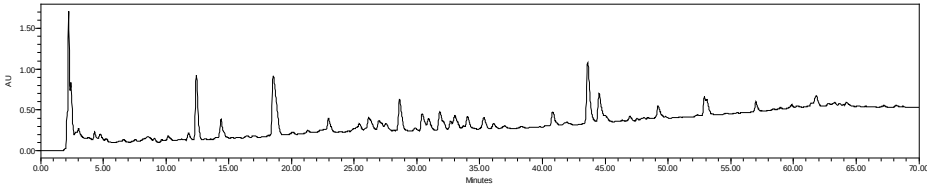
06/11/2008



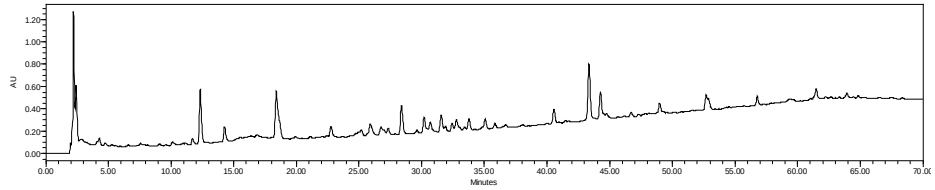
08/12/2008



10/07/2008



11/12/2008



02/19/2009

HLXL: *In Vitro* Assays

Since inflammation plays an important role in the pathophysiology of OA, not only with respect to pain and stiffness but also with respect to structural progression, therefore, the crude extract of HLXL, individual plant extracts along with fractionated components are subjected to two *in vitro* anti-inflammatory evaluations:

(1) COX 1 & COX2

(2) 5-Lipoxygenanse Assay (5-LPO)

At a concentration of 10 μg/mL, COX-2 inhibition assays were carried out for 12 extracts. (% inhibition > 70% was considered active)

code	name	% inhibition
Extract 1	*Glycyrrhiza uralensis*	83
Extract 2	*Corydalis yanhusuo*	83
Extract 3	*Centiana macrophylla*	65
Extract 4	*Boswellia carterii*	31
Extract 5	*Paeonia lactiflora*	70
Extract 6	*Notopterygium incisum*	50
Extract 7	*Angelica sinensis*	62
Extract 8	*Angelica pubescens*	86
Extract 9	*Cinnamomum cassia*	68
Extract 10	*Ligusticum chuanxiong*	65
Extract 11	*Salvia miltiorrhiza*	55

COX2/COX1 Activity of HLXL

Compound Name	% Inhibition of COX-2	% Inhibition of COX-1	Selectivity (COX-2/COX-1)
Betulinic acid (10 μM)	95	57	1.67
Isoliquiritigenin (10 μM)	70	34	**2.06**
Reseveratrol (10 μM)	66	98	**0.67**
Phenethyl trans-ferulate (10 μM)	90	81	1.11
Celecoxib (6.55 μM)	93		
Celecoxib (45 nM)	60		
Indomethacin (62.5 μM)		99	
Indomethacin (49 nM)		49	

COX-2 *In Vitro* Assay

(1) Ultrafiltration LC-MS Screening for COX-2 Ligands

(2) Functional COX-2 Assay for Agonists or Antagonists

oCOX-1 and hCOX-2 functional assay result of compounds in HLXL

Compound code	Compound name	IC_{50} on oCOX-1 (μM)	IC_{50} on hCOX-2 (μM)
MA5-74-25	Senkyunolide O	25	**5**
MA5-74-20	Roburic acid	5	**9**
MA4-121-8	Phenethyl-trans-ferulate	18	**31**
MA5-74-19	Falcarindiol	> 100	**15**
FW02	Cryptotanshinone	> 100	**23**
MA5-74-5	Acetyl-11-keto- β-boswellic acid	8	85
MA5-74-2	β-Boswellic acid	15	> 100
MA5-74-3	acetyl-11-keto-α-boswellic acid	9	> 100
MA5-74-4	Acetyl-β-boswellic acid	8	73
FW14	Betulinic acid	20	> 100

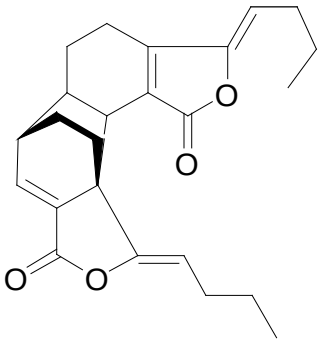
$Ki=IC_{50}*Km/(S+Km)=IC_{50}/2$

O = ovine

h = human

Potent COX-2 Inhibitors

Ligusticum chuanxiong

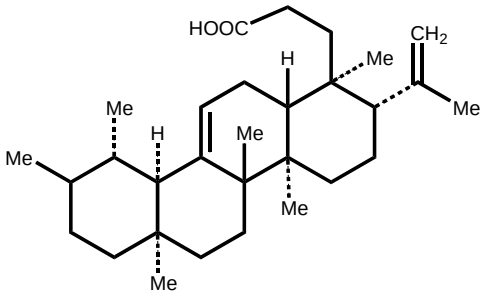


Senkyunolide O

IC_{50} oCOX-1 = 25 µM

IC_{50} **hCOX-2 = 5 µM**

Gentiana macrophylla

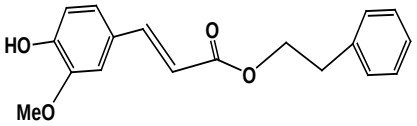


Roburic acid
MA5-74-20

oCOX-1 = 5 µM

hCOX-2 = 9 µM

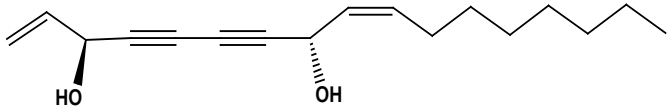
Notopterygium incisum



Phenethyl trans-ferulate
MA5-74-18

oCOX-1 = 18 µM

hCOX-2 = 31 µM

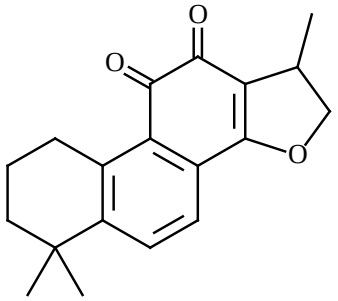


Falcarindiol
MA5-74-19

oCOX-1 > 100 µM

hCOX-2 = 15 µM

Salvia miltiorrhiza



Cryptotanshinone

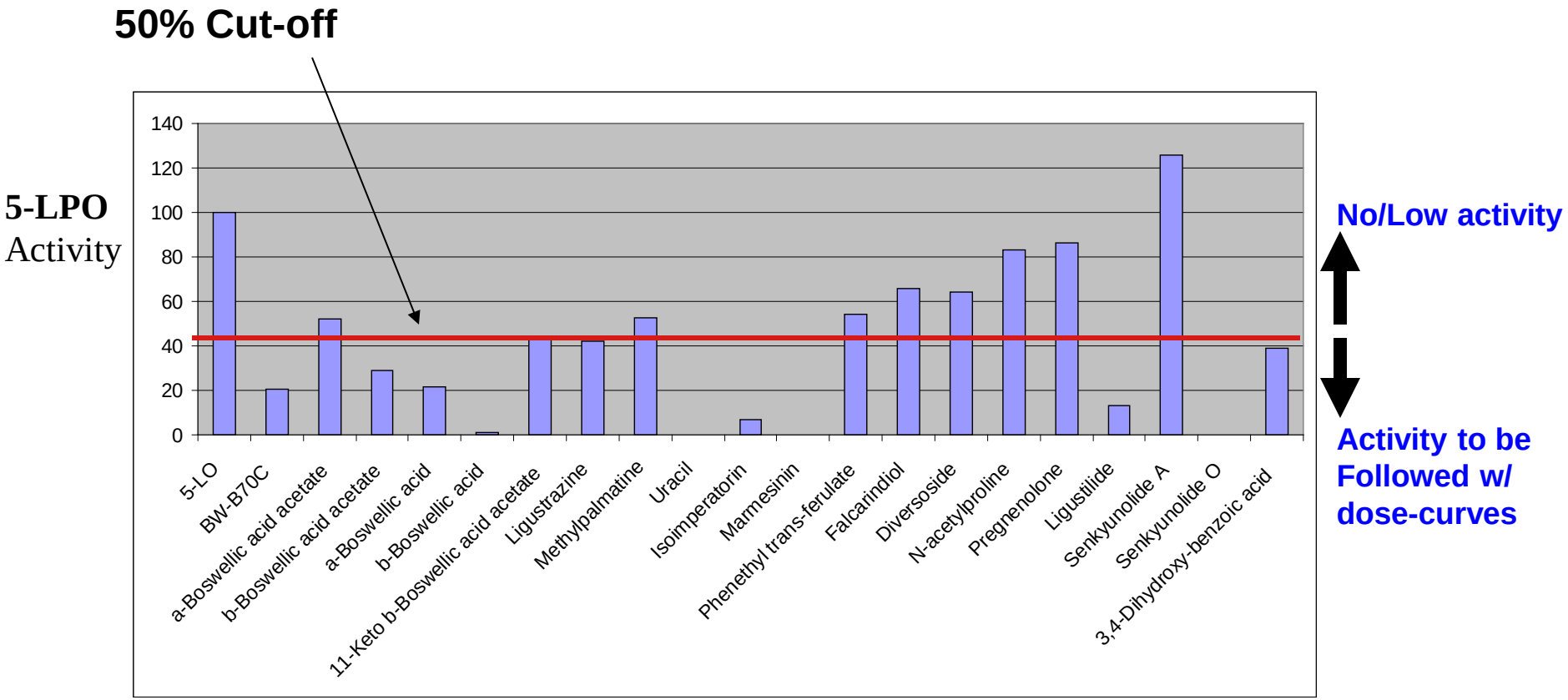
oCOX-1= > 100 µM

hCOX-2 = 23 µM

Functional COX-2 Assay

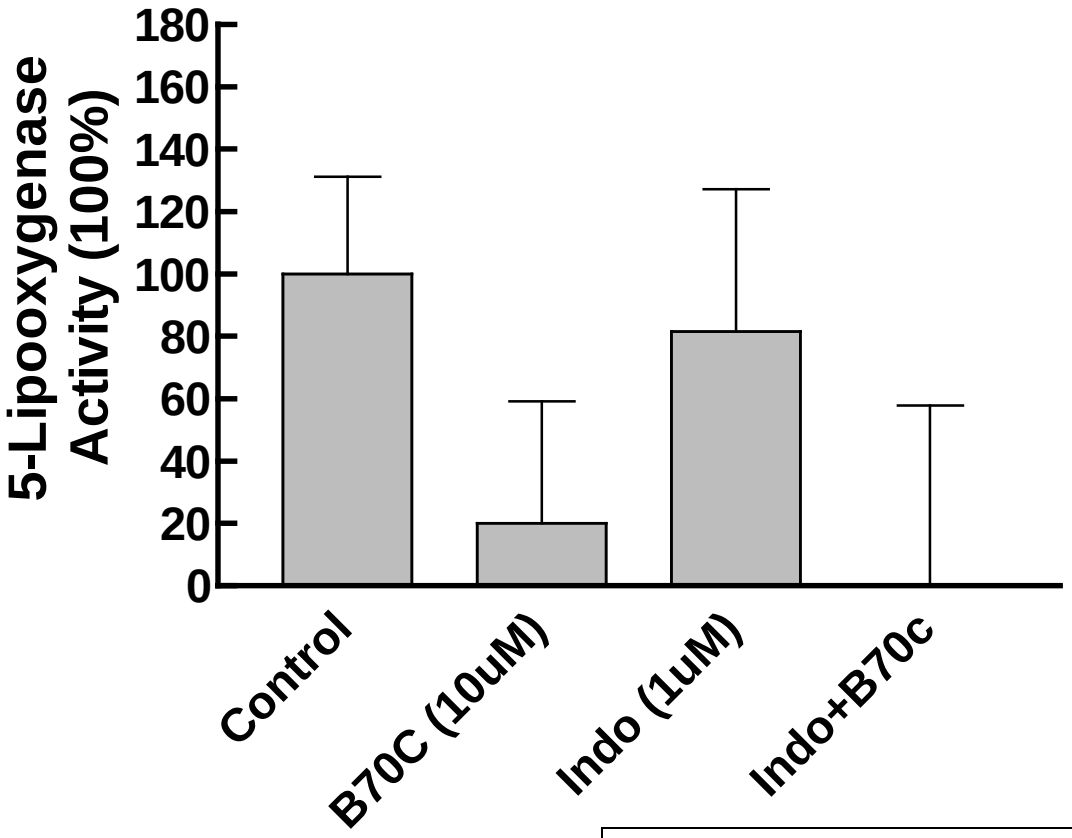
Plant Name	COX-2 inhibition (%)	Active compounds
Glycyrrhiza uralensis	83	Betulinic acid, Isoliquiritigenin, Resveratrol
Corydalis yanhusuo	83	
Centiana macrophylla	65	
	31	
Paeonia lactiflora	70	
Notopterygium incisum	50	Phenethyl trans-ferulate
Angelica sinensis	62	
	86	
Cinnamomum cassia	68	
	65	
Salvia miltiorrhiza	55	

5-LPO Active Isolates



All Compounds = 10μM

Lack of 5-LO inhibition by indomethacin (Indo), a COX inhibitor

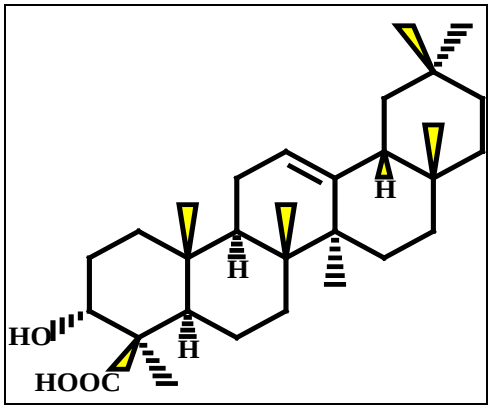


Positive control: BW B70c, a known selective 5-LO inhibitor

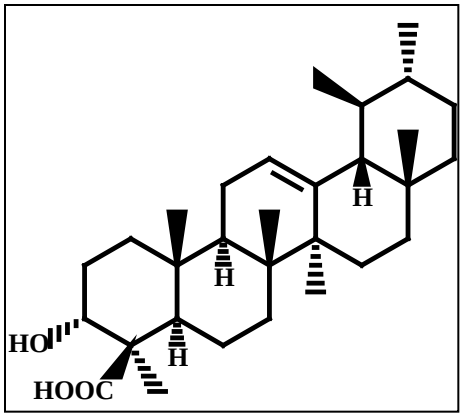
5-LPO Inhibition IC_{50} Values

Compounds	IC50 ± SEM	% Maximum inhibition
β-boswellic acid acetate	0.23±2.57 μM	90%
α-boswellic acid	3.71±13.2 μM	64%
β -boswellic acid	2.70±3.14 μM	100%
11-keto β-boswellic acid acetate	31.3±2.10 μM	100%
Marmesinin	1.80±0.43 μM	68%
Ligustilide	1.50±3.80 μM	100%
p-Hydroxyphenethyl-anisate	0.36±1.54 μM	100%
Isoimperatorin	0.055±1.5 μM	82%
Senkyunolide O	0.57±1.99 μM	100%
3,4-Dihydroxy-benzoic acid	8.71±3.10 μM	44%

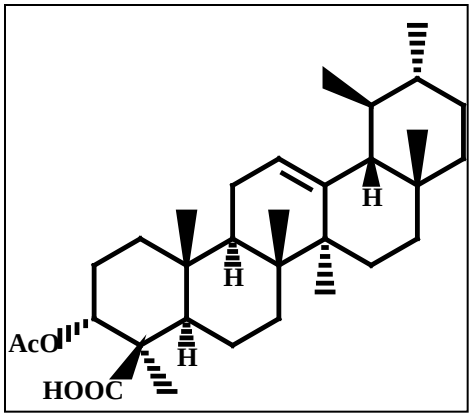
5-LPO Active Isolates



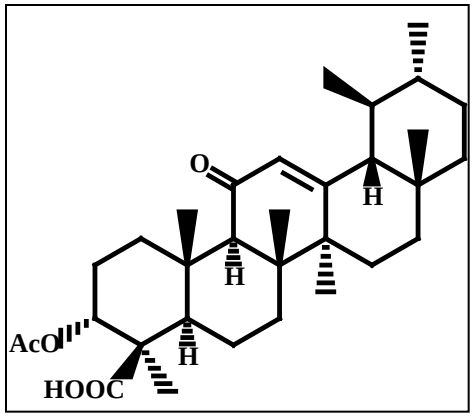
α-Boswellic acid



β-Boswellic acid

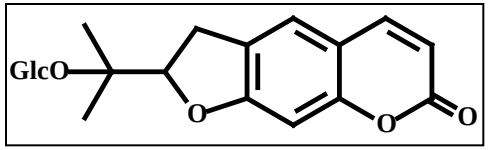


Acetyl-β-boswellic acid

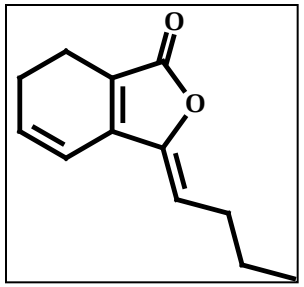


Acetyl-11-keto-β-boswellic acid

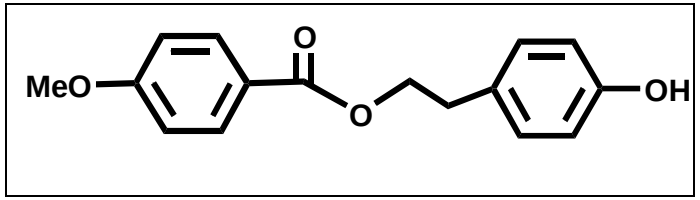
5-LPO Active Isolates



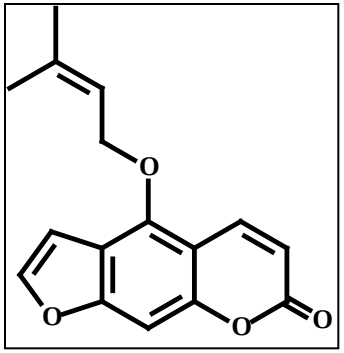
Marmesinin



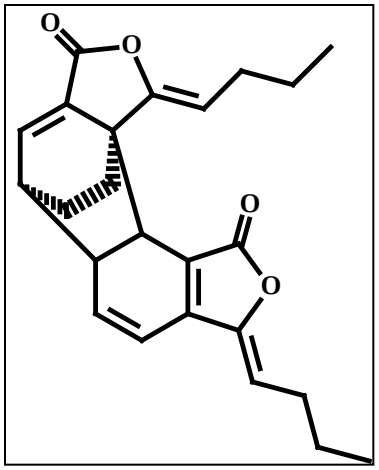
Ligustilide



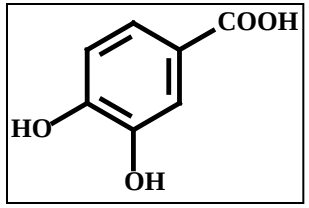
p-Hydroxyphenethyl-anisate



Isoimperatorin

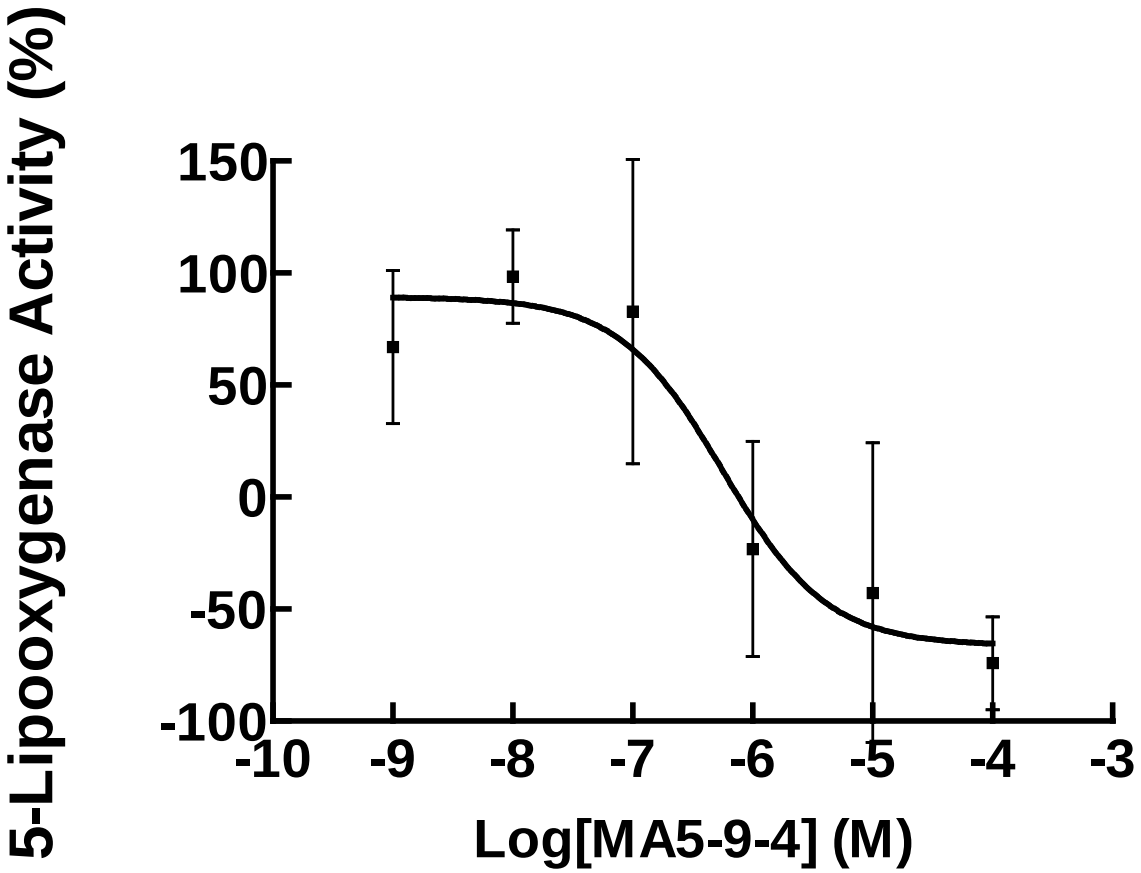


Senkyunolide O



3, 4-Dihydroxybenzoic acid

Dose-dependent Inhibition of 5-LPO by Senkyunolide O



Conclusions

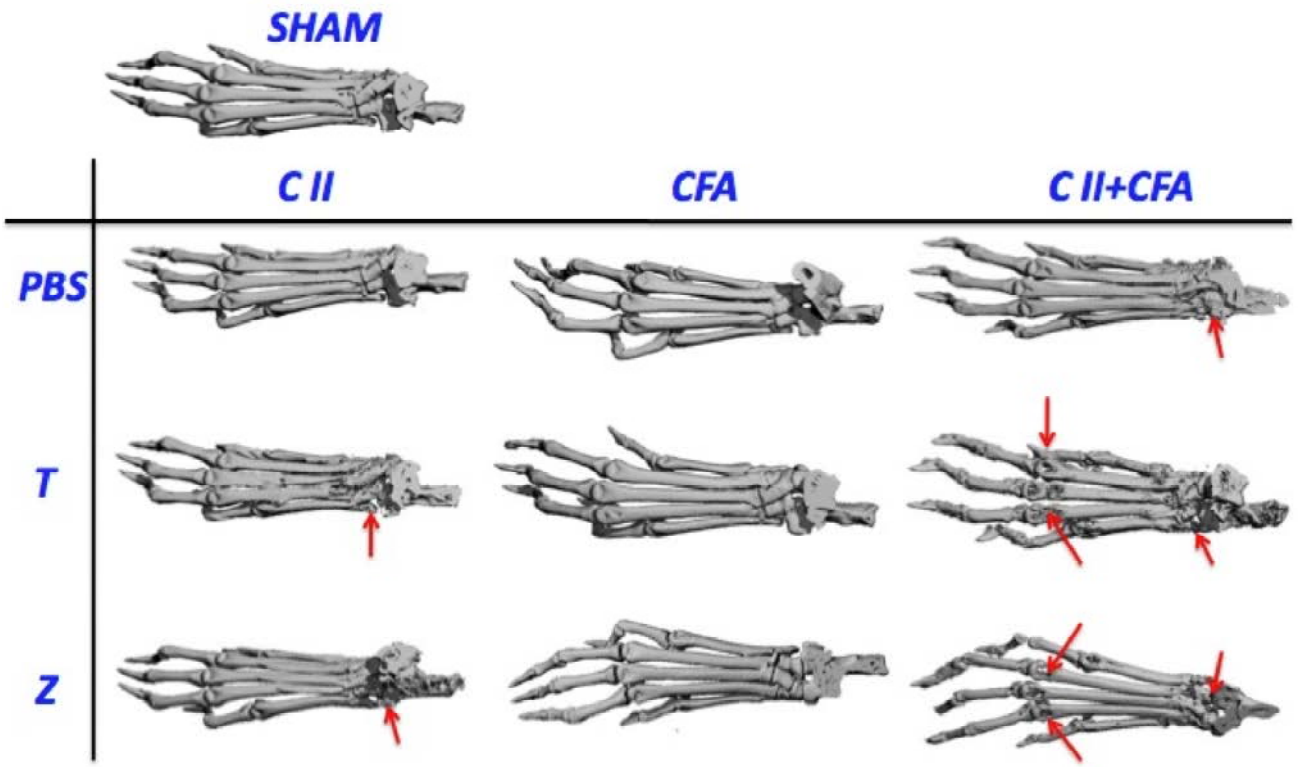
(1) More than 56 bioactive compounds have been obtained and characterized from the 11 component plants of HLXL

(2) Recently, human COX2 enzyme became commercially available which enabled us to conduct functional COX-2 assays and led to the confirmation of nine inhibitors with IC_{50} values in the range of 5-31 μM (senkyunolide O, roburic acid, phenethyl-trans-ferulate, falcarindiol, and cryptotanshonone)

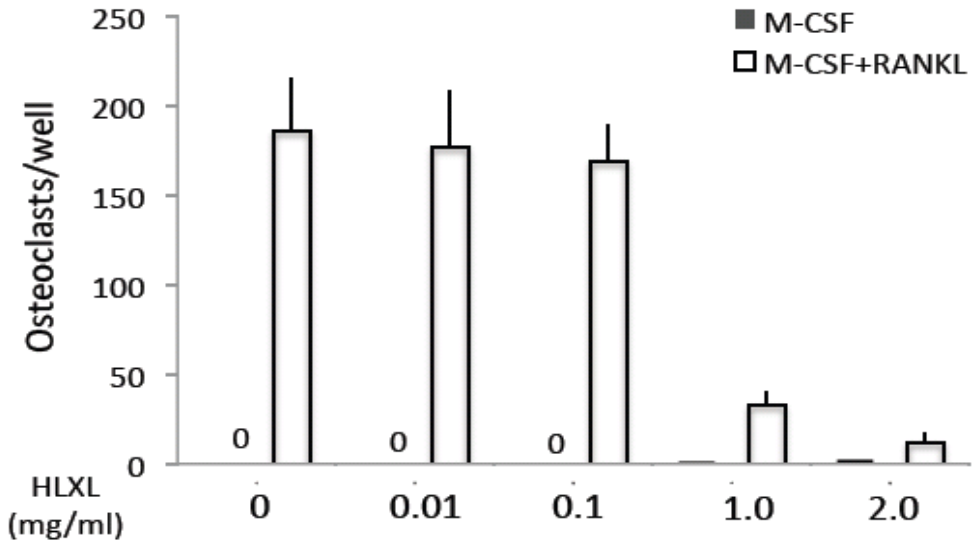
(3) Evaluation of the isolates in a 5-LPO assay led to the identification of five boswellic acid derivatives and 8 other compounds as having potential anti inflammatory activity. Functional Assay provided the IC_{50} values of 10 potent 5-LPO inhibitors: α-boswellic acid, β-boswellic acid, acetyl-β-boswellic acid, acetyl-11-keto-β-boswellic acid, marmesinin, ligustilide, p-hydroxyphenethyl-anisate, isoimperatorin, senkyunolide O, and 3, 4,-dihydroxy-benzoic acid.

(4) As evidenced by the *in vitro* binding and functional assays, the overall efficacy of HLXL may have been contributed in part by these bioactive compounds.

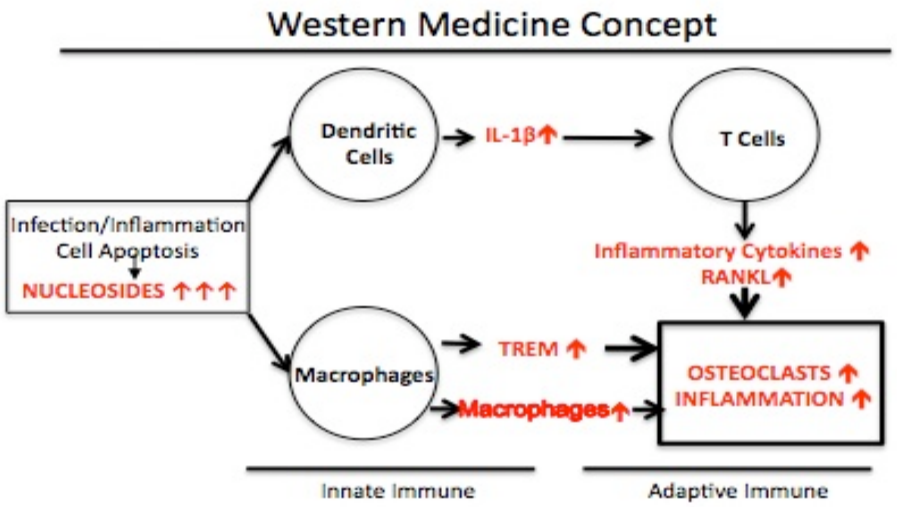
Effect of HLXL Bone destruction and joint deformity in nucleoside-treated CIA mice

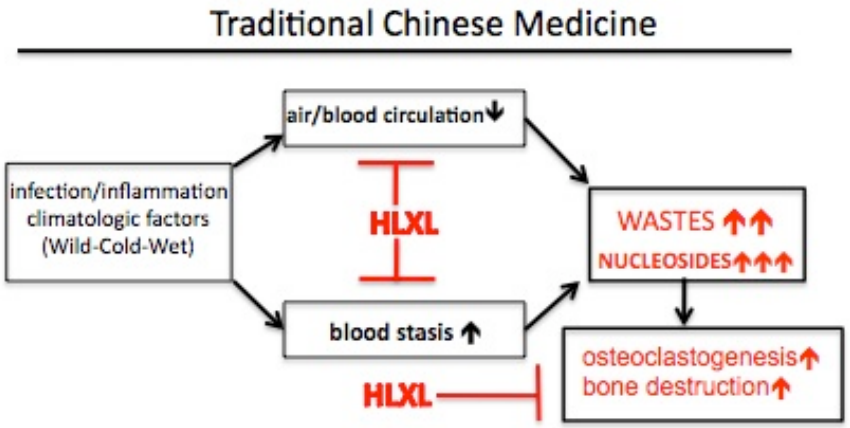


The Effects of HLXL on Osteoclasts

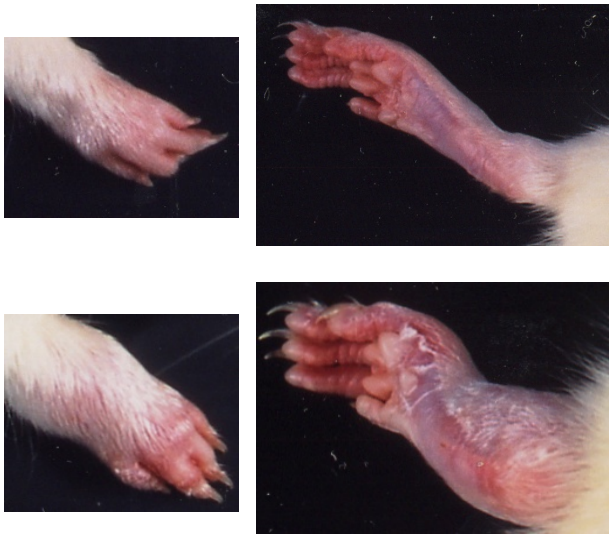


Accumulated nucleosides are an important molecule of linking Western and Chinese Medicine in the pathogenesis of arthritis. Not only nucleosides activate the innate immune system accelerating inflammation and bone destruction, but they also are the constitutive elements of blood stasis involved in bone destruction





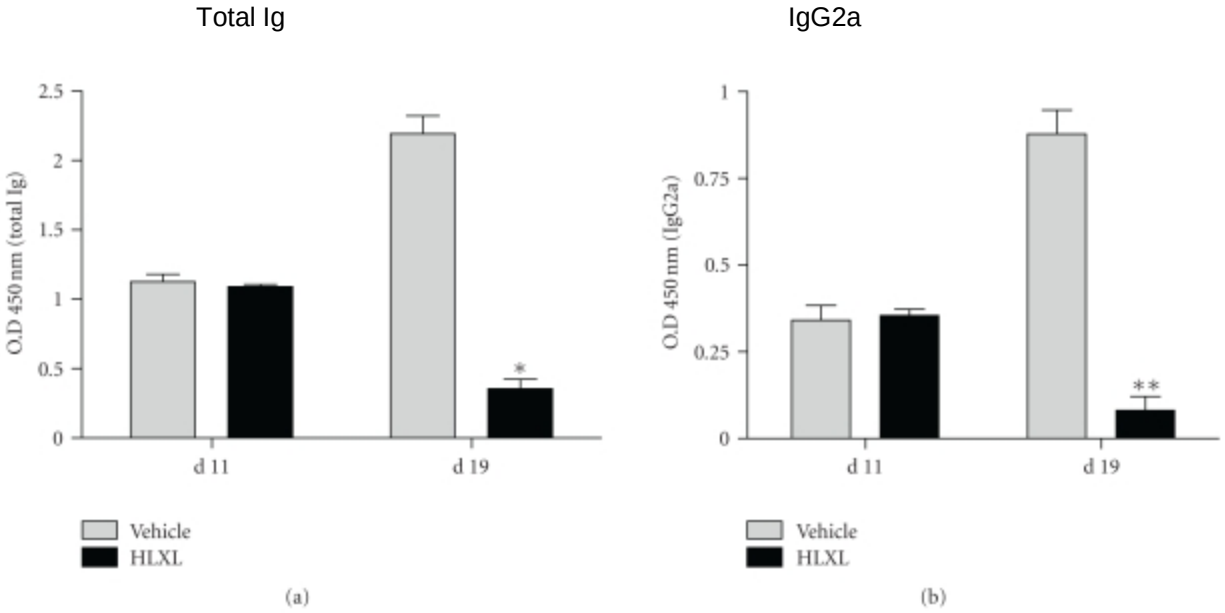
In Vivo Study: Adjuvant Arthritis (AA)



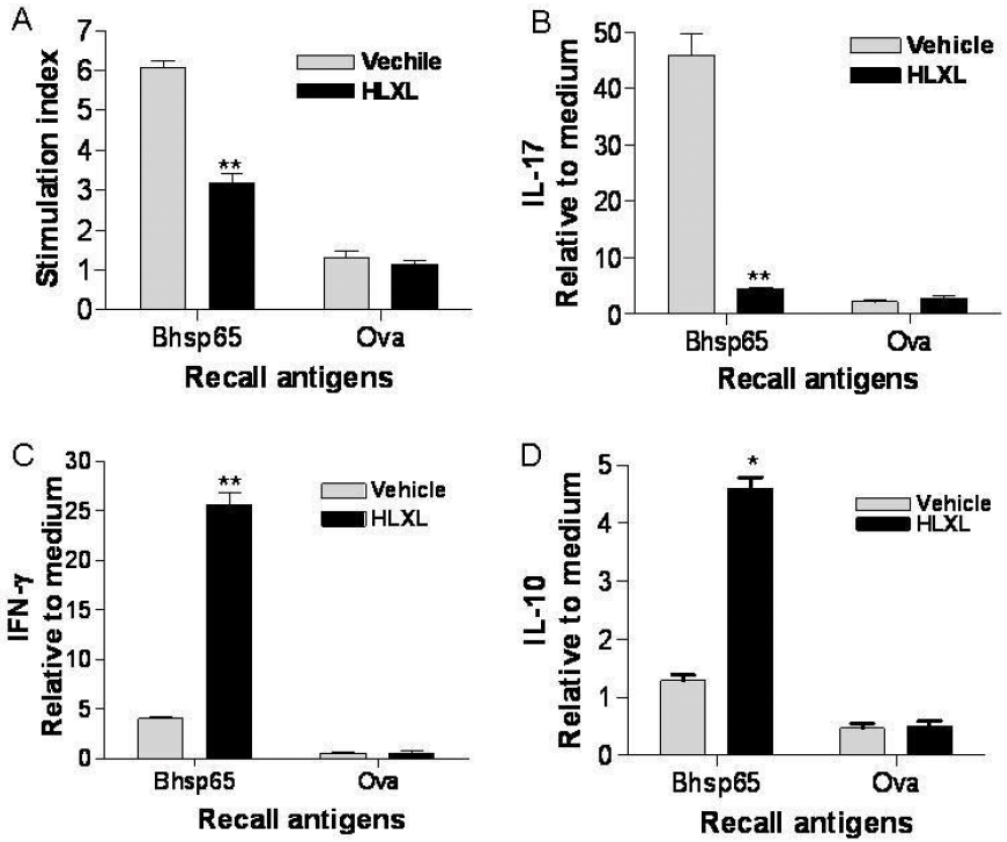
Fore paw Hind paw

AA is an animal model of human arthritis
Induced in the Lewis (LEW) rat (RT-1l) by injection of heat-killed *Mycobacterium tuberculosis* H37Ra.
Arthritic Lewis rats raise T cell response to mycobacterial hsp65 (Bhsp65).
The arthritogenic T helper 1 (Th1) cells are directed against the epitope 177-191/ 180-188 of Bhsp65.

HLXL Inhibited the Antibody Response to Bhsp65 of LEW Rats with AA



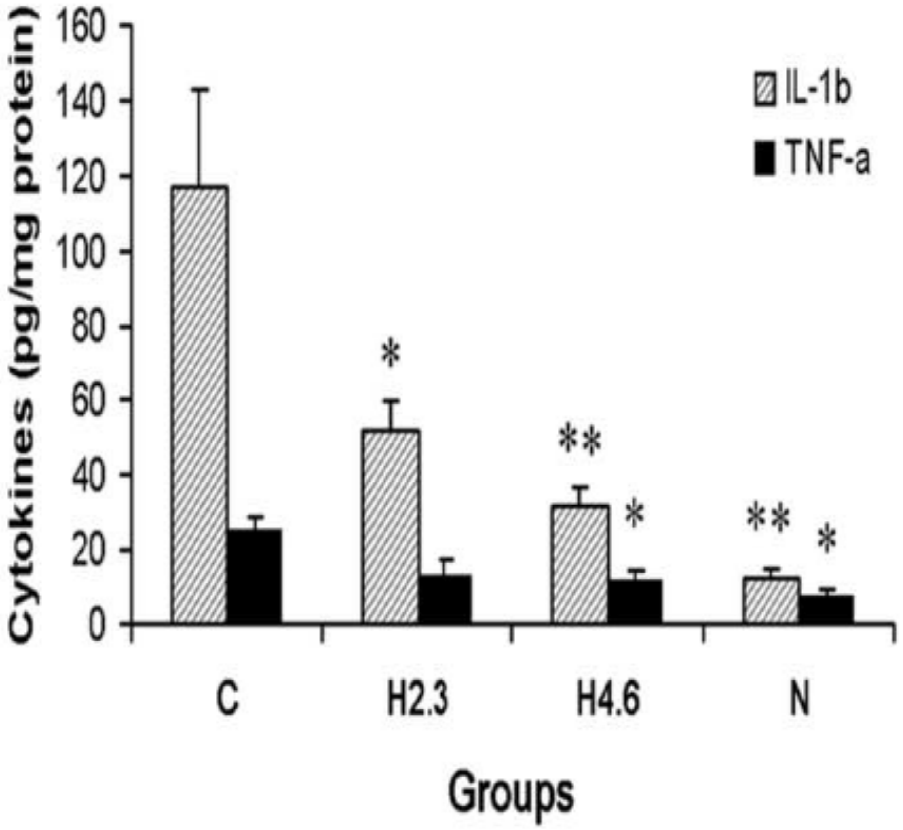
HLXL modulates the T cell proliferative and cytokine responses to Bhsp65 of arthritic LEW rats.



LNC of arthritic rats harvested on d 7 after initiation of the daily feeding of HLXL or water were tested for their T cell proliferative (A) and cytokine response (B-D) to antigenic re-stimulation with Bhsp65 in vitro. Ova served as the control antigen.

Effect of HLXL on IL-1β and TNF-α levels (pg/mg protein, Mean ± S.E.) 25 days post-CFA injection.

Tissue was obtained from four groups of rats: group N (no arthritis + vehicle treatment, n=4), group H4.6 (arthritis + HLXL treatment at 4.60g/kg/day, n=7), group H2.3 (arthritis + HLXL treatment at 2.30g/kg/day n=6), and group C (arthritis + vehicle treatment n= 6). Data showed that both IL-1β and TNF-α increased significantly in local tissue following development of arthritis. However, after HLXL treatment, local tissue IL-1β and TNF-α decreased significantly, *p<0.05 and **p<0.01, compared to the



Cytokines work together to regulate immune system

* Interleukin IL-1β also influence TH1/TH2 immune responsiveness

* IL-10 : Anti-inflammatory cytokine

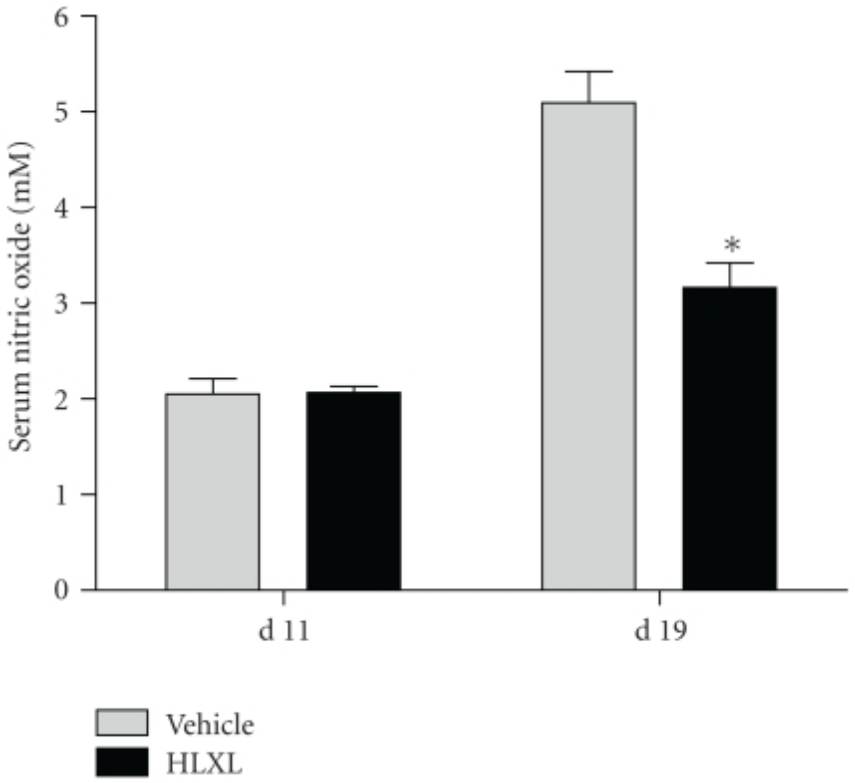
* IL-17 : Pro-inflammatory cytokine

* IFN-γ : Synergies with TNF-α and TNF-β to inhibit many cell types proliferation is also involved in processes of bone growth and inhibits bone resorption by partial inhibition of the formation of osteoclasts

Conclusions

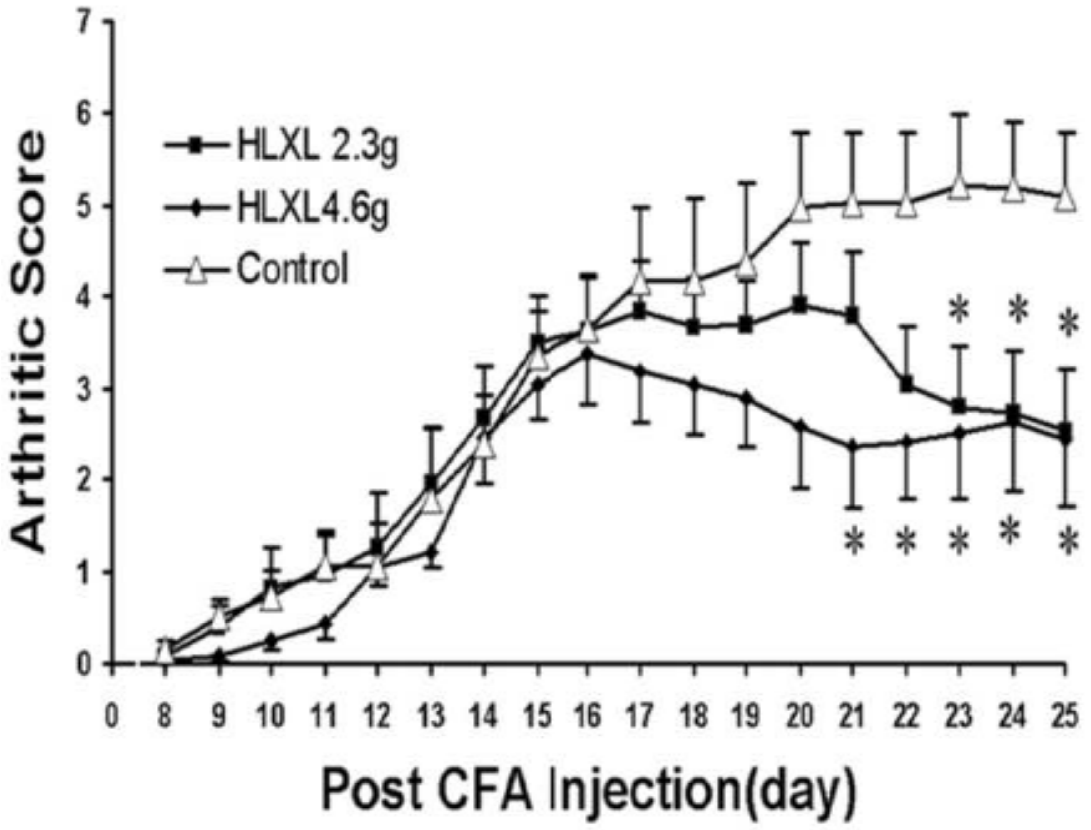
The anti-arthritic activity of HLXL might involve modulation of the antigen-specific T cell response by altering the level of response and/or deviating the cytokine response from a pro-inflammatory (Th1) to an anti-inflammatory type (Th2). This altered T cell response also facilitates the generation of antibodies to BHsp65 that are protective against arthritis.

Effects of HLXL on the Level of serum NO in LEW AA Rats



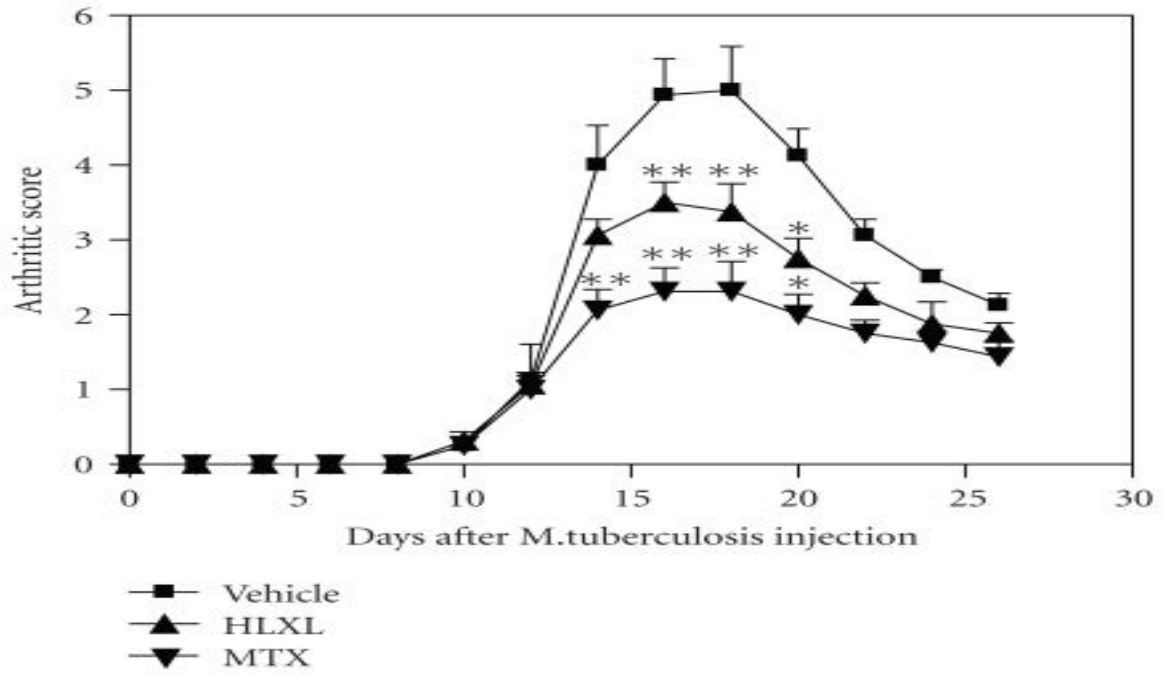
Effect of HLXL on Arthritis in Rats

(Arthritic Scores: Mean ± S.E., n = 8/group)

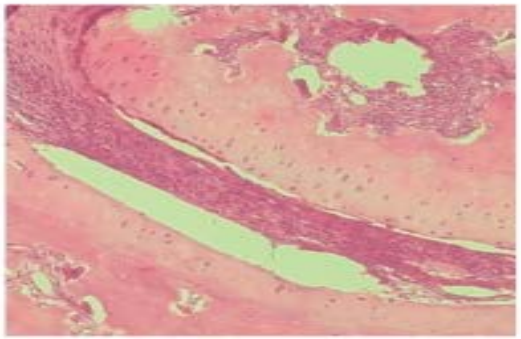


Effect of HLXL on Arthritis in Rats

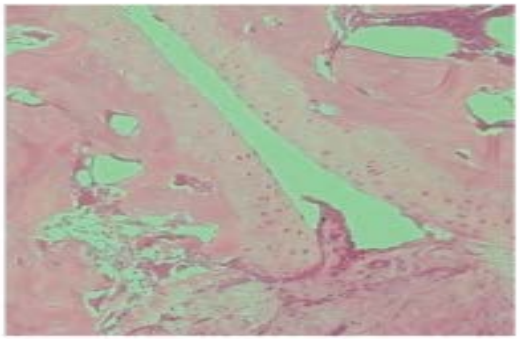
(Arthritic Scores: Mean ± S.E., n = 4/group)



(a)

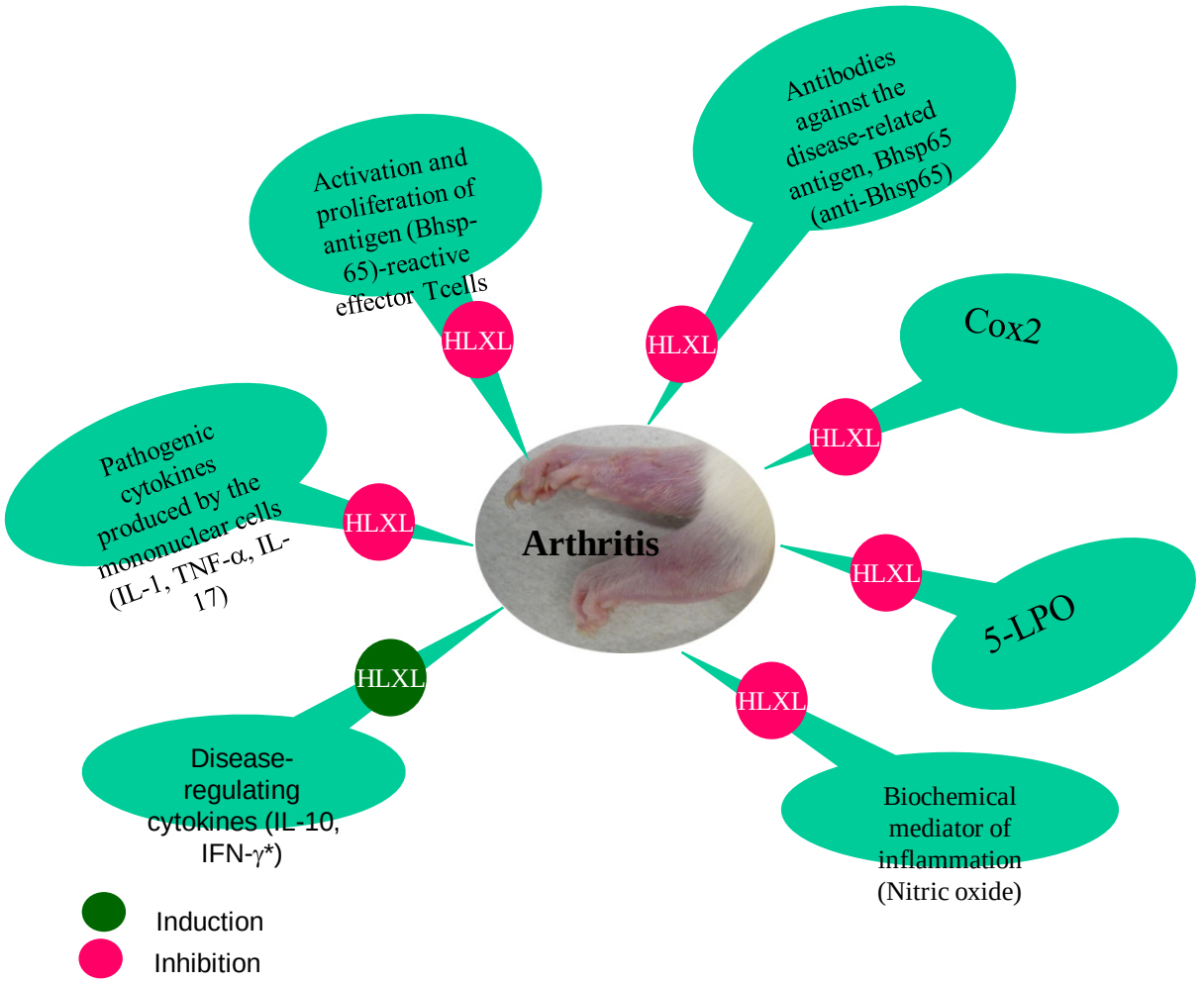


(b)



(c)

HLXL: Mechanism of Actions



Progress to Date: Clinical Study

IRB approval April 2007, first assessment visit May 2007.

13 participants randomized (7 male)
Mean age = 60 (45 – 70) years

100% compliance to assessment visits

100% compliance to dosing

No adverse events

Phase II study (128 Patients)

TCM for Treatment of Human Diseases

1. A large number of bioactive natural products with medium potencies vs highly potent single chemical entity

2. A multi-components and multi-targeted vs single targeted approach

3. Re-discovery the wisdom of traditional Chinese medicine

Summary

- **Autism represents an immunological and inflammatory disorder with definable biomarkers, mainly targeting GI and Brain**
- **Application of clinical measures to address the abnormalities identified by the biomarkers of inflammation immune system and would be a viable approach for treatment of Autism**
- **Evidence based TCM with immune-modulatory and anti-inflammatory activities such as HLXL may have the potential as an alternative treatment for Autism**

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