Biotherapeutic Immunomodulation as a Countermeasure to Hyperinflammatory Tissue Damage : A Double-Blind, Phase 1 Human Study of Osteoarthritis, With Surprising Efficacy

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Swiss Academy of Pharmaceutical Sciences (SAPhW)
PharmaLunch

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MAJOR TOPIC FOCUS

- **Main advantage to therapeutic use of stress response gene products**
- **Representative clinical outcomes of stress-response modulation**
- **Inflammation and heat shock protein-mediated regulation**
- **Clinical demonstration of stress-response therapy in osteoarthritis**
- **Bioactive compounds contained in or induced by proprietary formulations described here**

IMMUNOREGULATION WITH HEAT SHOCK PROTEINS (HSP)

Equal or superior in clinical efficacy to small molecule pharmaceuticals.

MAIN ADVANTAGE

HSPs strengthen normal homeostatic processes, and do NOT interfere with inflammatory signaling cascades. They have negligible adverse side effects/toxicity.

ALSO SCE provides a solution to regulate HSP's which is low cost to produce.

- Sustainable with minimum infrastructure.

- Based on 100% natural compounds

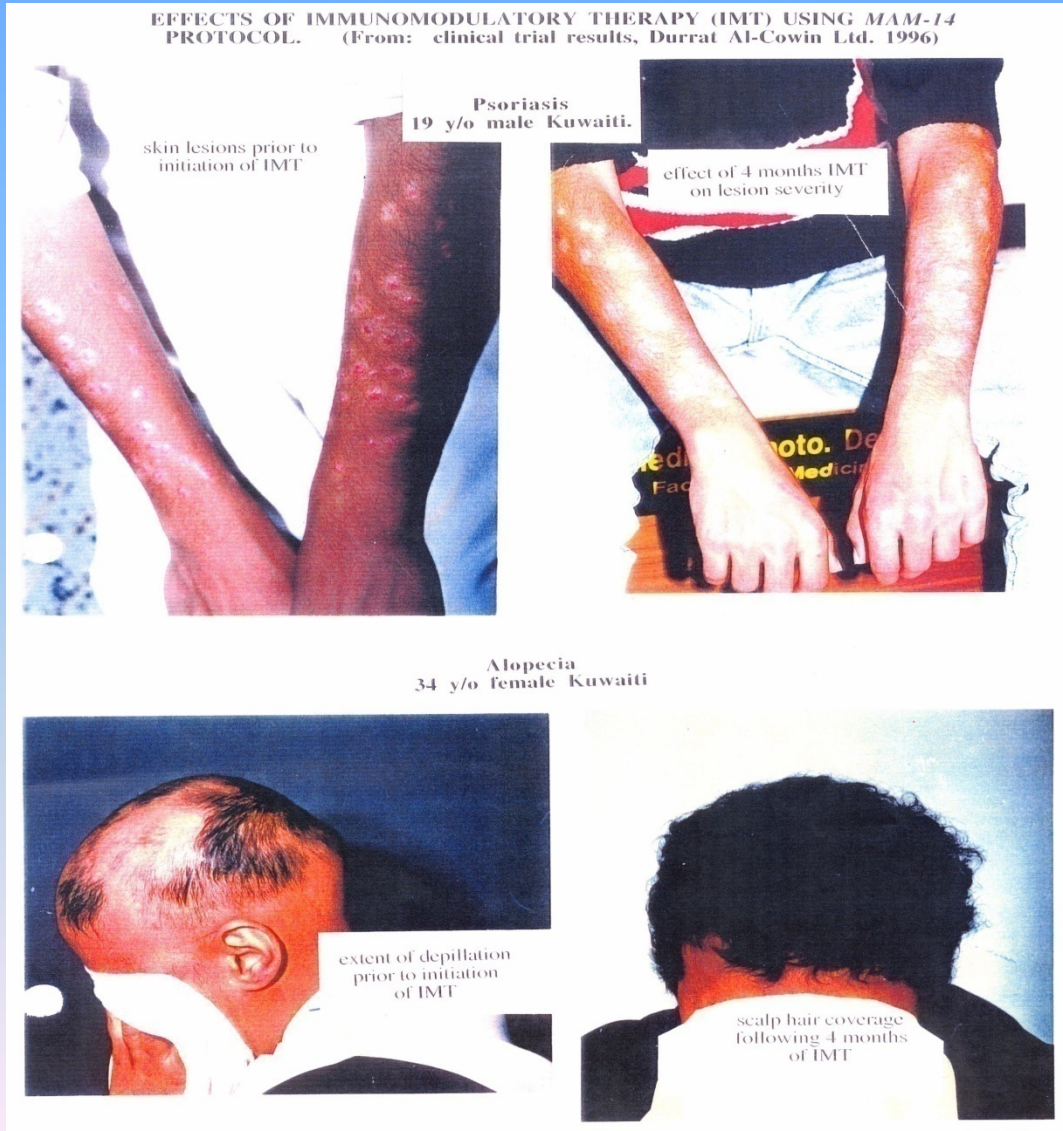
VISUAL REPRESENTATIVE CLINICAL OUTCOMES OF STRESS RESPONSE MODULATION

MAM-14 Immunotherapy for psoriasis and alopecia

UPPER FRAMES

Case 1 : Psoriasis

The patient, a 19 year-old Kuwaiti male, afflicted with severe psoriasis on both forearms is shown in the upper left. Four months after initiation of treatment, all lesions had subsided leaving smooth, normal skin with some residual depigmentation remaining (upper right).



LOWER FRAMES

Case 2 : Alopecia Areata

The patient, a 34 year-old Kuwaiti female, afflicted with severe alopecia with extensive scalp depilation is shown at the lower left. Four months after initiation of treatment, the disease process is clearly suppressed as evidenced by return of normal hair growth (lower right).

INFLAMMATION AND HEAT SHOCK PROTEIN-MEDIATED REGULATION

DISEASE INITIATORS (Challenge)

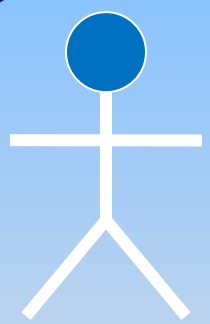
Microorganisms (bacteria, viruses)

Toxic substances (including dietary)

Genetic defect

Injury

Normal aging



BODY RESPONSE

Stress Response Gene Expression

Regulatory

Activation of immune and antioxidant defenses

OUTCOME

Immune defense normal, Immune regulation normal.
Typical result : Recovery.

Immune defense overwhelmed (Not sufficiently robust).
Immune regulation normal.
Typical result : Infectious disease; cancer.

Immune defense normal or high, Immune regulation insufficient.
Typical result : Autoimmunity and other inflammatory syndromes.

CLINICAL DEMONSTRATION OF STRESS-RESPONSE THERAPY IN OSTEOARTHRITIS (OA)

Mahmoud FF, Al-Awadhi AM, Haines DD. Amelioration of human osteoarthritis symptoms with topical 'biotherapeutics': a phase I human trial. *Cell Stress Chaperones*. 2014 Nov;20(2): 267-276.

20 OA patients receiving topical sour cherry seed extract containing inducer of heme oxygenase-1 (HO-1), applied to index knee, 2x daily, 8 weeks.

MAJOR OUTCOMES

Significant HO-1-dependent decreases at week 8 noted for :

- **Joint pain**
- **Serum c-reactive protein**
- **CD3+ IL8+ T cells**
- **CD3+TNF-α+ T cells**
- **CD3+IFN-γ+ T cells**
- **CD3+il1α+ T cells**
- **CD3+IL-1β+**

Cell Stress and Chaperones
DOI 10.1007/s12192-014-0553-0

ORIGINAL PAPER

Amelioration of human osteoarthritis symptoms with topical 'biotherapeutics': a phase I human trial

Fadia F. Mahmoud · Adel M. Al-Awadhi · David D. Haines

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Abstract Osteoarthritis (OA) treatments presently rely on analgesics, which manage pain but fail to restore imbalances between catabolic and anabolic processes that underlie OA pathogenesis. Recently, biologic (biotherapeutic) drugs, which alter the activity of catabolic agents such as nitric oxide and inflammatory cytokines in ways, allowing tissue regeneration, were evaluated for efficacy in OA treatment. These studies failed to demonstrate dramatic abatement of OA symptoms by these drugs, but suggested strategies by which biologic agents might be used to treat OA. The present review summarizes current understanding of OA pathogenesis and evolving treatments. Preliminary evaluations of a novel biotherapeutic strategy are presented here. Twenty OA patients receiving sour topical cherry seed extract (SCE), an inducer of heme oxygenase-1 (HO-1), a major physiological protectant against oxidative stress exhibited significantly decreased joint pain and activation of CD4+ T cells expressing inflammatory cytokines ($p<0.05$), significantly decreased peripheral blood C-reactive protein (CRP), and increased leukocyte HO-1 ($p<0.05$) in comparison with ten placebo-treated patients. SCE inhibits joint-damaging inflammatory mediator production. This agent therefore meets the main criterion for classification as a "biotherapeutic," or "biologic" agent. The negligible toxicity and low cost of such materials make them promising contributors to OA treatment, sustainable within resource limitations of a wide range of patients.

Keywords Biotherapeutic agent · Osteoarthritis · Heme oxygenase-1 · Sour cherry · Cytokines · Inflammation

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Introduction

Osteoarthritis (OA), a degenerative age-related disease that affects the joints, is the most common human musculoskeletal disorder and a leading cause of disability in elderly populations worldwide (Aggarwal et al. 2013). OA onset is typically triggered by sustained biomechanical trauma, resulting in chondrocyte-mediated cartilage destruction.

Oxidative stress, created by this degradative process, promotes emergence of senescent osteoarthritic osteoblasts, which in turn enhance dysregulation of proinflammatory signaling and apoptotic depletion of functional joint cells, causing insufficient cartilage repair and aberrant remodeling of the extracellular matrix (Chevalier et al. 2013; Clerigues et al. 2012, 2013). Tissue damage is exacerbated by trauma-related dysregulation of normal maintenance of healthy joint homeostasis (Dieppe and Lohmander 2005). This disruption promotes increasingly severe inflammation (synovitis) (Volpi and Maccari 2005), leading to adverse changes in joint fluid composition, breakdown of extracellular matrix material, and impairment of normal tissue repair.

The pathomechanisms of OA are facilitated by progressively elevated levels of the inflammatory cytokines tumor necrosis factor alpha (TNF-α), and the interleukins (IL) IL-1β, IL-6, and IL-8, produced primarily by macrophages and T lymphocytes, systemically and in affected joint tissue (Attur et al. 1998). Downstream signaling cascades of these

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Figure 1. Major pathomechanisms contributing to OA-associated articular tissue damage. **Mechanical trauma and endogenous oxidative stressors increase synoviocyte, T cell and macrophage expression of the inflammatory cytokines IL1-β, TNF-α, and IL-6**

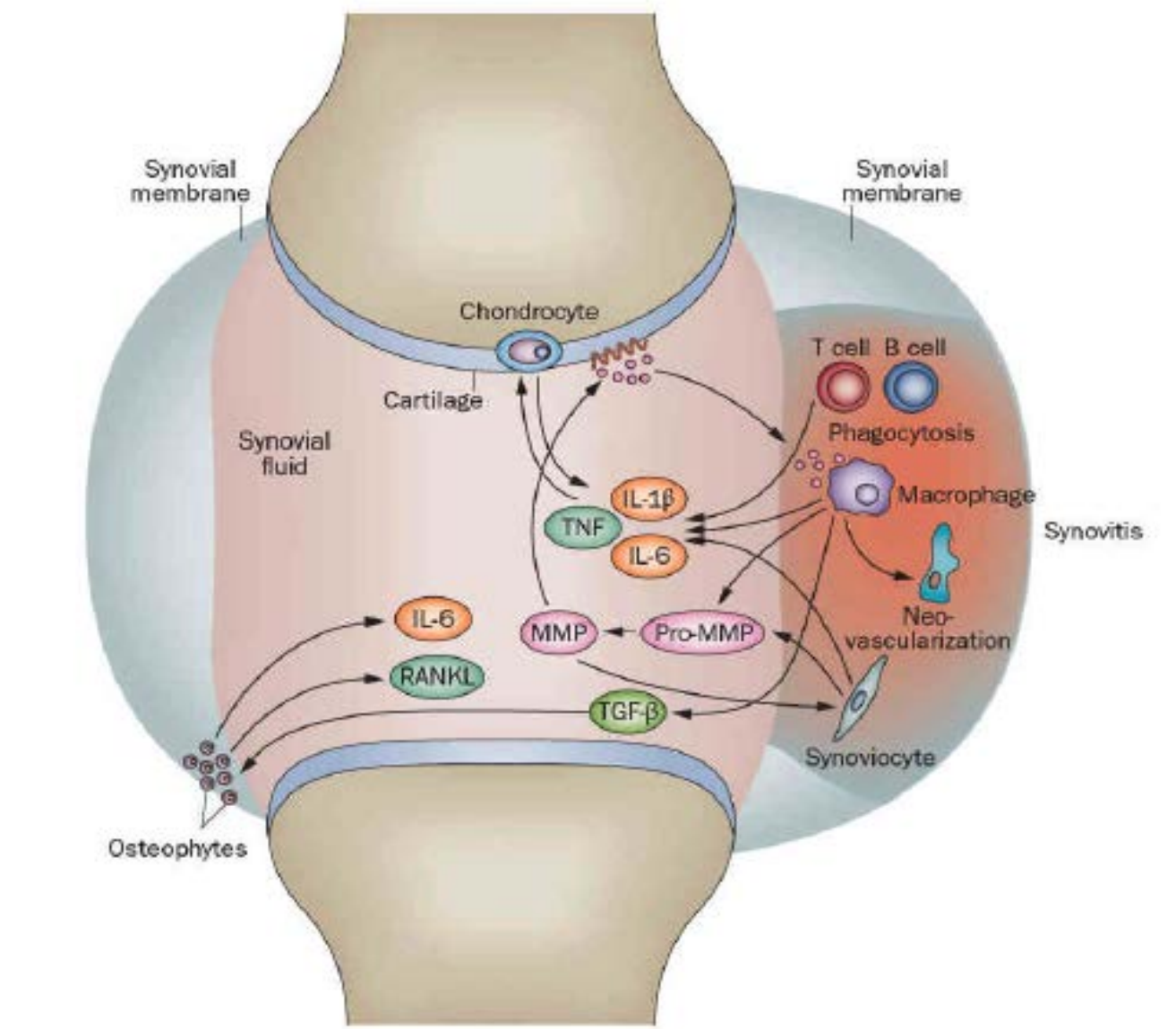


Figure 2. Suppression of OA-associated pain with topical SCE preparation

Outcomes are shown for two groups of OA patients: 20 administered 5ml *SCE*-containing skin cream, and 10 receiving placebo emollient applied topically to skin on the suprapatellar portion of index knee (the knee selected for pain assessment) twice daily for 2 months. Pain was self-assessed at baseline, and following the 2-month treatment regimen, with results reported as pain score ± SEM on the 100-mm visual analogue scale (VAS) WOMAC pain subscale.

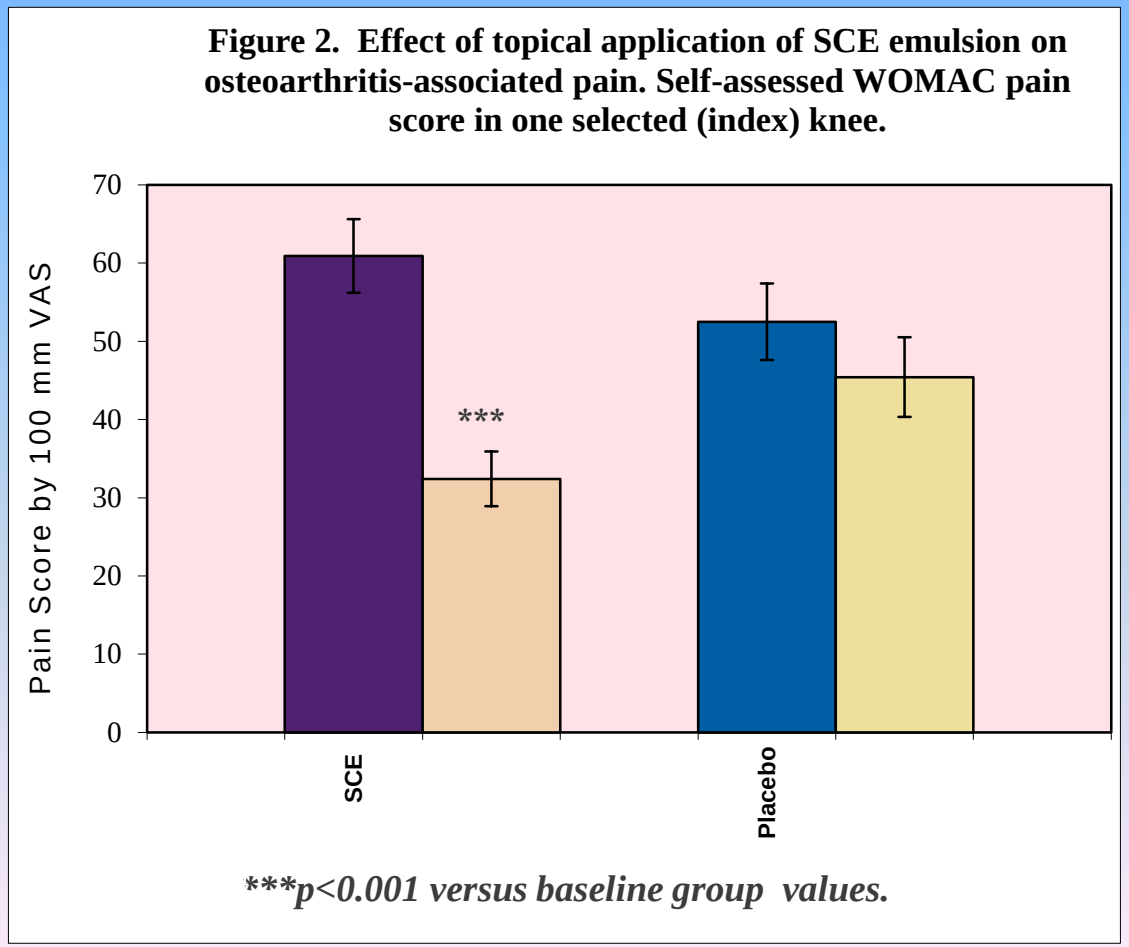


Figure 3. SCE treatment-associated heme oxygenase-1 (HO-1) production increase by PBMC. Lysates made from PBMC Ficoll-Hypaque-extracted from freshly collected peripheral blood of topical SCE-treated subjects (n = 20); and placebo-treated subjects (n = 10) at baseline and following 2 months of treatment, are evaluated by ELISA for average content of the enzyme, reported in ng/ml HO-1 protein ± SEM.

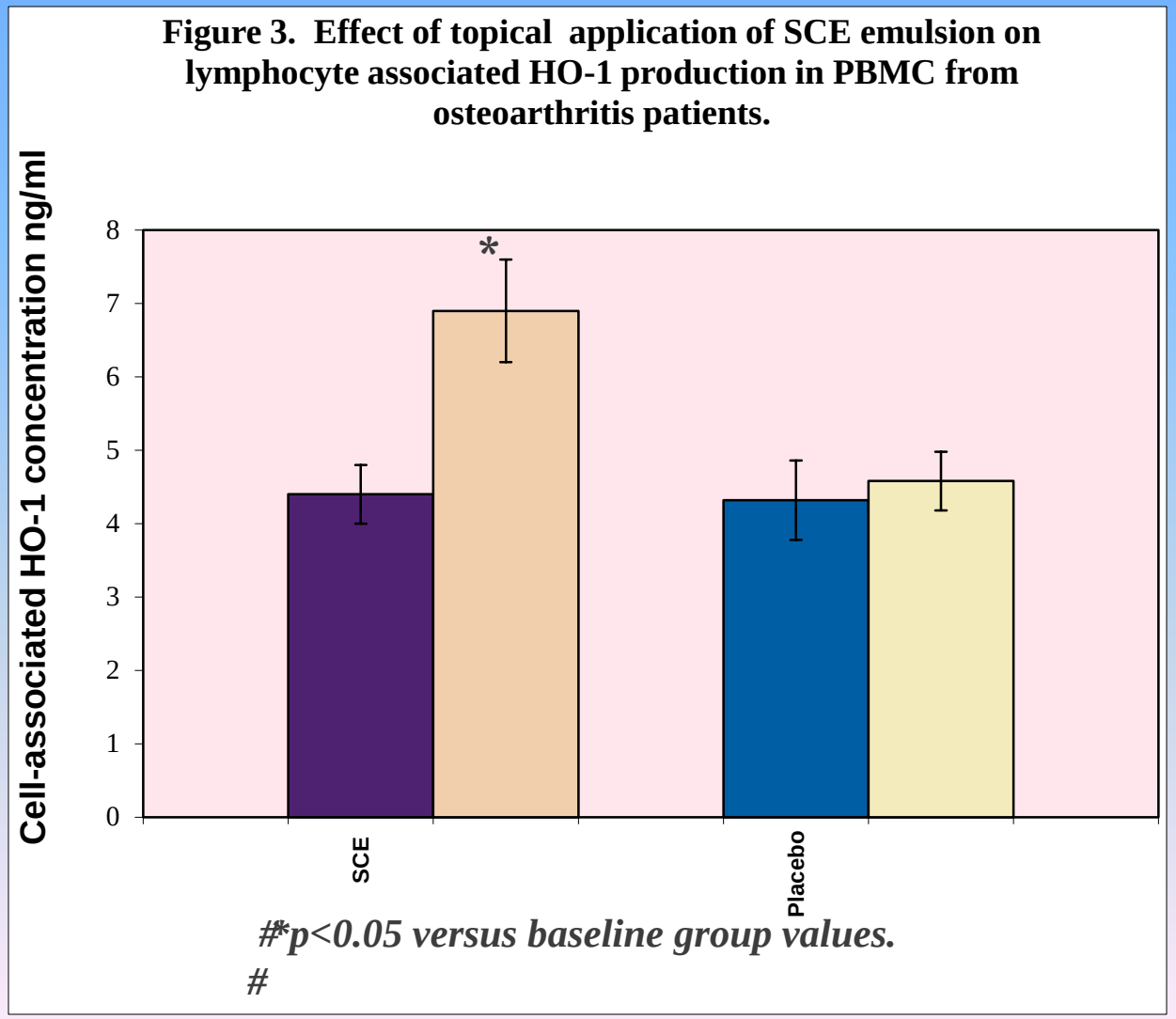


Figure 4. SCE treatment-associated decrease in serum c-reactive protein (CRP). Defibrinated serum extracted from non-anticoagulated peripheral blood collected at baseline and following 2 months of treatment was evaluated by ELISA for CRP content in test subjects treated topically with SCE-containing skin cream (n = 20); and a control group (n = 10) treated with a placebo emollient. Outcomes are reported as average concentration of CRP in serum ± SEM.

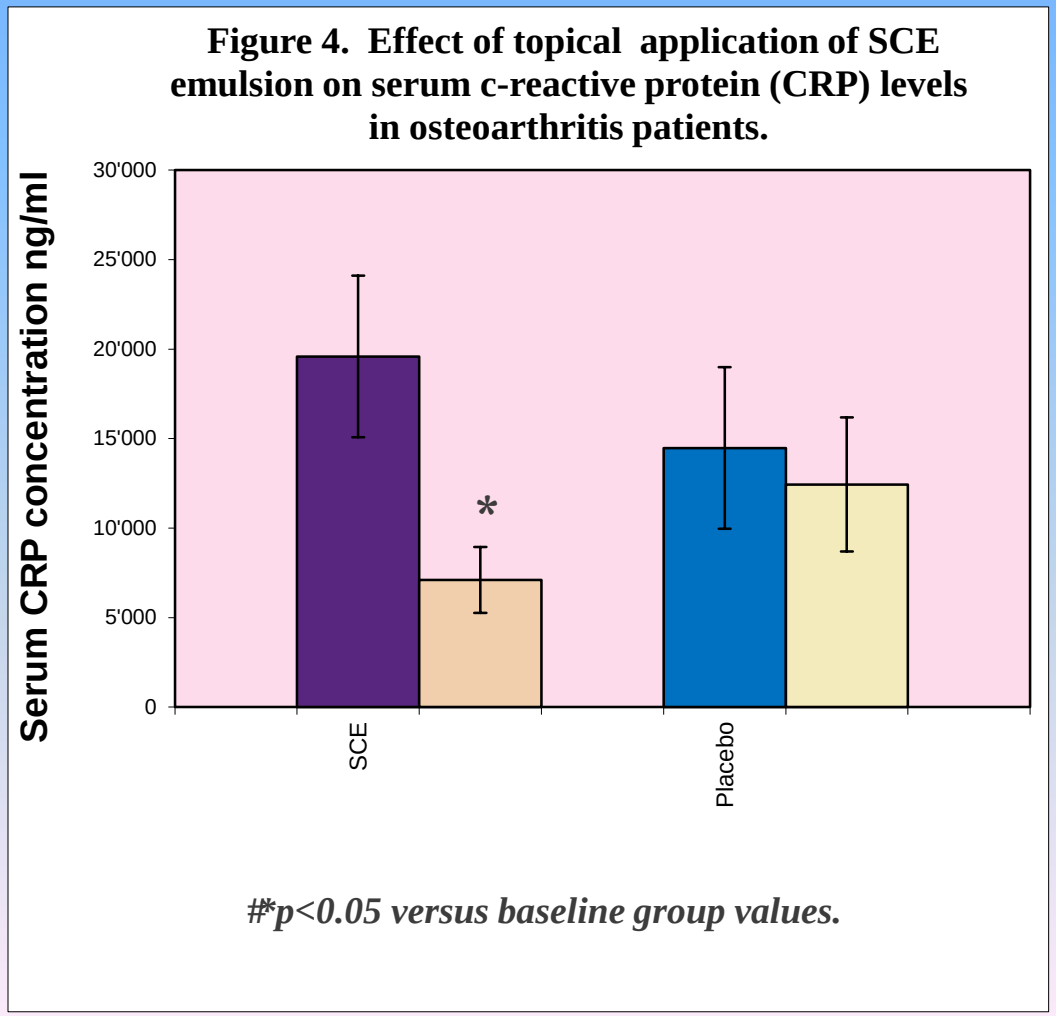
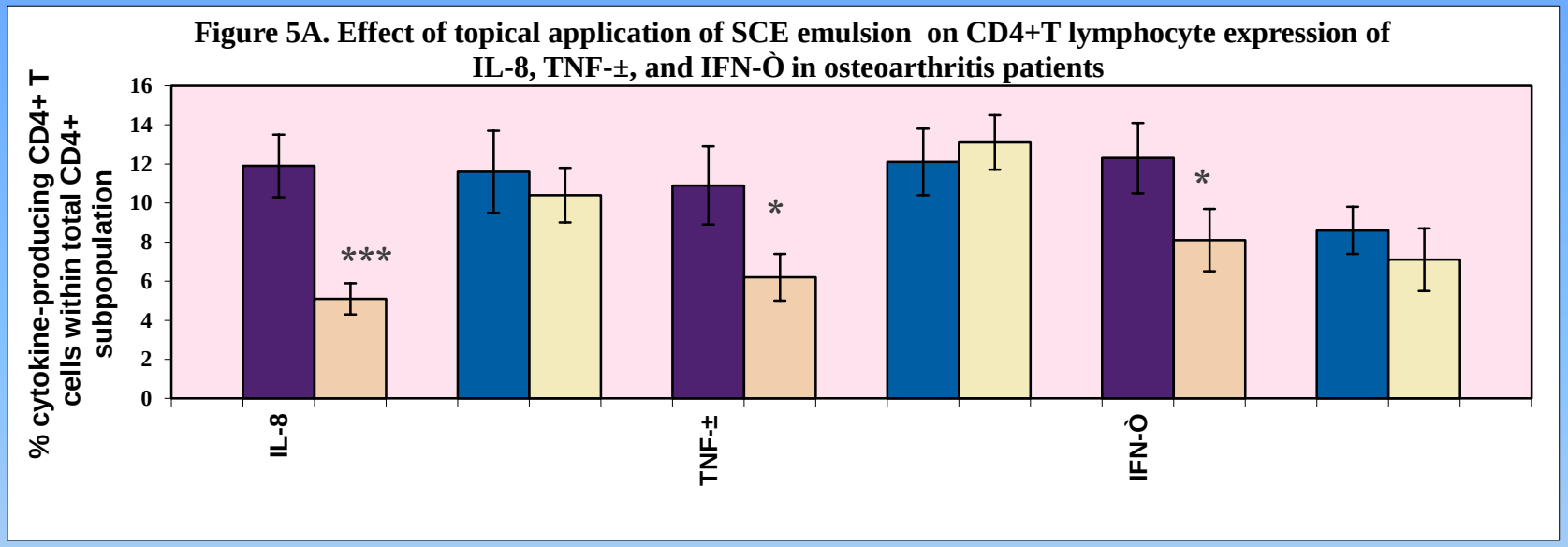
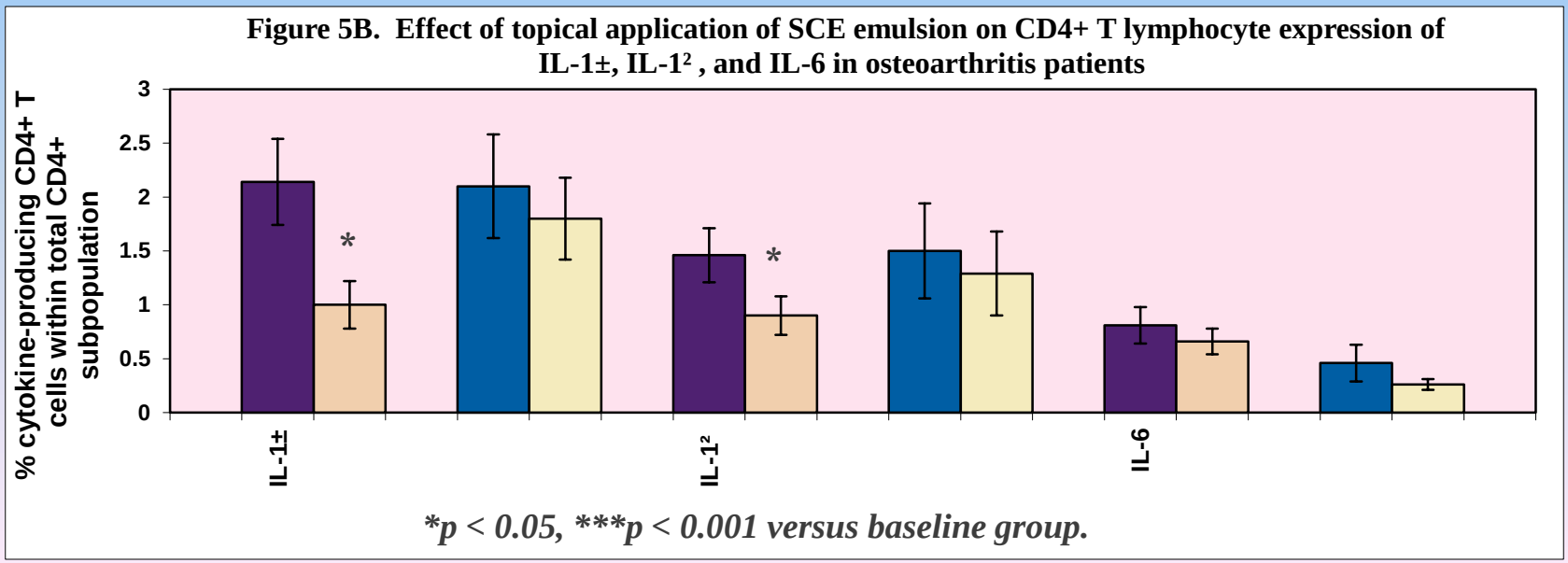


Figure 5. SCE-mediated suppression of CD3+CD4+IL-8+, CD3+CD4+TNF-a+, CD3+CD4+IL-1a and CD3+CD4+IL-1b+ representation in peripheral blood mononuclear cells (PBMC). Analyzed by 2-color flow cytometry

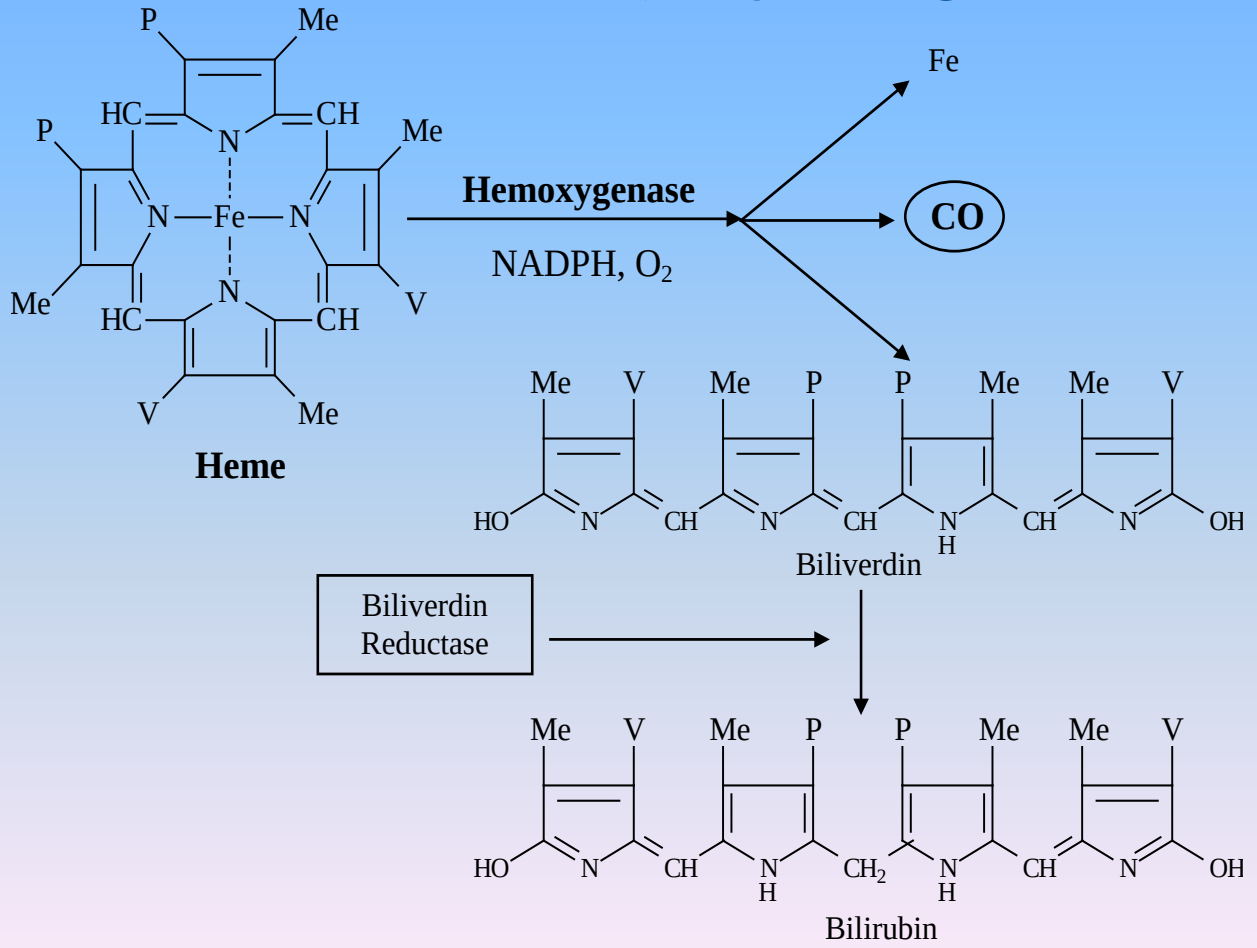




HEME OXYGENASE-1 (HSP-32)

Major effects of sour cherry seed flavone fraction are mediated by this enzyme

Heme oxygenase activity: reaction stoichiometry. Heme oxygenases degrade heme by cleavage of the heme ring at its alpha-methene bridge, producing carbon monoxide (CO), ferrous ion (Fe2+) and biliverdin, which is further reacted by biliverdin reductase to bilirubin (a major endogenous antioxidant).



Major Therapeutic Components of Sour Cherry Seed Solid Fraction (Representative Selection)

Flavones and Related Phytochemicals

Resveratrol	Anti-inflammatory with multiple beneficial effects. Observed to extend lifespan in some organisms.
Anthocyanidins	Anti-allergic/anti-inflammatory. Aids in antioxidant activity of vitamin C. Along with kaempferol and myricetin, it is shown to reduce risk of pancreatic cancer in smokers.
Gallocatechins	Form water-soluble anthocyanins when complexed with sugars. Powerful antioxidants with ability to protect cells in both water- and fat-soluble environments.
Kaempherol	Polyphenol antioxidants with unique ability to repair the vitamin E radical. Demonstrated to be anticarcinogenic and chemopreventive.
	A polyphenol that increases energy expenditure and oxygen consumption via multiple pathways. Major effect is increased thermogenesis and fat loss without increasing cortisol levels, or negatively modulating various adrenergic receptors. In other words, this component contributes to effective weight control without adverse side effects of other commonly uses weight loss supplements.

Major Therapeutic Components of Sour Cherry Seed Oil (Representative Selection)

Triglyceride Free Fatty Acids

Linoleic acid	Decreases severity of atopic eczema and inhibits chemically-induced cancer in mice. Stabilizes skin barrier.
Oleic acid	Excipient compound with strong emulsifying and solubilizing properties.

Aldehydes

Hexanal	Imparts natural fruity odor. Used as flavoring by food industry.

Vitamin E Isomers

±-tocopherol	Most powerful vitamin E isoforms. Inhibits skin damage by slowing oxidation of lipids and other skin components.
´-tocopherol	Complements ±-tocopherol cytoprotective activity.
´-tocotrienol	Stronger than other vitamin E isofoms in some cases (e.g. H_20_2 neutralization).

Prototypic Product based on Sour Cherry Seed Oil

Distributors:
University of Debrecen
Faculty of Pharmacy
Debrecen
Phone: 36-52-453586
and
JONACO Kft.
Nyírtass, 4522
Dozsa Gyorgy Str. 47

Olabella®

Olabella cream contains natural compounds (sour cherry seed kernel oil) and is perfect for all skin types, especially dry skins. It helps to deeply hydrate and regenerate the original structure of the skin. Skin will be visibly softer, smoother and silkier after using Olabella. Compositions with antioxidant properties intensify the microcirculation of skin vessels, and reduce the signs of aging. The natural flavonoids of the sour cherry seed kernel oil improve circulation. They make the wall s of the skin vessels strong and elastic. Furthermore, the revitalizing antioxidant vitamin E and ^{2} -tochopherol prevent free radical damages, and help to rebuild the protective barrier of the skin. The urea component of the cream inhibits the loss of water in the rehydrated skin cells.

Directions: Twice daily, softly massage the cream into the skin (jar for face lifting, tube for body care) with circular motions of fingers.

Ingredients: Lanolin, Stearinic acid, Cetostearolum, Paraffinum liquidum, Triethanolamine, Methylparaben, Aqua, Urea, Prunus cerasus seed kernel oil, Perfume.

Active ingredients of Prunus cerasus seed kernel oil: ^{3} -sitosterol, ^{2} -tochopherol, squalen, vitamin-E, unsaturated free fatty acids, esters.

Manufacturer: Central Pharmacy of the University of Debrecen, 4032, Debrecen, Nagyerdei krt. 98, Hungary. Phone: 36-52-453586

Competitive Advantage
Content of Major Therapeutic Components of Sour Cherry Seed Oil versus Competing Products

Comparison with Shea Butter

Shea butter is a lipid-soluble paste extracted from the seed of a slow-growing hardwood tree indigenous to parts of Africa. It is rich in vitamin E isoforms, making it a highly attractive cosmetic base.

Sour cherry oil has the following major advantages over Shea butter :

- **Shea butter collection is labor-intensive and the supply is limited, increasing its basic cost.**
- **The tocopherol content of Shea butter is lower than sour cherry seed oil and is also widely variable depending on its geographic source.**

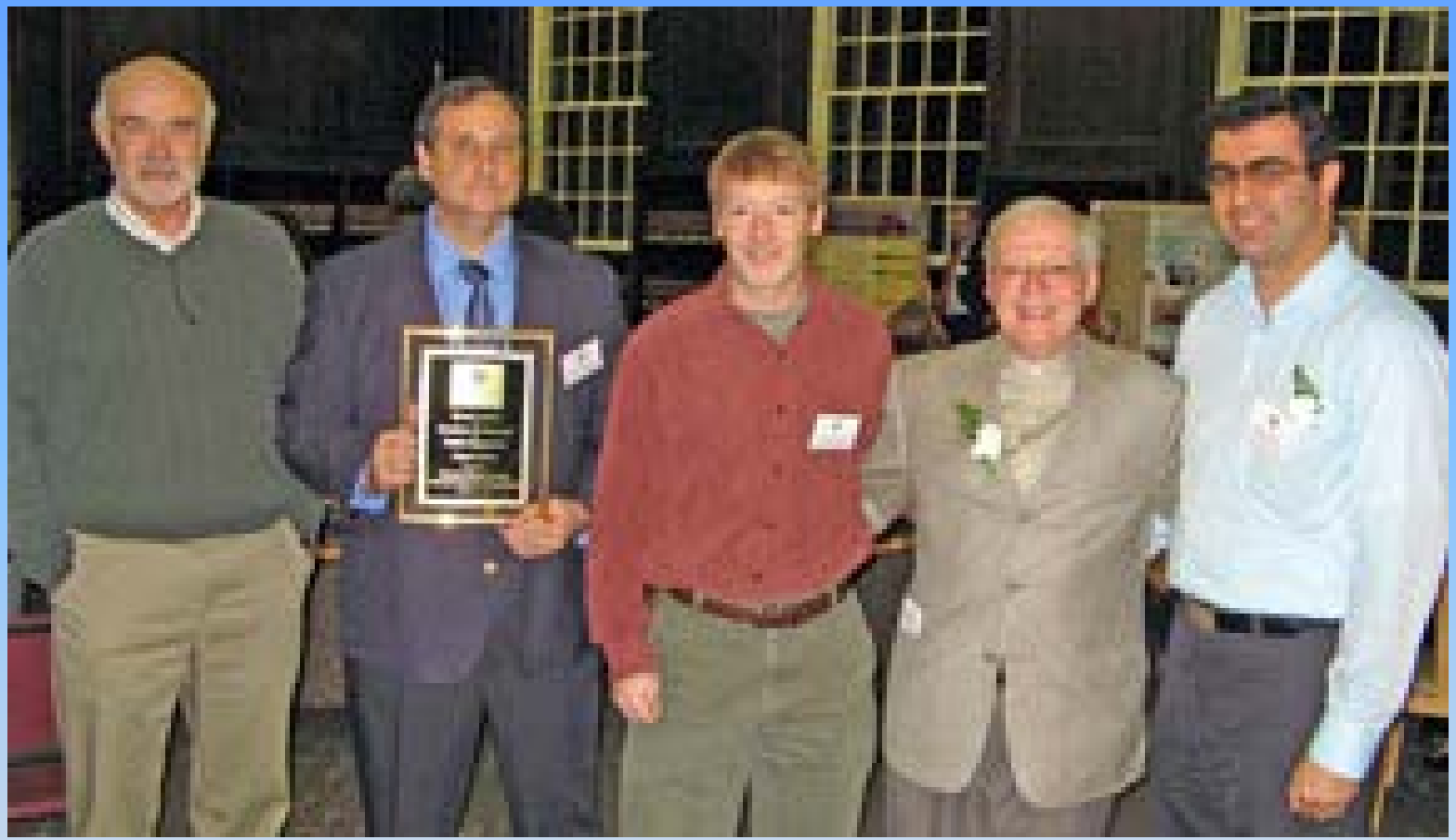
Protective Compound	Shea Butter	Sour Cherry Seed Oil
±-Tocopherol	2.9-41.4 mg/100g	52-53 mg/100g
Total Tocopherol Content	2.9-80.5 (mean = 22) mg/100g	80-85 mg/100g

LEAD INVESTIGATORS
for Development of Sour Cherry Seed-Based Products

Dr David D. Haines, of the Department of Molecular and Cell Biology at the University of Connecticut, and Visiting Professor at the Faculty of Pharmacy Health Science Centre at the University of Debrecen in Hungary. He is a former U.S. Army chemical weapons officer and a veteran of the 1991 Gulf War in Kuwait.

Dr Fadia Mahmoud is a senior lecturer at the Department of Medical Laboratory Sciences at Kuwait University. She conducted her post doctoral work on the Human Genome Project at Yale Medical School.

PROGRAM RESEARCH AFFILIATES



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