Metagenics Institute

Modulation of Inflammatory Responses by Select Plant-Derived Ingredients

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Training Objective

- Understand the science behind select plant-derived ingredients
  - Bioavailability
  - Biologic mechanisms of action (MOA)
  - Potential clinical applications

Scientific Takeaways

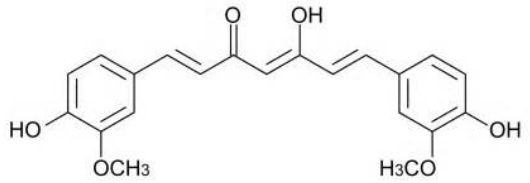
- These plant-derived ingredients are:
  - Highly *bioavailable*
    - Well-absorbed and reaches target tissues
  - Exceptionally *well-characterized*
    - Have defined biologic mechanisms of action (MOA)
  - Known to reduce systemic *inflammation and pain*
    - Via multiple MOAs

Ingredient Overview

Curcumin
Xanthohumol
Boswellia serrata
Ginger

Curcuminoids: Isolated Constituents

- Primary active constituents in turmeric root (*Curcuma longa*)
- Turmeric has culinary and medicinal uses



Curcumin/Turmeric Root

- Family: Zingiberaceae (Ginger family)
- “Orally, turmeric is used for osteoarthritis, rheumatoid arthritis (RA), dyspepsia, abdominal pain, Crohn's disease and ulcerative colitis, coronary artery bypass graft (CABG) surgery, hemorrhage, diarrhea, flatulence, abdominal bloating, loss of appetite, jaundice, hepatitis, Helicobacter pylori (H pylori), peptic ulcers, irritable bowel syndrome, and liver and gallbladder conditions. It is also used for headaches, bronchitis, common cold, respiratory infections, hyperlipidemia, lichen planus, radiation mucositis, fibromyalgia, fatigue, leprosy, fever, amenorrhea, pruritus, surgical recovery, and cancer, including colorectal cancer and prostate cancer. Other uses include depression, Alzheimer's disease, anterior uveitis, diabetes, edema, worms, kidney inflammation, systemic lupus erythematosus (SLE), tuberculosis, cystitis, and joint pain.”

- Therapeutic Research Center (formerly Natural Medicines Comprehensive Database)

Curcumin/Turmeric Root Actions

- **Analgesic**: Reduces pain including neuropathic pain
- **Anti-arthritic**: Reduces joint inflammation, Reduces MMPs (involved in joint destruction)
- **Anti-inflammatory**
- **Antioxidant**

- Other:
  - Gastrointestinal, Respiratory effects, Anti-Alzheimer's, Antidepressant, Anticancer, Antidiabetic, Cardiovascular, Antithrombotic

- Source: Therapeutic Research Center

Standard Curcumin Preparations

- Poorly bioavailable
  - Not well absorbed
  - Animal studies have shown that the majority of curcumin (up to 85%) passes through the GI tract

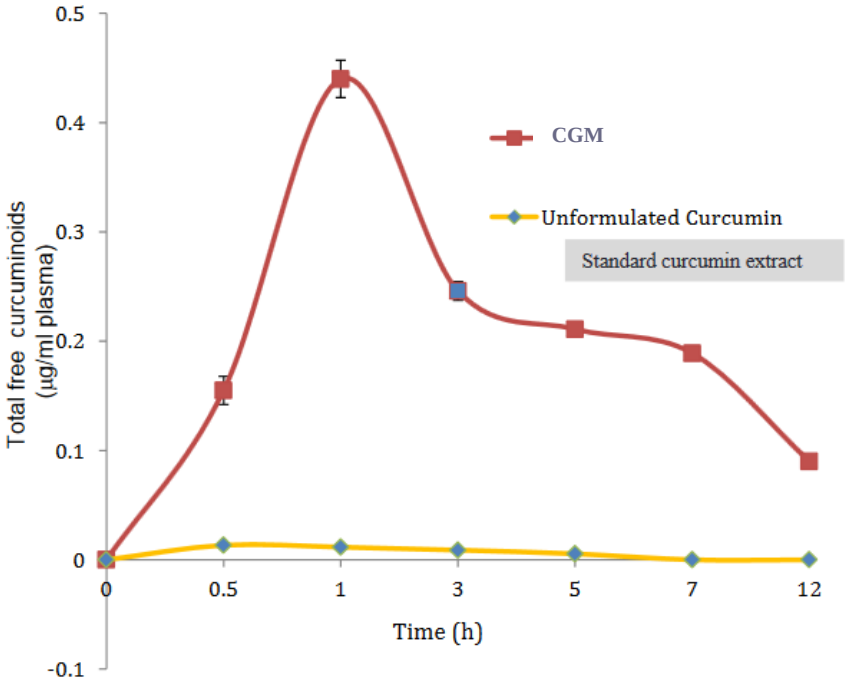
- Absorption improves slightly when consumed with lipids/fat or piperine (in peppercorns)

Curcumin as “CGM”

- Curcumagalactomannoside (CGM)
- Combines curcumin with galactomannan fibers (from fenugreek seeds)
- Patented (but *not* an exclusive ingredient)
- Bioavailability
  - Enhanced absorption of curcuminoids into the bloodstream
  - Exceptional delivery to target tissues

CGM: Human Plasma Levels

- Studies have shown that CGM is exceptionally well-absorbed compared to standard curcumin preparations
- Plasma curcuminoids were assayed after a single 500mg dosage



- Sudheeran P., *J Clin Psychopharmacol.* 2016; 36(3):236-43.

CGM: Human Plasma

- Plasma curcuminoids were also assayed after 30 days of twice daily 500mg administrations

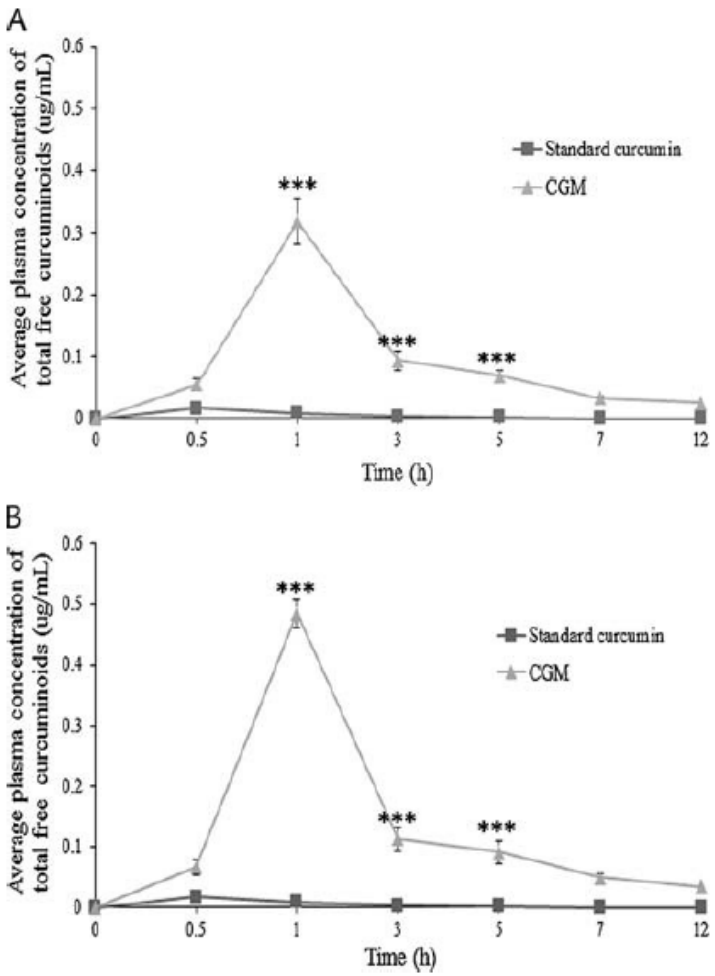


FIGURE 1. Plasma free curcuminoids concentration by time plots after the single-dose (500 mg once per day, A) and repeated-dose (500 mg twice daily for 30 days, B) CGM and standard curcumin consumption. Data are expressed as mean (SD). *** indicates $P < 0.001$ for values of CGM-treated group compared with those of standard curcumin group.

- Sudheeran P., *J Clin Psychopharmacol.* 2016; 36(3):236-43.

CGM: Rat Plasma Levels

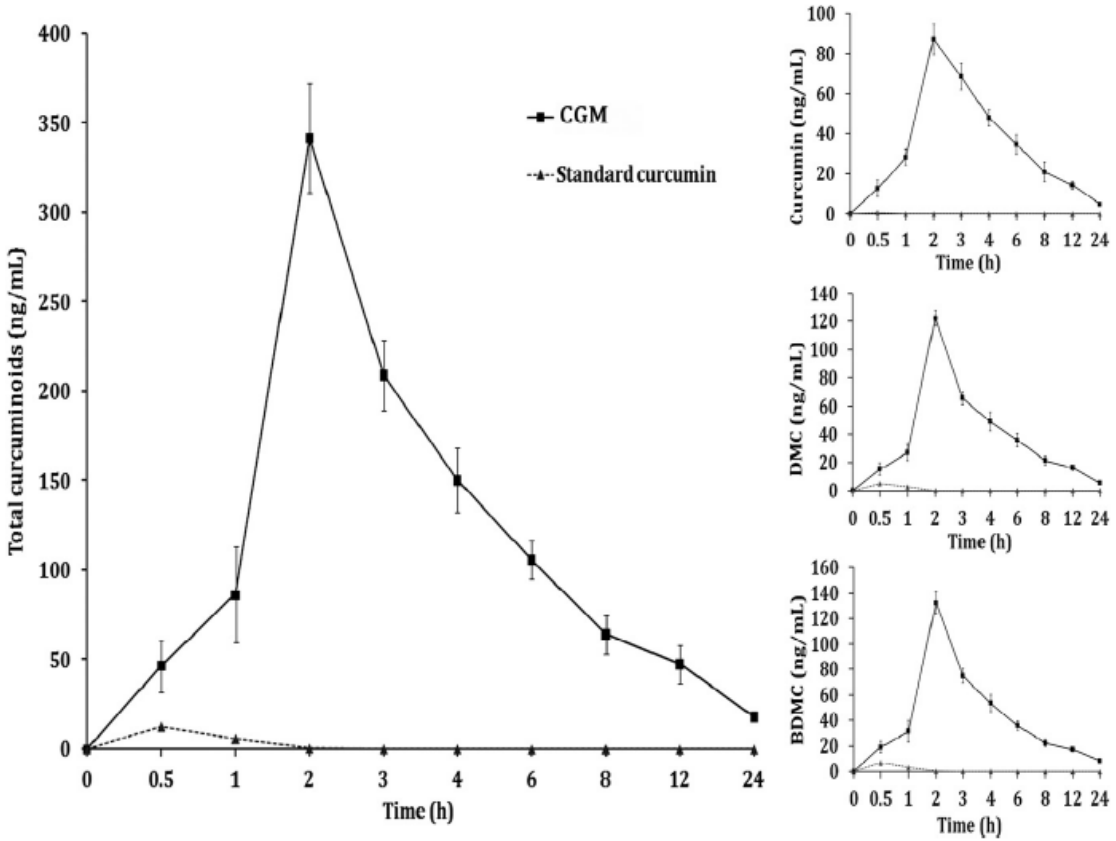


Fig. 2 – Plasma concentration – time curve of total free curcuminoids and individual curcuminoids (curcumin, DMC and BDMC) of Wistar rats orally administered with standard curcumin or CGM. The values are presented as mean ± SD.

IM K., *J of Functional Foods*. 2015;14:215-225

CGM: Rat *Tissue* Levels

Table 1 – Pharmacokinetic parameters of CGM and standard curcumin in various biomatrices of rats up on oral administration.

Sample	Dose (mg/kg)	Tissue	Parameters						Folds increase to standard curcumin	Tissue/plasma ratio
			T_{max} (h)	C_{max} (ng/g)	$T_{1/2}$ (h)	C_{12} (ng/g)	C_{24} (ng/g)	AUC (ng/g-h)		
Standard curcumin	200	Plasma	0.5	12.52 ± 4.16	0.80	n.d.	n.d.	70.18	–	–
		Liver	1.0	9.65 ± 5.08	1.50	n.d.	n.d.	7.77	–	0.77
		Kidney	1.0	6.89 ± 3.13	1.00	n.d.	n.d.	12.21	–	0.55
		Heart	0.5	5.58 ± 2.21	1.40	n.d.	n.d.	8.70	–	0.44
		Spleen	0.5	5.70 ± 2.62	1.50	n.d.	n.d.	9.87	–	0.45
		Brain	1.0	1.40 ± 0.80	1.50	n.d.	n.d.	2.41	–	0.11
		Intestine	0.5	140,045.32 ± 56,000	2.00	1343.00 ± 210.00	54.00 ± 11.00	351,277	–	11,185.73
CGM	200	Plasma	2.0	341.57 ± 30.88*	3.70	47.61 ± 11.00*	18.02 ± 0.01*	1758.00*	25.05	–
		Liver	2.0	445.52 ± 83.00*	3.00	n.d.	n.d.	867.60*	111.66	1.30*
		Kidney	2.0	240.10 ± 47.25*	2.75	n.d.	n.d.	882.20*	72.25	0.70*
		Heart	2.0	391.76 ± 102.50*	3.3	n.d.	n.d.	476.90*	54.82	1.14*
		Spleen	2.0	229.72 ± 42.20*	3.25	n.d.	n.d.	543.00*	55.02	0.67*
		Brain	2.0	343.00 ± 64.70*	3.40	n.d.	n.d.	838.50*	347.93	1.00*
		Intestine	0.5	462,412.51 ± 88,000*	7.00	135,377.76 ± 41,000*	53,006.15 ± 9700*	4396,000*	12.51	1353.79*

AUC, area under the concentration-time curve; C_{max}, maximum tissue concentration; T_{max}, time to reach C_{max}; $T_{1/2}$, half-life; the fold increase is calculated as $AUC_{CurQfen}/AUC_{Standard\ curcumin}$. The values are given as mean ± SD (n = 3), where * denotes $p < 0.001$, when values of CGM are compared with the values of standard curcumin.

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CGM: Rat Brain Levels

- CGM effectively crosses the blood-brain barrier (BBB)

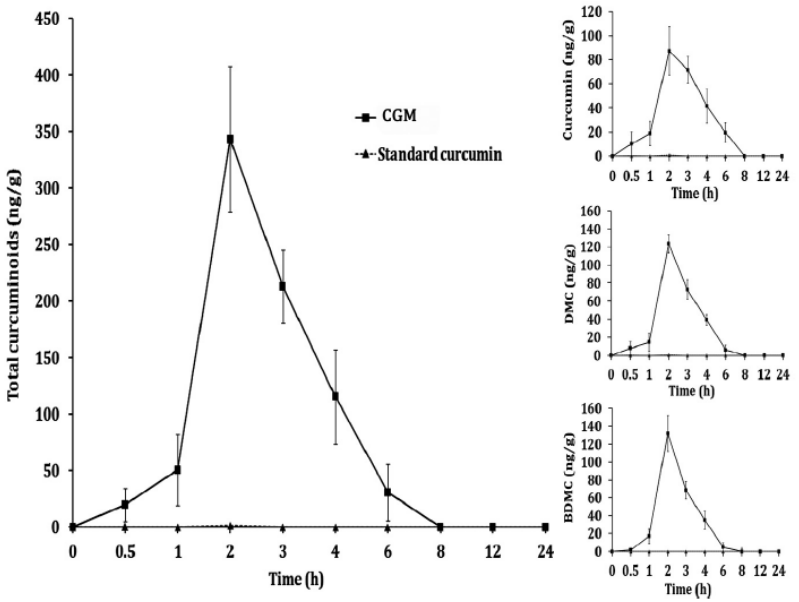

Fig. 3 – Brain concentration – time curve of total free curcuminoids and individual curcuminoids (curcumin, DMC and BDMC) of Wistar rats orally administered with standard curcumin or CGM. The values are presented as mean ± SD.

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Curcumin/CGM Key Takeaways

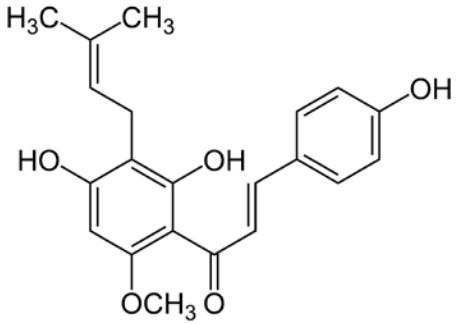
- Most pertinent therapeutic actions:
  - **Analgesic**
  - **Anti-arthritic**
  - **Anti-inflammatory**
  - **Antioxidant**

- CGM is highly bioavailable
  - Readily enters the blood *and* tissues

Xanthohumol: An Isolated Constituent

- Xanthohumol is one of many active constituents in hops flowers (*Humulus lupulus*)

- Hops flowers have commercial (used to flavor and preserve beer) and medicinal uses



Hops Flowers

- Family: Cannabaceae (same family as Cannabis)
- “Orally, hops are used for anxiety, insomnia and other sleep disorders, restlessness, tension, excitability, attention deficit-hyperactivity disorder (ADHD), nervousness, and irritability. They are also used orally as an appetite stimulant, diuretic, a bitter tonic, to stimulate lactation, and for indigestion. Other oral uses include prostate cancer, breast cancer, ovarian cancer, menopausal symptoms, hyperlipidemia, tuberculosis, cystitis, intestinal cramps, mucous colitis, neuralgia, and priapism..”
- Source: Therapeutic Research Center

Hops Flower Actions

- **Anti-arthritic**
- **Anti-inflammatory**
- **Antioxidant**

- Other:
  - Anticancer, Cardiovascular risk reduction, Sedative, Nervine, Treats menopausal symptoms

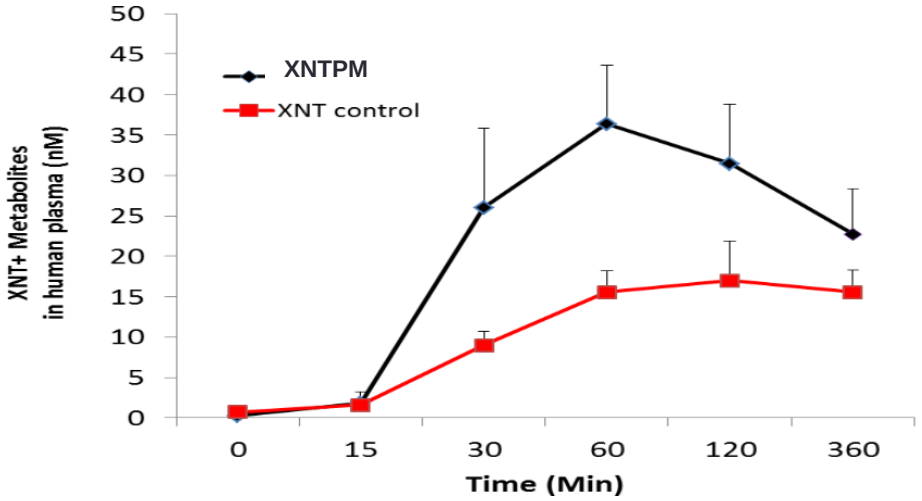
- Source: Therapeutic Research Center

Xanthohumol as “XNTPM”

- Xanthohumol bound to a protein matrix to increase bioavailability and stability
- *Is* an exclusive ingredient
- Is a Hops extract standardized to 2.5% xanthohumol

Xanthohumol Bioavailability

- Xanthohumol is generally not well-absorbed
- However, a study conducted at the FMRC showed that XNTPM had 81% higher bioavailability compared to a standard xanthohumol



Xanthohumol/XNTPM Key Takeaways

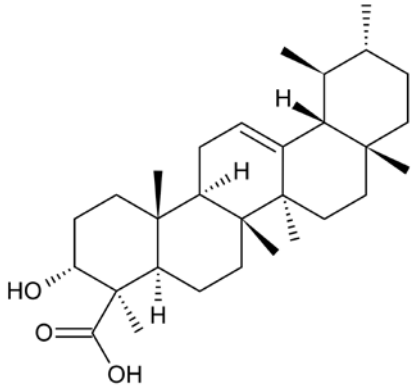
- Most pertinent therapeutic actions:
  - **Anti-arthritic**
  - **Anti-inflammatory**
  - **Antioxidant**

- XNTPM
  - Standardized to 2.5% xanthohumol
  - Better bioavailability

Boswellia serrata Extract

- Also known as Indian Frankincense
- Is a “gum resin” from the *Boswellia serrata* tree

- Extract contains a few active constituents
  - Beta boswellic acid (#1)
  - Alpha boswellic acids
  - Essential oils
  - Flavonoids - Quercetin



Boswellia serrata Extract

- “Orally, boswellia is used for brain injury, osteoarthritis, rheumatoid arthritis (RA), rheumatism, bursitis, and tendonitis. Other uses include ulcerative colitis, collagenous colitis, Crohn's disease, and abdominal pain. It is used for asthma, allergic rhinitis, sore throat, syphilis, painful menstruation, pimples, bruises, headache, diabetes, and cancer. It is also used as a stimulant, respiratory antiseptic, diuretic, and for stimulating menstrual flow.”

- Source: Therapeutic Research Center

Boswellia serrata Extract Actions

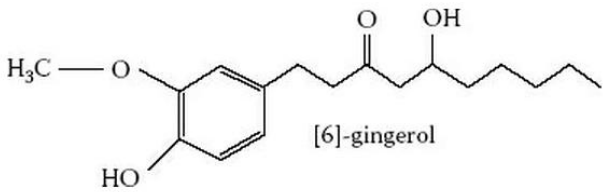
- **Analgesic**: Reduces pain
- **Anti-arthritic**: Inhibits 5-LOX; Decreases cartilage damage
- **Anti-inflammatory/Immunomodulatory effects**
- **Antioxidant**

- Other:
  - Anticancer, Anti-asthma effects, Anti-microbial

- Sources: Therapeutic Research Center, Herbal Medicine from the Heart of the Earth

Ginger Root Extract

- Contains several constituents
  - Gingerol
  - Gingerdione
  - Shogaol
  - Sesquiterpene and monoterpene volatile oils
- Culinary and medicinal uses



Ginger Root

- Family: Zingiberaceae (Ginger family)
- “Orally, ginger is used for motion sickness, morning sickness, colic, diarrhea, dyspepsia, flatulence, irritable bowel syndrome, chemotherapy-induced nausea, antiretroviral-induced nausea and vomiting, rheumatoid arthritis (RA), osteoarthritis, loss of appetite, post-surgical nausea and vomiting, dysmenorrhea, migraine headache, and for discontinuing selective serotonin reuptake inhibitor (SSRI) drug therapy. It is also used orally for anorexia, upper respiratory tract infections, cough, respiratory distress, bronchitis, diabetes, as a galactagogue, diaphoretic, and diuretic; and for treating stomachache, nausea, cholera, and bleeding. Fresh ginger is used orally for treating acute bacterial dysentery, baldness, malaria, orchitis, poisonous snake bites, and toothaches. Dried ginger is used for chest pain, low back pain, and stomach pain.”

- Source: Therapeutic Research Center

Ginger Root Actions

- **Analgesic**
- **Anti-arthritic**
- **Anti-inflammatory**
- **Antioxidant**

- Other:
  - Anti-emetic, Anti-diabetic, Antibacterial, Antifungal, Cardiovascular

- Source: Therapeutic Research Center

Boswellia serrata & Ginger Key Takeaways

- Most pertinent therapeutic actions:
  - **Analgesic**
  - **Anti-arthritic**
  - **Anti-inflammatory**
  - **Anti-oxidant**

Biochemistry & Pharmacology Conventions

(Agonists and Antagonists)

Basic Biochemical Reaction

Substrate —Enzyme→ Product

For example:

Arachidonic acid —COX-2→ PGE_2

Pharmacology Basics

- Pharmacologic actions
  - **Agonists** – *Cause* an action
    - Activate receptors to produce a biological response
  - **Antagonists** – *Block/Inhibit/Reduce* an action
    - Reduce biological responses often by binding to receptors

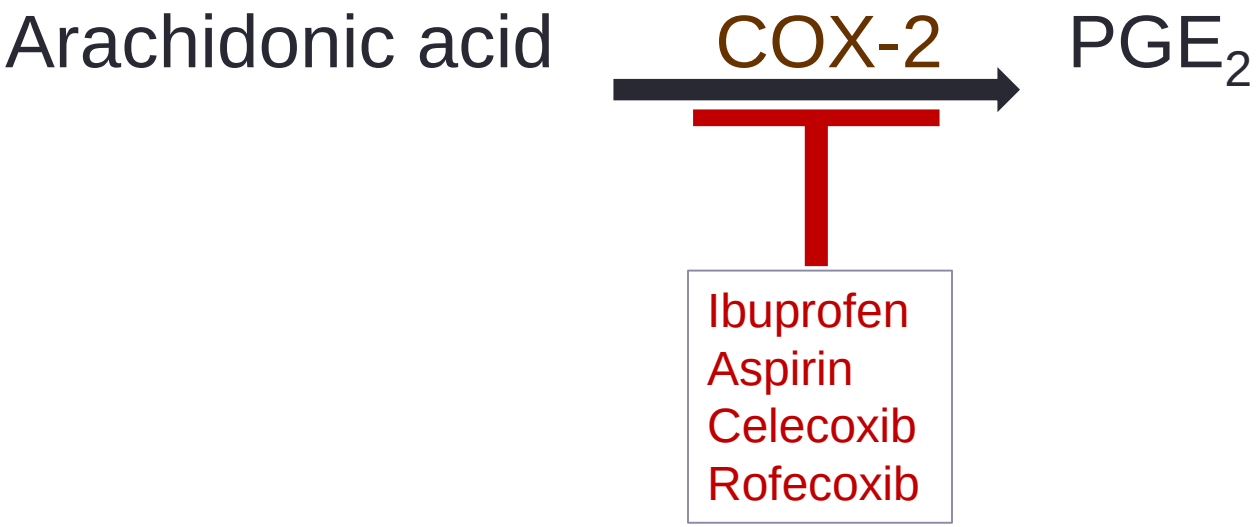
- These actions can be produced by
  - Synthetic or natural compounds
  - Pharmaceuticals, nutraceuticals, vitamins, plant-derived constituents (including many that are found in foods and culinary herbs)

Antagonists

- Antagonist example:

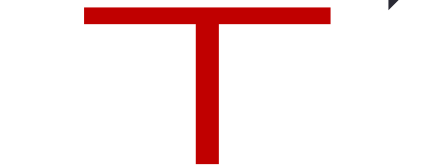
Ibuprofen, an NSAID, is well-known COX-2 inhibitor

Antagonists



Antagonists

Arachidonic acid → COX-2 → PGE_2



- Ibuprofen
- Aspirin
- Celecoxib
- Rofecoxib
- Curcumin
- Xanthohumol
- Boswellic acids
- Ginger extract

Inflammation & Pain Pathways

Mechanisms of Action (MOAs)

Key Mediators of Inflammation & Pain

- Enzymes
  - Phospholipase A2 (PLA2)
  - Cyclooxygenase (COX-1 and COX-2)
  - Lipoxygenase (LOX)
- Gene Expression Regulators
  - Nuclear factor-kappa B (NFkB)
- Pro-inflammatory cytokines
  - TNFα
  - IL-1β
  - IL-6
  - IL-12
- Chemokines
  - Neutrophil chemotactic factor (CXCL8, aka IL-8)
  - Monocyte chemoattractant protein 1 (MCP-1)
  - Interferon-γ activated protein (IP-10)

Key Mediators of Inflammation & Pain *Modulated* by Curcumin, Xanthohumol, Boswellic acids, and Ginger

- Enzymes
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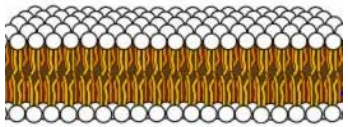
ALL are interfered with by:
Curcumin
Xanthohumol
Boswellic acids
Ginger

Mechanism of Action (MOA) Summary

	Curcumin	Xanthohumol	*Boswellia serrata*	Ginger
Inhibition of pro-inflammatory cytokines, chemokines, and transcription factors associated with inflammation and pain	✓ Inhibits NFkB ✓ Reduces serum levels of: TNFα IL-1β[2] IL-6 MCP-1 ✓ Diminishes chondrocyte production of CXCL8 (IL-8)	✓ Inhibits NFkB ✓ Reduces WBC production of: TNFα IL-12 MCP-1	✓ Inhibits NFkB	✓ Inhibits NFkB ✓ Diminishes synoviocyte production of IP-10
Inhibition of enzymes and prostaglandins associated with inflammation and pain	✓ Inhibits PLA2 ✓ Inhibits COX-2 ✓ Inhibits 5-LOX	✓ Inhibits COX-1 & COX-2	✓ Inhibits COX-1 & COX-2 ✓ Inhibits 5-LOX	✓ Inhibits PLA2 ✓ Inhibits COX-1 & COX-2 ✓ Inhibits LOX

Inflammation & Pain: Key Enzymes

- **Phospholipase A2 (PLA2)**
  - Liberates arachidonic acid (**ARA**) from the cell membrane



  - ARA is then available as a substrate for **COX-1**, **COX-2**, and **LOX** (as well as other enzymes)

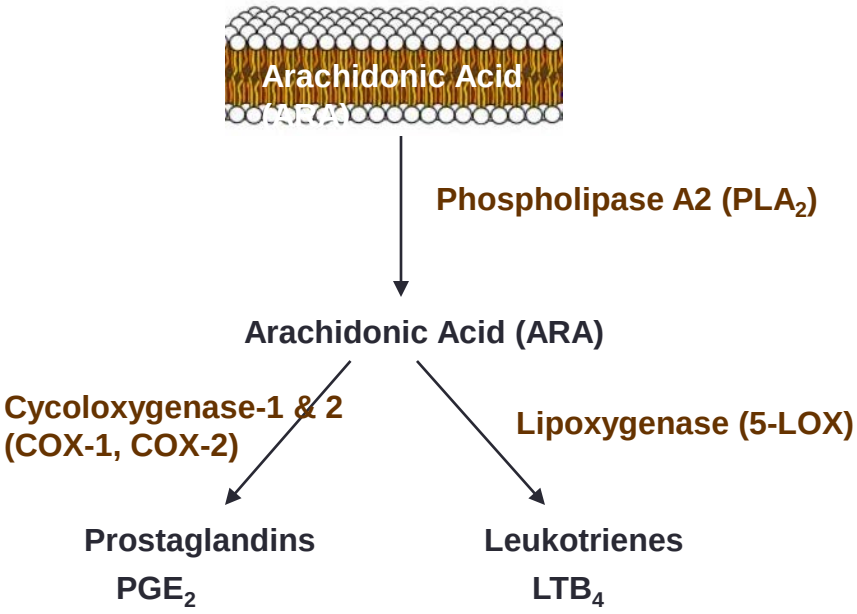
Inflammation & Pain: Key Enzymes

- **Cyclooxygenase (COX-1 and COX-2)**
  - Converts arachidonic acid (AA) to prostaglandins (including PGE_2)
  - **PGE_2**
    - Increases *pain perception*
    - Contributes to the *destruction of cartilage* in arthritic joints in both **rheumatoid arthritis (RA)** and **osteoarthritis (OA)**

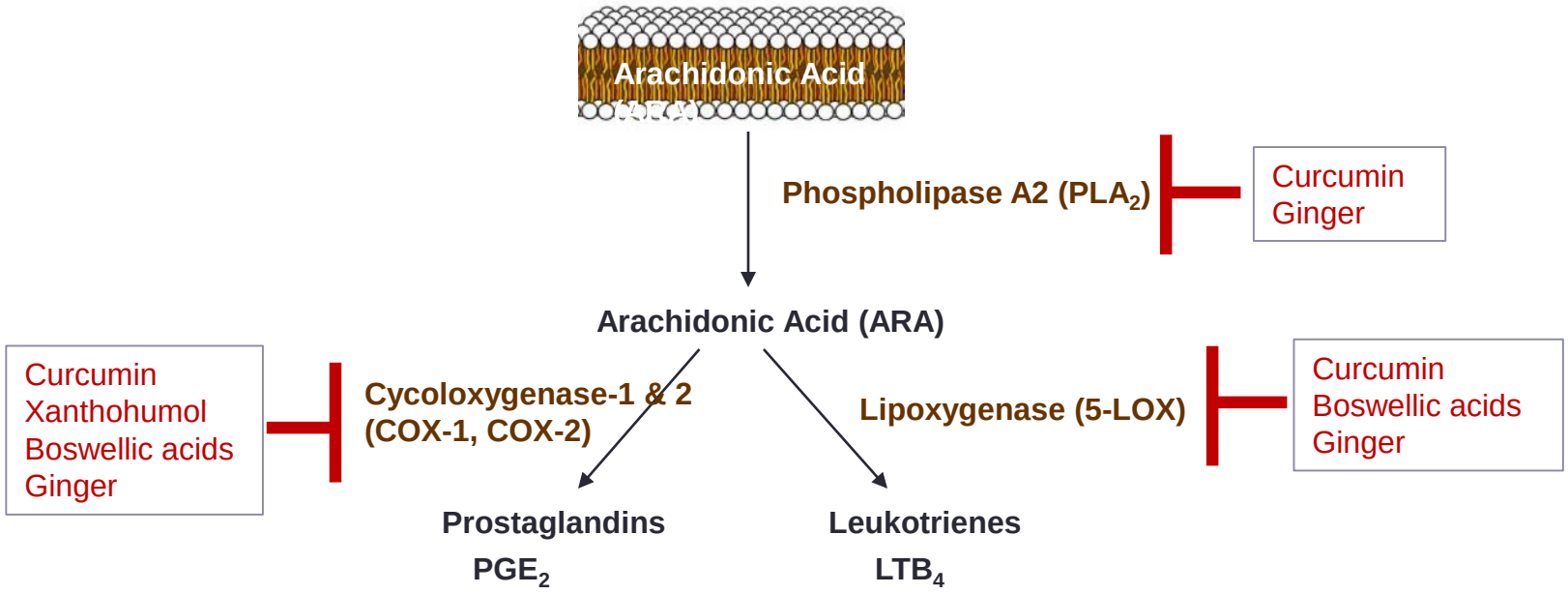
Inflammation & Pain: Key Enzymes

- **Lipoxygenase (LOX)**
  - Converts arachidonic acid (AA) to leukotrienes (including LTB4)
  - Overproduction of **leukotrienes** plays a role in inflammatory conditions like **asthma** and **allergic rhinitis**
  - Commonly used LOX inhibitors include medications used as analgesics for **OA** and **RA**, and treatments for **asthma**

Inflammation & Pain: Key Enzymes



Inflammation & Pain: Key Enzymes



Inflammation & Pain: Key Regulator of Gene Expression

- Nuclear factor-kappa B (NFkB)
  - Protein complex that controls DNA transcription/genetic expression
  - When activated, controls/regulates the expression of ~500 genes including:
    - Pro-inflammatory enzymes - PLA2, COX-1, COX-2, LOX
    - Pro-inflammatory cytokines - IL-1β, IL-6, IL-12, TNFα
    - Chemokines – CXCL8 (aka IL-8), MCP-1

    - For more go to: http://www.bu.edu/nf-kb/gene-resources/target-genes/

Nuclear factor-kappa B (NFkB)

Upstream **NFkB (Inactive)**

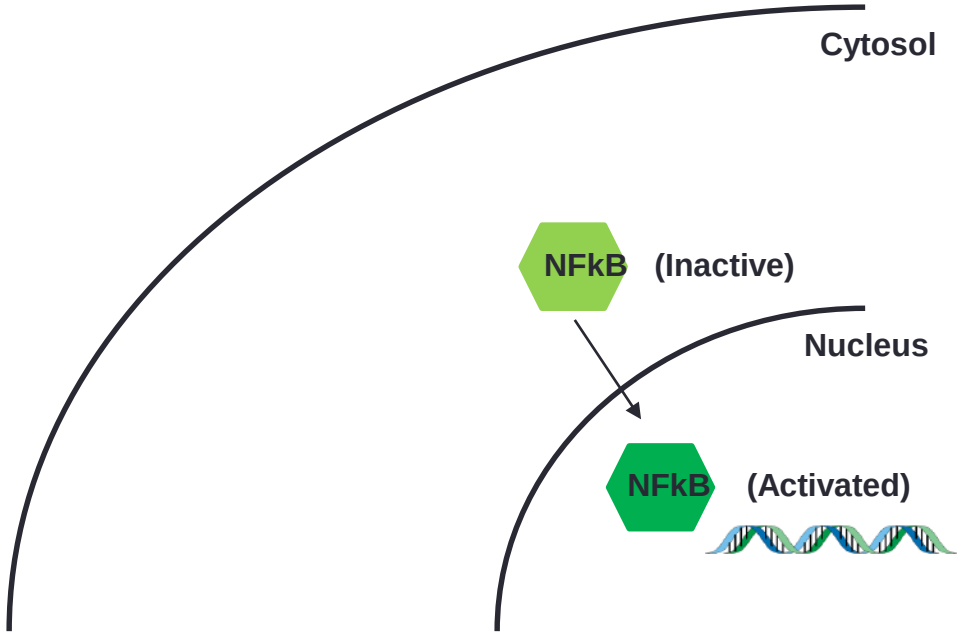
↓

NFkB (Activated)

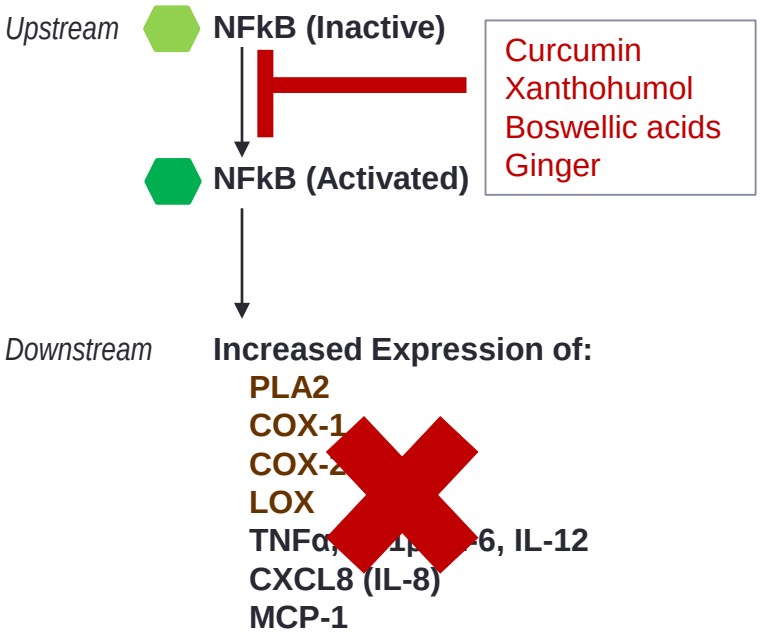
↓

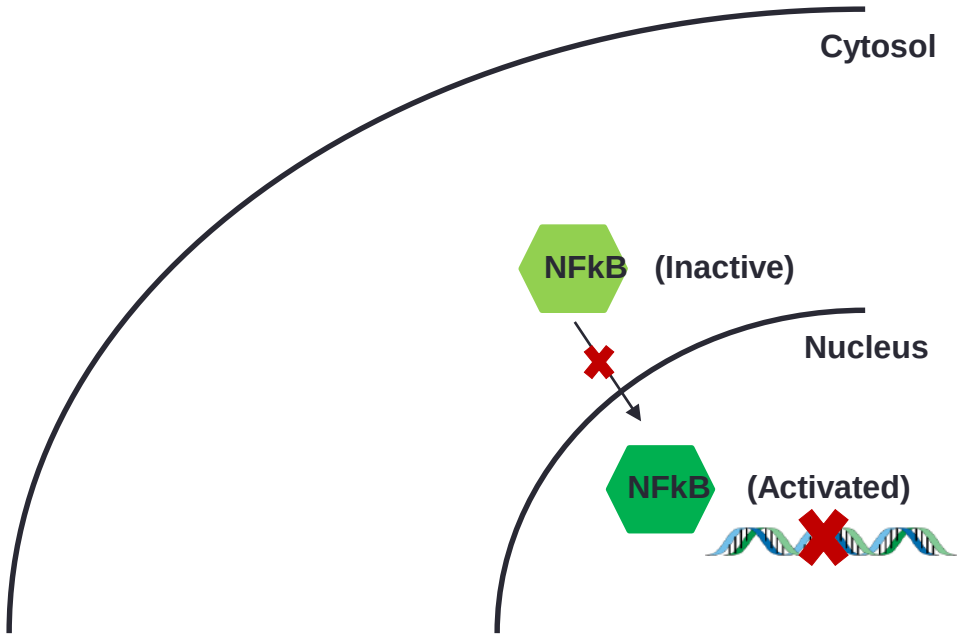
Downstream **Increased Expression of:**

- **PLA2**
- **COX-1**
- **COX-2**
- **LOX**
- **TNFα, IL-1β, IL-6, IL-12**
- **CXCL8 (IL-8)**
- **MCP-1**



Nuclear factor-kappa B (NFkB)

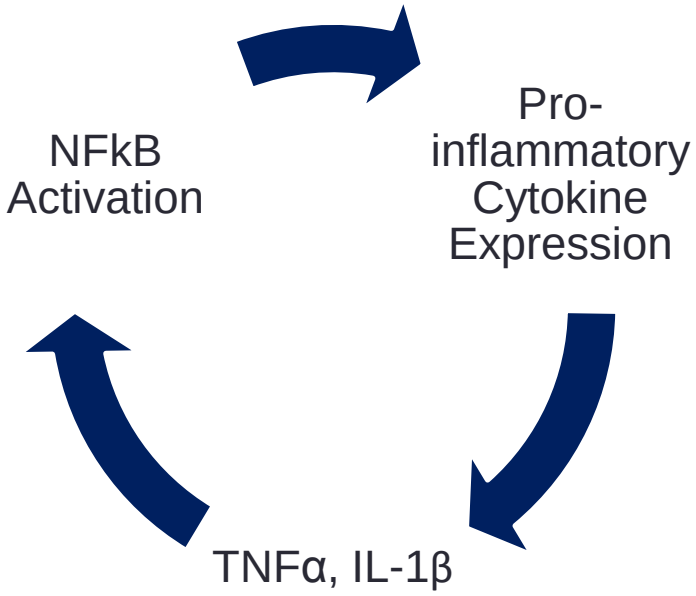




Inflammation & Pain: NFkB & Pro-inflammatory Cytokines

- Not only does NFkB increase the *expression* of pro-inflammatory cytokines

- Some pro-inflammatory cytokines increase the *activation* of NFkB
  - TNFα, IL-1β



TNFα Activates NFkB

Upstream

TNFα

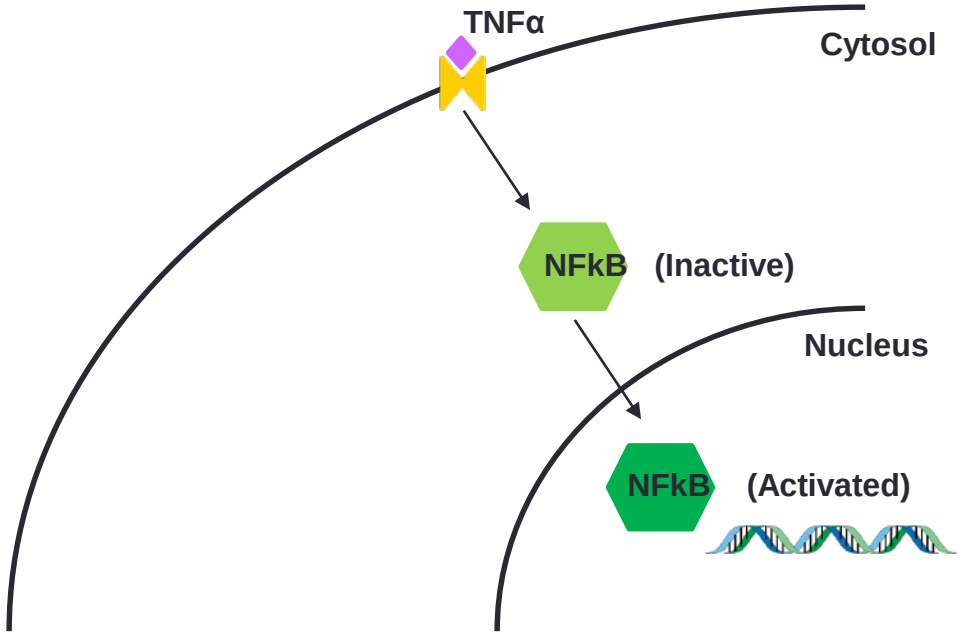
NFkB (Inactive)

NFkB (Activated)

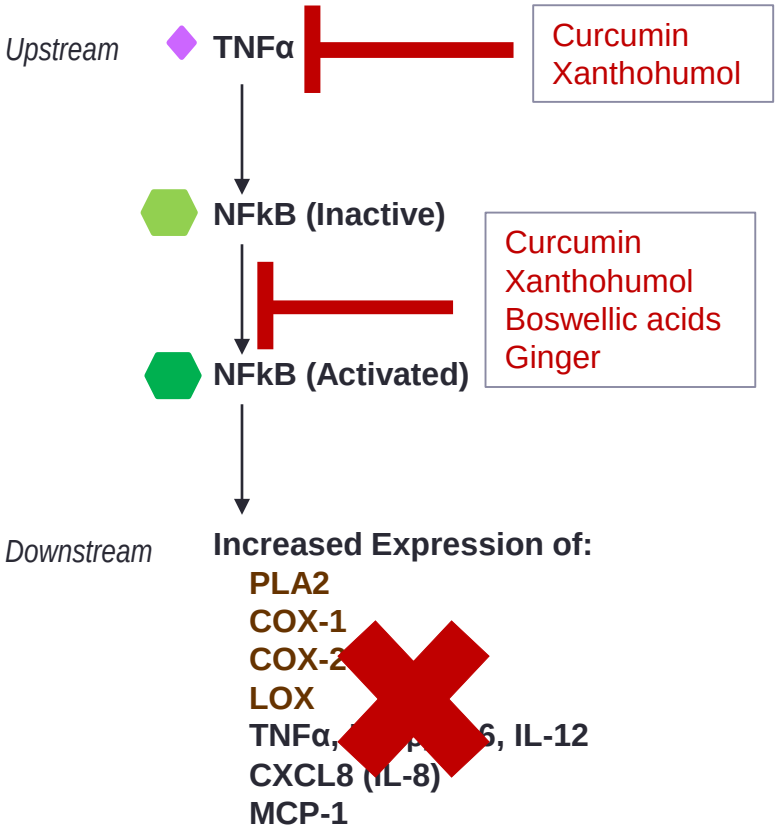
Downstream

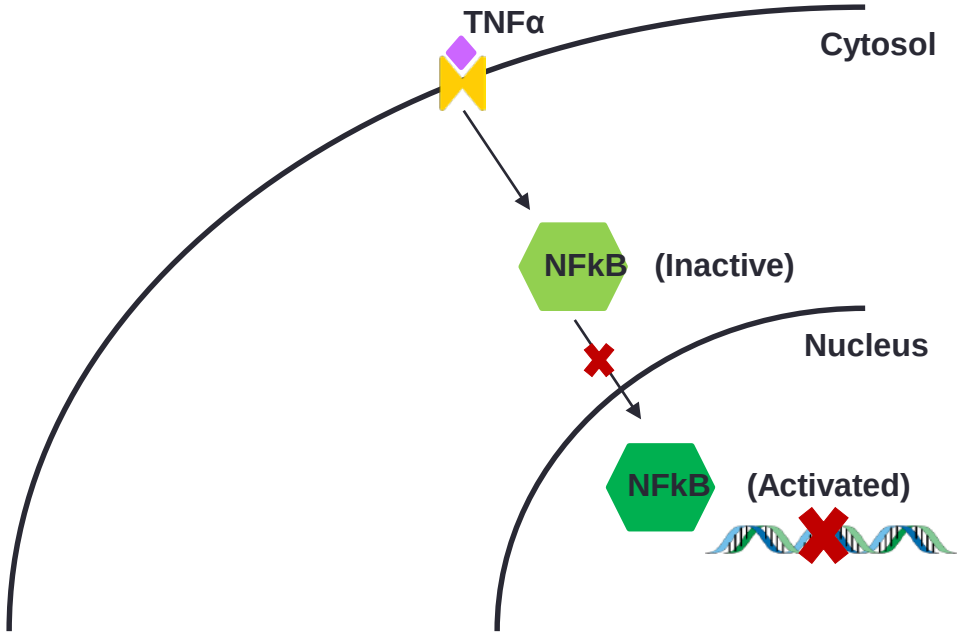
Increased Expression of:

- **PLA2**
- **COX-1**
- **COX-2**
- **LOX**
- **TNFα, IL-1β, IL-6, IL-12**
- **CXCL8 (IL-8)**
- **MCP-1**



Curcumin and Xanthohumol Reduce TNFα Expression





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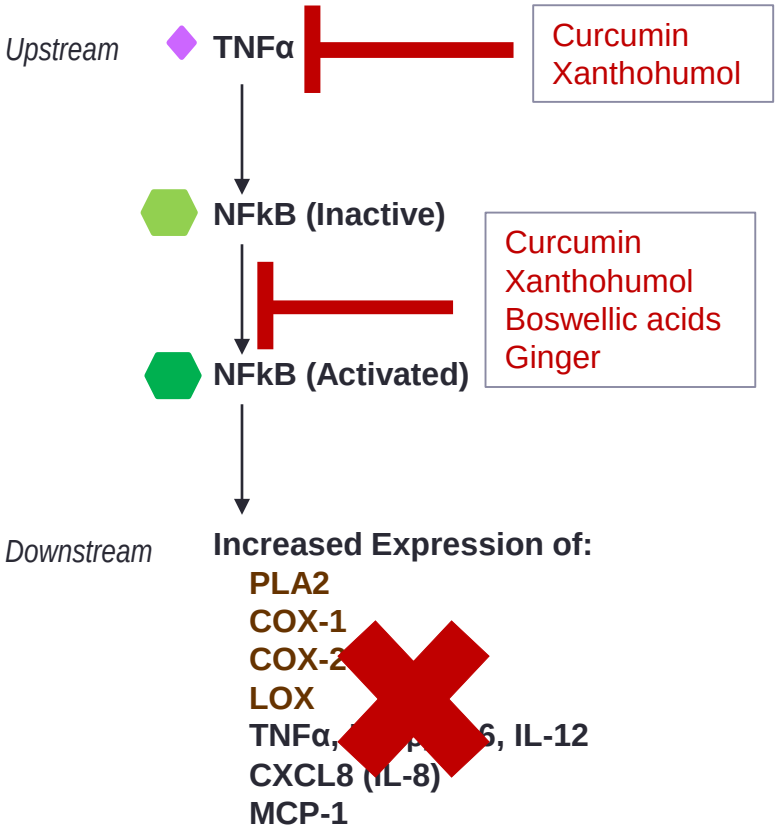
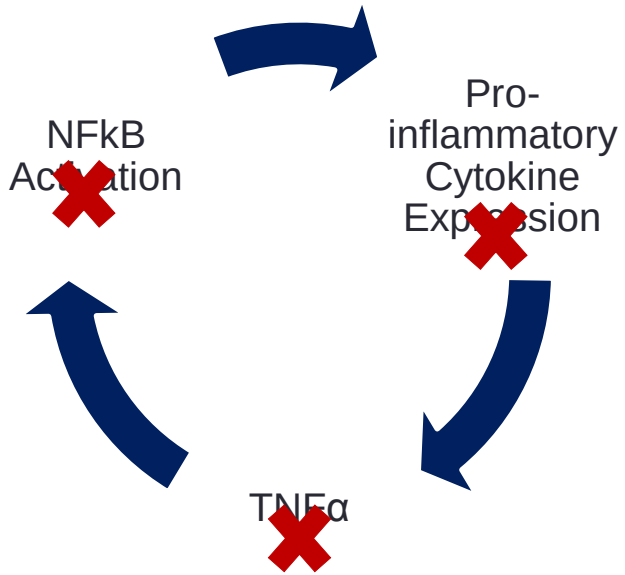




Inflammation & Pain: Chemokines

- Inflammatory chemokines
  - Chemokine ligand 8 (CXCL8, aka IL-8)
  - Monocyte chemoattractant protein 1 (MCP-1)
  - Interferon-γ activated protein (IP-10)

- Recruit white blood cells to local sites of inflammation
  - Promote *joint pathology* in patients with arthritis

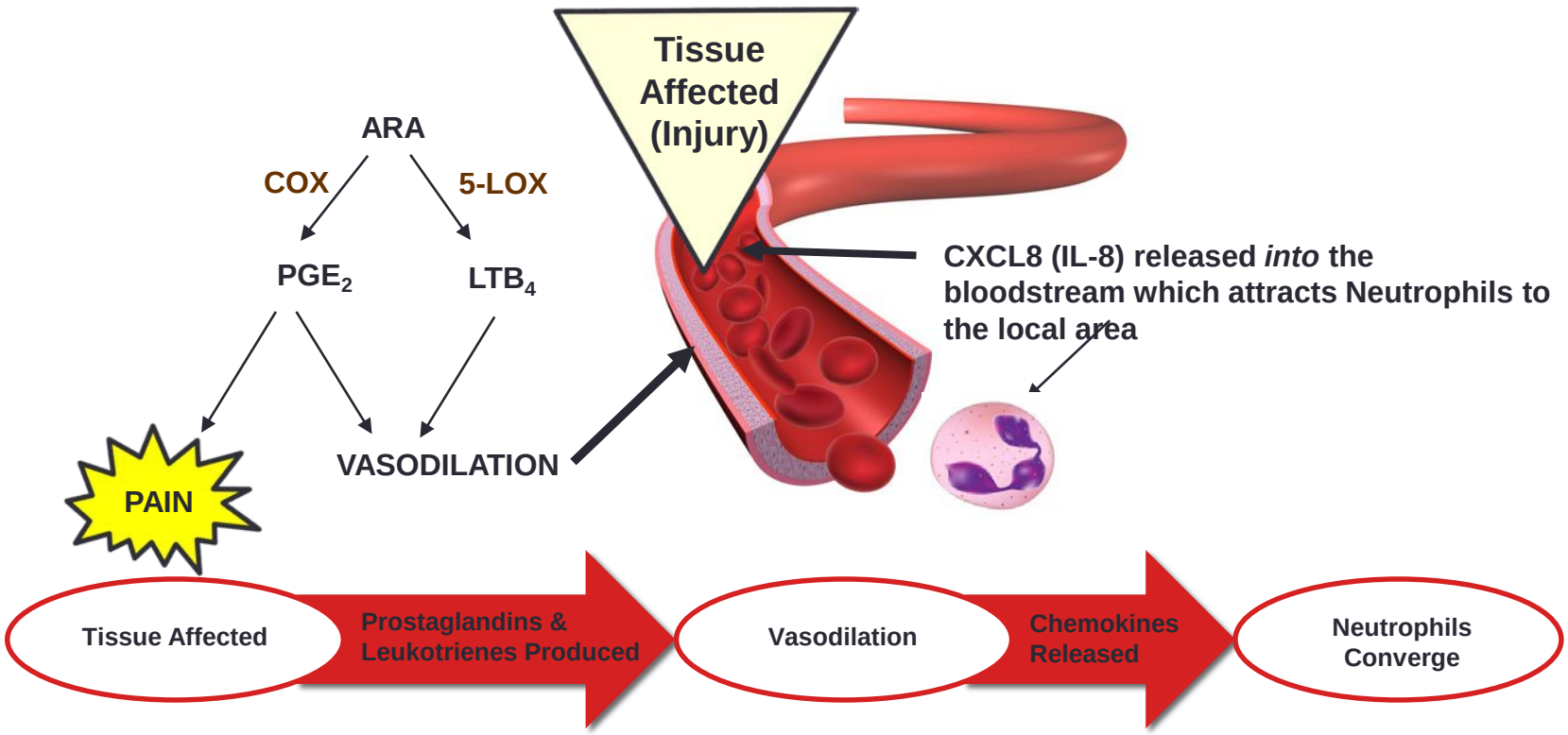
Inflammation & Pain: Chemokines

- Curcumin
  - Reduces serum levels of MCP-1
  - Reduces chondrocyte production of CXCL8

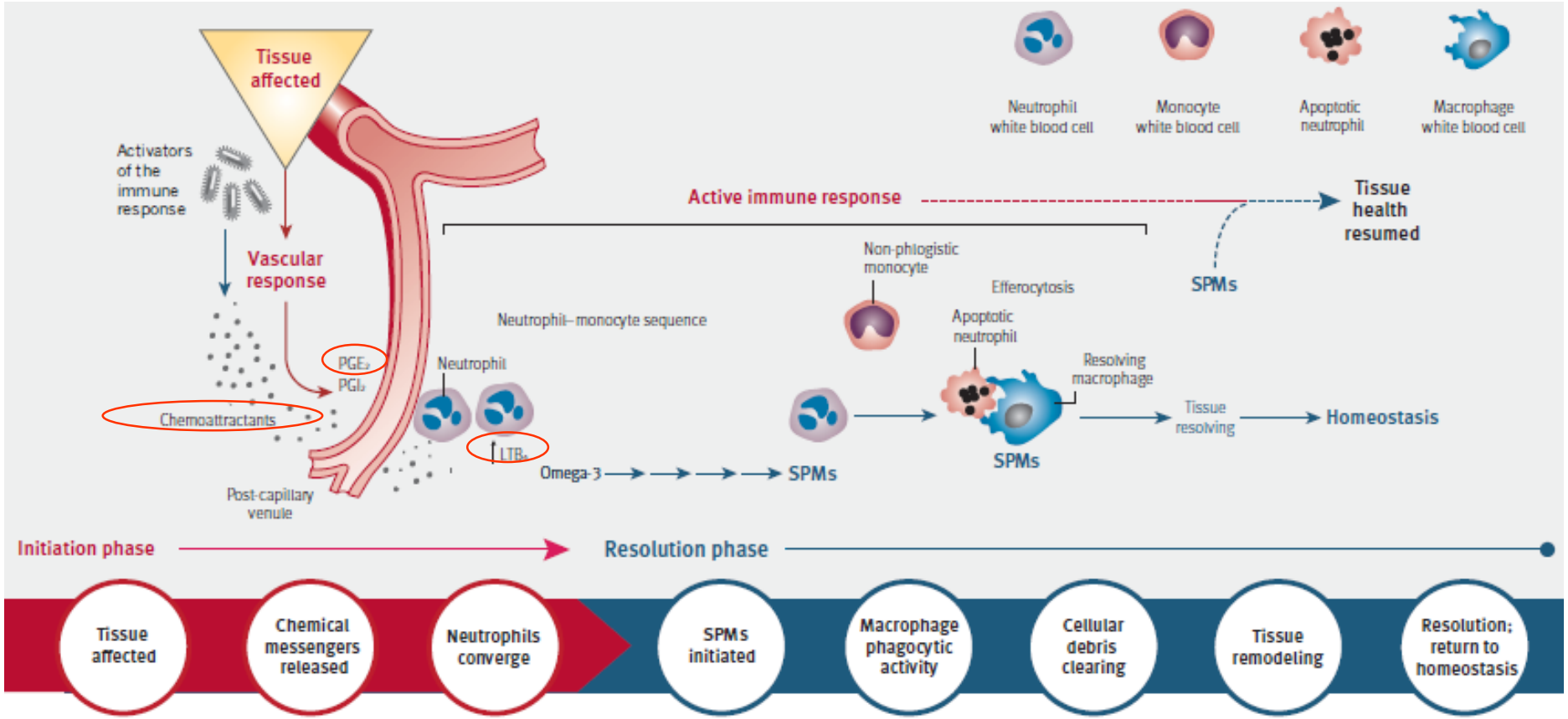
- Xanthohumol
  - Reduces macrophage production of MCP-1

- Ginger
  - Reduces IP-10, specifically in activated human synoviocytes

Immune Response Causing the *Initiation* of Inflammation & Pain



This Combination of Ingredients Acts *Upstream* of SPMs



Mechanistic Takeaways

- Curcumin + Xanthohumol + *Boswellia serrata* + Ginger:
  - Inhibit several key mediators of inflammation & pain
    - NFkB – Upregulates cytokines, chemokines, pro-inflammatory enzymes
    - Pro-inflammatory cytokines
    - Chemokines – WBC recruitment
    - Enzymes involved in prostaglandin and leukotriene production
  - Act upstream and downstream on inflammation & pain pathways

Mechanism of Action (MOA) Summary

	Curcumin	Xanthohumol	*Boswellia serrata*	Ginger
Inhibition of pro-inflammatory cytokines, chemokines, and transcription factors associated with inflammation and pain	✓ Inhibits NFkB ✓ Reduces serum levels of: TNFα IL-1β[2] IL-6 MCP-1 ✓ Diminishes chondrocyte production of CXCL8 (IL-8)	✓ Inhibits NFkB ✓ Reduces WBC production of: TNFα IL-12 MCP-1	✓ Inhibits NFkB	✓ Inhibits NFkB ✓ Diminishes synoviocyte production of IP-10
Inhibition of enzymes and prostaglandins associated with inflammation and pain	✓ Inhibits PLA2 ✓ Inhibits COX-2 ✓ Inhibits 5-LOX	✓ Inhibits COX-1 & COX-2	✓ Inhibits COX-1 & COX-2 ✓ Inhibits 5-LOX	✓ Inhibits PLA2 ✓ Inhibits COX-1 & COX-2 ✓ Inhibits LOX

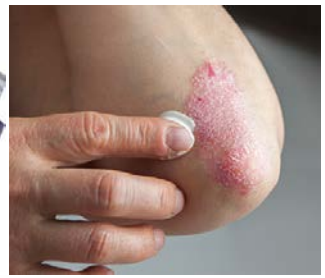
Clinical Applications

Clinical Applications

Target	Pharmaceutical Antagonist	Botanical Antagonist	Clinical Relevance
COX-1 & COX-2	NSAIDs	Curcumin, Xanthohumol, Ginger	• Arthritis (OA, RA, JRA, AS, psoriatic, gout) • Musculoskeletal pain (back, etc.) • Tendonitis and bursitis • Menstrual cramps • Postoperative pain • Headache and migraine
LOX	Leukotriene inhibitors	Curcumin, *Boswellia serrata*, Ginger	• Arthritis (OA, RA, JRA, AS, gout) • Musculoskeletal pain • Tendonitis and bursitis • Menstrual cramps • Asthma prevention and treatment
NFkB	Glucocorticoids	Curcumin, Xanthohumol, *Boswellia serrata*, Ginger	• Inflammatory, autoimmune and allergic disorders (including asthma)
TNFα	TNFα inhibitors	Curcumin	• Arthritis (RA) • Psoriasis





Scientific Takeaways

- CGM and XNTPM are *highly bioavailable*
- Curcumin, Xanthohumol, *Boswellia serrata, and* Ginger *reduce* systemic inflammation and pain via several well-defined *mechanisms*
- These ingredients could potentially be used as an *alternative to* or *in conjunction* with other treatments for inflammation and pain