

perluxan®

A UNIQUE BOTANICAL ANTI-INFLAMMATORY AGENT



Click to view:

[PRODUCT OVERVIEW](#)

[TECHNICAL OVERVIEW](#)

[CLINICAL STUDY OVERVIEW](#)

[WHITE PAPER](#)

Contact Info

Perluxan®

t (866) 963-2007
www.perluxan.com

Gregory Bonfilio

t (707) 766-7060
gbonfilio@pharmachemlabs.com

Proprietary Nutritionals, Inc.

a subsidiary of Pharmachem Laboratories, Inc.

265 Harrison Avenue
Kearny, NJ 07032 USA
t (519) 647-2071
f (201) 622-1415
www.pnibrands.com



© 2009 Pharmachem Laboratories, Inc.

Perluxan® is a registered trademark of Pharmachem Laboratories.

The statements in this document have not been evaluated by the FDA. This product is not intended to diagnose, treat, cure or prevent any disease.

perluxan®

A UNIQUE BOTANICAL ANTI-INFLAMMATORY AGENT

PRODUCT OVERVIEW

Features

Standardized

- A proprietary extract of hops cones with a specific content of alpha acids
- Designed to be virtually free of phytoestrogenic and sleep inducing compounds

Researched

- Documented multiple mechanisms of action in human *in vitro* and *in vivo* studies
- The subject of extensive ongoing research and human clinical studies

Safe & Effective

- A GI friendly alternative for the temporary relief of minor everyday aches and pains
- Supports joint health and mobility
- Well tolerated and absorbed
- Fast acting

Versatile

- Anti-inflammatory
- Anti-oxidative
- Bone resorption inhibitive



Contact Info

Perluxan®

t (866) 963-2007
www.perluxan.com

Gregory Bonfilio

t (707) 766-7060
gbonfilio@pharmachemlabs.com

Proprietary Nutritionals, Inc.

a subsidiary of Pharmachem Laboratories, Inc.

265 Harrison Avenue
Kearny, NJ 07032 USA
t (519) 647-2071
f (201) 622-1415
www.pnibrands.com



© 2009 Pharmachem Laboratories, Inc.
Perluxan® is a registered trademark of Pharmachem Laboratories.

The statements in this document have not been evaluated by the FDA. This product is not intended to diagnose, treat, cure or prevent any disease.

Introduction

The female flower cone of the hops plant (*Humulus lupulus* L.), a climbing vine native to North America, Europe and Asia, is primarily used to add flavor to beer. Numerous cultivars of hops are selectively utilized by brewers to balance the sweetness of malt with bitterness, adding flowery, fruity and herbal aromas. The first cultivation of hops was documented in 736 and hops was introduced to the US in 1629. Today's leading production centers in North America include the Yakima Valley in Washington and Willamette Valley in Oregon.



Figure 1: hops cultivation (left), Mt. Hood cone (middle) and production (right)

Hops contains a spectrum of bioactive ingredients with numerous traditional therapeutic applications, including the treatment of anxiety and insomnia due its mild sedative and relaxing effects, and its use during menopause for its high phytoestrogen content. However, it was not until recently that the anti-inflammatory activity of certain hops fractions, primarily derived from resinous extracts, was discovered. The so called alpha acids, isolated and enriched by state-of-the-art supercritical carbon dioxide extraction technology, have shown great potential in helping to support the body's natural response to inflammation.

The Search for a Botanical Alternative to Improve Joint Health

During the late 1990's, a handful of research companies began looking for natural ways to improve joint health and ease minor pain. Extensive literature reviews of 230 botanicals yielded about 20 candidates for proof-of-principle studies (*in vitro* IC₅₀ and IC₈₀ tests) to investigate their potential of eventually becoming a natural, efficacious alternative.

Of those 20 prospects, one ingredient was identified as having the highest chance of success. This ingredient, a standardized extract of hops previously known for its antioxidant properties, was subsequently tested at the respected William Harvey Institute in the UK using the gold-standard William Harvey Whole Blood Assay. The powdered extract used in the research was specifically designed to contain high amounts of naturally occurring alpha acids and was characterized by a good solubility and bioavailability (measured in CACO-2 cell lines) when compared to the other botanicals. The extract was also made to be virtually free of both phytoestrogens and sleep-inducing compounds, to maximize its safety and efficacy in joint health.

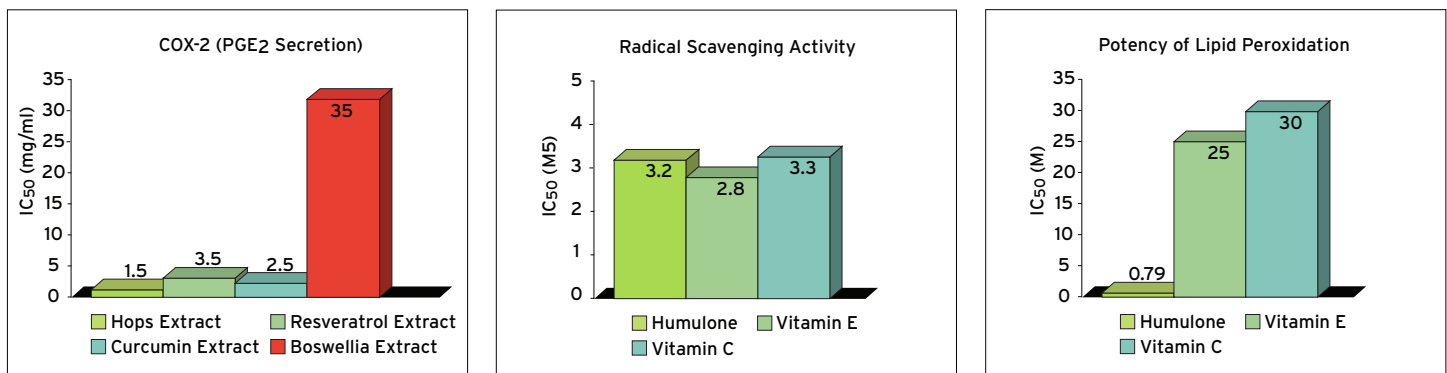


Figure 2: COX-2 selectivity (left) and high radical scavenging (middle) and outstanding lipid peroxidation protecting (right) activity of Humulone.

Note: The IC₅₀ values represent the concentration required to produce 50% inhibition of the corresponding activity. The lower the IC₅₀ value for the activity, the less of the corresponding extract required to inhibit and the higher its potency.

After convincingly passing these crucial milestones and revealing its mechanism-of-action, the hops extract was subsequently put to the only true test, a placebo controlled clinical trial to investigate its effect on joint discomfort in human subjects.

The Effect of a Standardized Extract of Hops in Humans

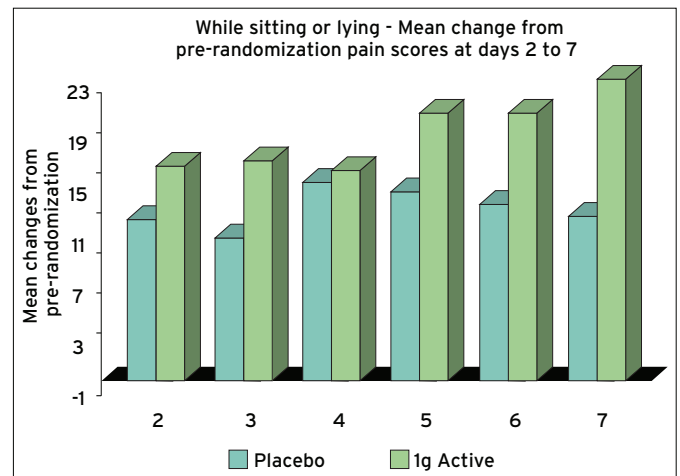
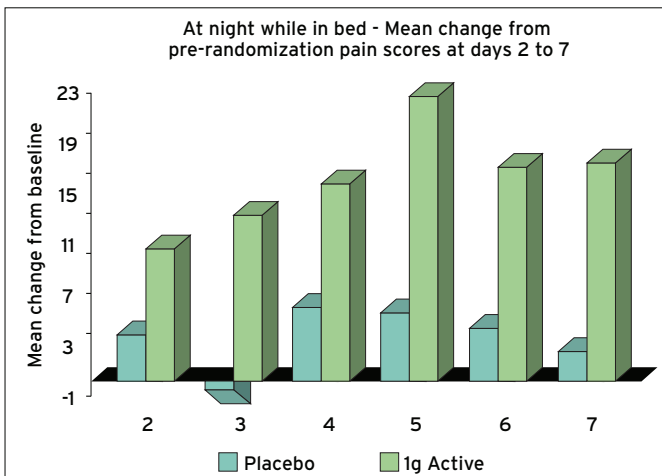
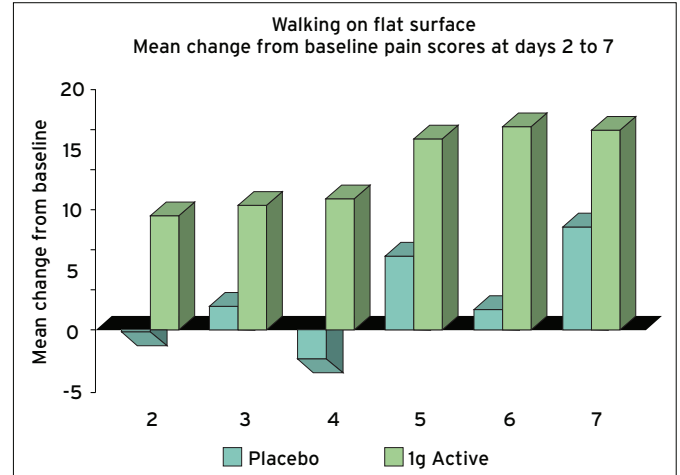
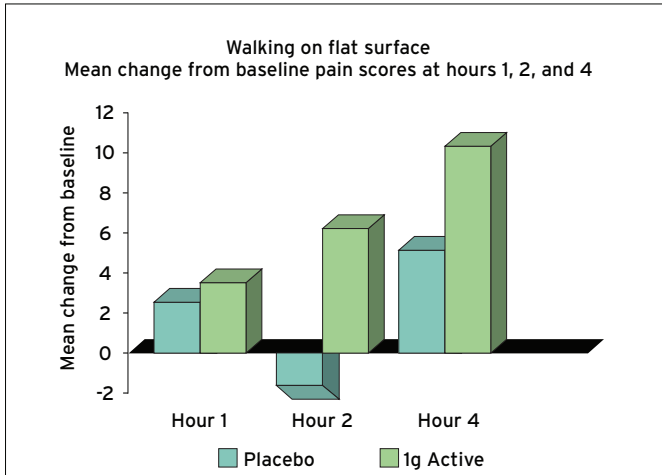
The effect of a standardized supercritical carbon dioxide hops extract containing 30% alpha acids (Perluxan®) on individuals with osteoarthritis of the knee was investigated in a randomized, double-blind, placebo-controlled study.

Thirty six (36) individuals ingested either placebo or Perluxan® in capsule form, and the effect of the ingredient to improve pain relief after 14 days of supplementation (posttest) was measured by WOMAC (symptom assessment questionnaire) and compared to starting values (pretest). The WOMAC is a validated instrument designed specifically for the assessment of lower extremity pain and function in Osteoarthritis (OA) of the knee or hip and is a reliable and sensitive instrument for the detection of clinically important changes in health status. This study investigated pain severity scores during everyday activities such as Walking on a Flat Surface, Up and Down Stairs, While in Bed, Sitting or Lying and Standing Upright.

The subjects' ages ranged from 39 to 74 years; the average age was 57 years. The majority of subjects were Caucasian (92%) and female (78%). The critical success factor for treatment of pain was a self-determined fast acting pain relieving effect of the product to motivate the individual to continuously take the supplement.

Perluxan® intake showed a fast acting effect on mean pain relief and significant improvement over placebo could be measured after only two (2) hours following the first dose. By the end of the second week, it was clear that Perluxan® helped to relieve minor pain during normal daily activities and may have improved joint mobility in the active group.

Pain Relief During Everyday Activities



It was concluded that 14 days of oral Perluxan® supplementation significantly improved parameters of osteoarthritis pain. The effectiveness of Perluxan® was also supported by the extremely limited use of rescue medication in the treatment groups in comparison to placebo. The comparison of pre- and post-supplementation blood work and the adverse side effect monitoring also showed that Perluxan® was well-tolerated and lead to no gastrointestinal discomfort.

To learn more about the fascinating history of hops, the anti-inflammatory effect of Perluxan® and the scientific data supporting the proposed mechanisms-of-action, please contact Pharmachem Labs.

Product Data Sheet

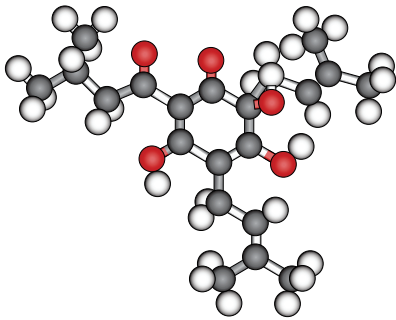
Product	Perluxan® SD	
Botanical Name	<i>Humulus lupulus</i> L.	
Plant Part Used	Cones	
Extraction	Supercritical carbon dioxide	
Carriers	Maltodextrin, food starch, silica	
Content (HPLC)	NLT 30% alpha and iso-alpha acids (humulone, cohumulone, adhumulone, iso-cohumulone and iso-adhumulone)	
	NMT 10% beta acids (lupulone, colupulone)	
Appearance	Free-flowing powder	
Color	Light green to yellow	
Aroma	Sweet	
Taste	Bitter	
Delivery	Tablets, capsules	
Total Plate Count	LT 10,000 CFU/g	
Yeast & Mold	LT 100 CFU/g	
Salmonella	negative	
E. coli	negative	
Staphylococcus aureus	negative	
Pseudomonas aeruginosa	negative	
Heavy Metals	As	<0.1ppm
	Cd	<0.1ppm
	Cs	<0.1 ppm
	Pb	<0.5ppm
	Hg	<0.1ppm

Non-GMO / Free of milk, egg, fish, shellfish, tree nuts, peanuts, wheat/gluten and soy
Hops (*Humulus lupulus* L.) is GRAS as a food ingredient (21 CFR 182.20)
U.S. Patent Nos. 7,144,590, 7,279,186, 5,604,263 and patents pending
Grown and made in the USA

perluxan®

A UNIQUE BOTANICAL ANTI-INFLAMMATORY AGENT

TECHNICAL OVERVIEW



Molecular structure of humulone

Perluxan® is a standardized hops extract containing a minimum of 30% alpha and iso-alpha acids. Perluxan® has been specifically developed to promote joint health and temporarily relieve minor daily aches and pain. This technical sheet summarizes the evidence of Perluxan®'s scientifically proven effects, as well as that of related hops fractions, resulting from years of clinical research.

Please contact us for copies of the original studies and additional technical information on Perluxan®.

Contact Info

Perluxan®

t (866) 963-2007
www.perluxan.com

Gregory Bonfilio

t (707) 766-7060
gbonfilio@pharmachemlabs.com

Proprietary Nutritionals, Inc.

a subsidiary of Pharmachem Laboratories, Inc.

265 Harrison Avenue
Kearny, NJ 07032 USA
t (519) 647-2071
f (201) 622-1415
www.pnibrands.com



© 2009 Pharmachem Laboratories, Inc.
Perluxan® is a registered trademark of Pharmachem Laboratories.

The statements in this document have not been evaluated by the FDA. This product is not intended to diagnose, treat, cure or prevent any disease.

TABLE OF CONTENTS

I.	Bioavailability	3
II.	Mechanism-of-Action (<i>in vitro</i> and <i>ex vivo</i> Studies)	4
IIa.	Inhibition of Nitric Oxide	5
IIb.	Inhibition of COX-2 Gene Expression	6
IIc.	Inhibition of COX-2 by Humulone ...	7
IId.	COX-2 Inhibition and Selectivity in Comparison to Other Botanicals and Common Drugs	8
	Hops Extract vs. Ibuprofen in Cyclooxygenase Models	9
IIe.	Selective COX-2 Inhibition	10
IIf.	COX-2 Selectivity in Head-to-Head Comparison to Ibuprofen (<i>in vitro</i>) ..	11
IIg.	COX-2 Selectivity in Head-to-Head Comparison to Ibuprofen (<i>ex vivo</i>) ..	12
IIh.	COX-2 Inhibition and Safe Use in Joint Health	13
IIi.	Inhibition Lipid Peroxidation and Radical Scavenging Activity	14
IIj.	Antioxidative Capacity	14
III.	Human Clinical Trial	15
	Human Clinical Trial Tables	16
	Product Data Sheet	17

I. Bioavailability

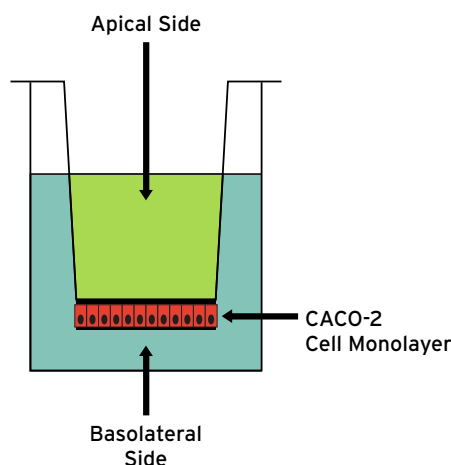
Source: E. Kuhrts: *Permeability Study of Hops Fractions Across CACO-2 Cell Monolayers*.
Lipoprotein Technologies internal file, 2003.

The bioavailability of herbal extracts is usually limited and the uptake of the proposed active ingredients is prerequisite to a beneficial effect after oral consumption.

The bioavailability of the hops extract has been tested using “*in vitro* models.” Simulated digestive processes, isolated intestinal cells and intestinal segments, and brush-border and basolateral membrane vesicles represent models for studying specific characteristics and the regulation of complex processes associated with digestion and absorption. These models have been used to investigate the effects of chemical speciation, food matrix and processing, and dietary components on the digestive stability, accessibility, and intestinal transport and metabolism of hops ingredients from foods and supplements.

The hops extract has a solubility of 0.1% at pH = 7.4. This solubility is reported as the total active principles of the extract in solution. The permeability of both components of the hops extract, humulone and iso-humulone, in the CACO-2 cell line is considered high. The absorption in this kind of model is typically 90-100% absorption potential *in vivo*.

Scheme of the CACO02 Permeability Assay

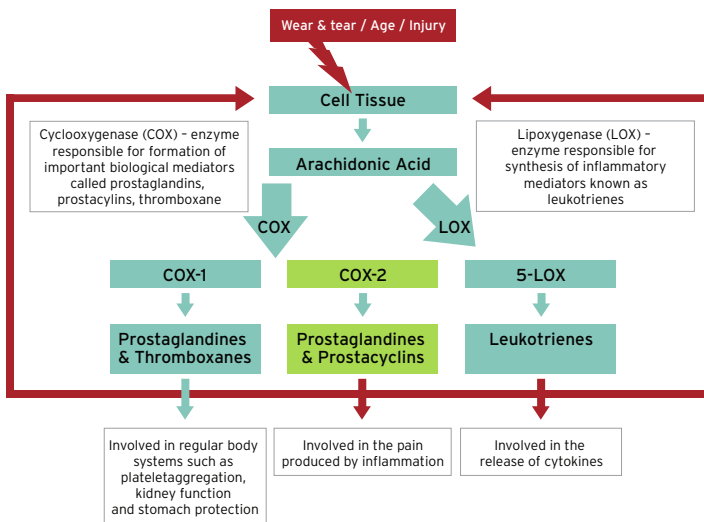


Test agent is incubated for 2 hr on either side of the monolayer (apical and basolateral), and the concentration of the test agent on both sides is measured by HPLC or LC/MS. High permeability predicts good human oral bioavailability. High asymmetry index indicates possible PGP efflux.

II. Mechanism-of-Action (*in vitro* and *ex vivo* Studies)

The potential mechanisms-of-action which may explain how Perluxan® benefits joint health have been studied in several *in vitro* and *ex vivo* studies. Research has confirmed that Perluxan® is selectively inhibiting iNOS, PGE₂ and COX-2, key pathways in the management of pain caused by inflammation.

The role of COX and LOX in inflammation and pain

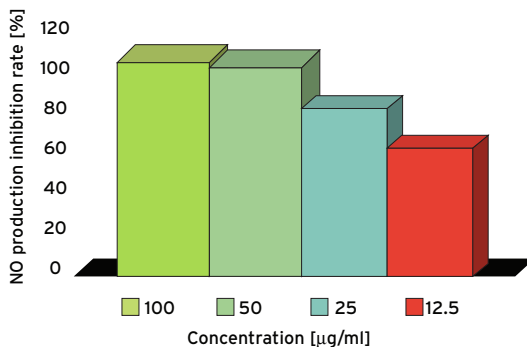


Ila. Inhibition of Nitric Oxide

Source: F. Zhao, H. Nozawa, A. Daikonnya, K. Kondo, S. Kitanaka: *Inhibitors of nitric oxide production from hops (Humulus lupulus L.)*. *Biol Pharm Bull* 2003, 26(1):61-5.

Nitric oxide (NO) plays an important role in many inflammatory responses and is also involved in carcinogenesis. In the present study, we investigated the inhibitory effect of hops extracts from *Humulus lupulus L.* on both the production of NO and the expression of inducible NO synthase (iNOS) in mouse macrophage RAW 264.7 cells. The production of NO was induced by a combination of lipopolysaccharide (LPS) and IFN-gamma, and determined by Griess assay. The expression of iNOS was detected by Western blotting. The LPS/IFN-gamma-induced production of NO and expression of iNOS were significantly inhibited by the ethyl acetate soluble fraction of *Humulus lupulus L.* Through bioactivity guided fractionation, humulene, five chalcones, 2,2-di-(3-methyl-2-butyleyl)-4,5-dihydroxy-cyclopent-4-en-1,3-dione, lupulone and three of its derivatives were isolated from the ethyl acetate soluble fraction. The chalcones, including xanthohumol, significantly inhibited the production of NO by suppressing the expression of iNOS.

Ethyl acetate soluble fraction of *Humulus lupulus L.*

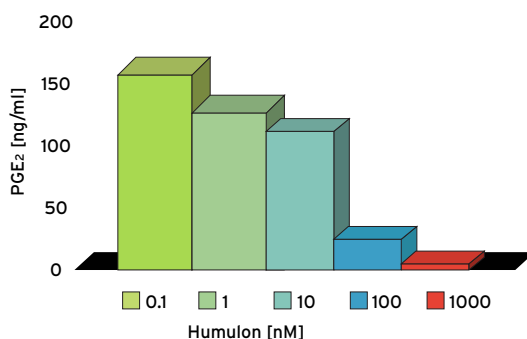


IIb. Inhibition of COX-2 Gene Expression

Source: K. Yamamoto, J. Wang, S. Yamamoto, H. Tobe: *Suppression of cyclooxygenase-2 gene transcription by humulon of beer hop extract studied with reference to glucocorticoid. FEBS Lett 2000, 465(2-3):103-106.*

In murine osteoblastic MC3T3-E1 cells which produced prostaglandin E₂ (PGE₂) as a bone resorption factor, the cyclooxygenase-2 (COX-2) induction by tumor necrosis factor alpha (TNF-alpha) was suppressed by dexamethasone with an IC₅₀ of 1nM. Humulone isolated from hops extract for beer brewing was reported previously as an inhibitor of bone resorption [Tobe, H. *et al.* (1997) *Biosci. Biotech. Biochem.* 61, 158-159]. It was shown that the compound suppressed the TNF-alpha-dependent COX-2 induction with an IC₅₀ of as low as about 30nM as demonstrated experimentally by catalytic activity assay, Northern blot analysis and promoter analysis. Reporter gene experiments suggested that humulone blocked the COX-2 expression mediated by NF-kappaB and NF-IL6, but the intracellular glucocorticoid receptor was not involved. The catalytic activity of COX-2 was inhibited by humulone with an IC₅₀ of as high as 1.6 microM. These results showed that humulone suppressed COX-2 induction at the step of transcription.

Effects of humulon on TNF α -mediated cyclooxygenase-2 induction

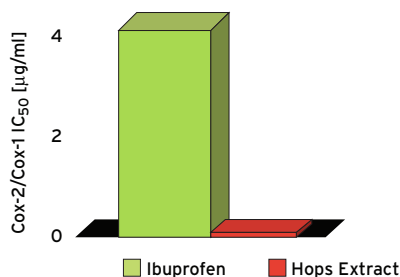


IIc. Inhibition of COX-2 by Humulone

Source: J.-C. Lee, J.K. Kundu, D.-M. Hwang, H.-K. Na, Y.-J. Surh: *Humulone inhibits phorbol ester-induced COX-2 expression in mouse skin by blocking activation of NF- κ B and AP-1: I B kinase and c-Jun-N-terminal kinase as respective potential upstream targets.* *Carcinogenesis* 2007, 28(7):1491-1498.

Humulone, a bitter acid derived from hop (*Humulus lupulus* L.), possesses antioxidative, anti-inflammatory and other biologically active activities. Although humulone has been reported to inhibit chemically induced mouse skin tumor promotion, the underlying mechanisms are yet to be elucidated. Since an inappropriate over-expression of cyclooxygenase-2 (COX-2) is implicated in carcinogenesis, the effects of humulone on COX-2 expression in mouse skin stimulated with the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA) has been investigated. Topical application of humulone (10 μ mol) significantly inhibited TPA-induced epidermal COX-2 expression. Humulone also diminished TPA-induced DNA binding of nuclear factor- κ B (NF- κ B) and activator protein-1 (AP-1). Humulone suppressed TPA-induced activation of NF- κ B and AP-1 and subsequent expression of COX-2 by blocking upstream kinases IKK and JNK.

In vitro for Cox-1/Cox-2 inhibitory potency and selectivity using a CACO-2 cell



IId. COX-2 Inhibition and Selectivity in Comparison to Other Botanicals and Common Drugs

Source: M. Lemay, M.A. Murray, A. Davies, H. Roh-Schmidt, R.K. Randolph: *in vitro* and *ex vivo* cyclooxygenase inhibition by a hops extract. *Asia Pac J Clin Nutr.* 2004, 13(Suppl):S110.

While there has been much research on botanical materials as potential pain-relieving COX inhibitors, it has not yet been demonstrated that oral consumption of botanical agents can inhibit COX-2 activity in humans. In particular it would be of interest to determine whether any botanical anti-inflammatory has COX-1-sparing activity, in order to reduce the risk of gastrointestinal side effects. This two-stage study was designed to first screen a variety of botanicals *in vitro*, and then to select one or more promising agents to test in healthy adult volunteers *in vivo*.

Method: Seventeen botanical agents, putative anti-inflammatories or pain-relievers all, were evaluated *in vitro* for COX-1 and COX-2 inhibitory potency and selectivity using a CACO-2 cell line with ibuprofen as an active control.

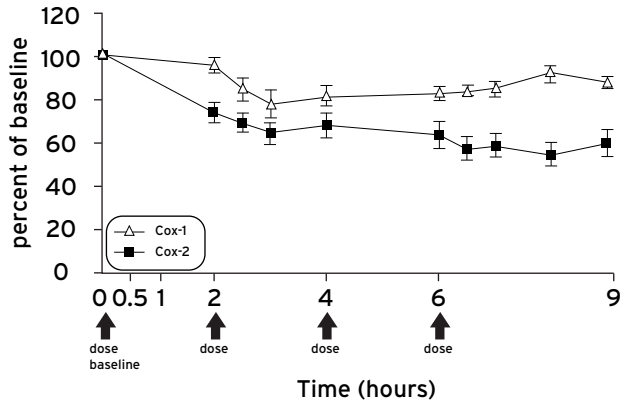
A promising compound, hops extract high in alpha acids, showed a COX-2/COX-1 IC₅₀ selectivity ratio of 0.06, compared to 4.2 for ibuprofen. Two different formulations of a standardized hops extract were compared with ibuprofen in a double-blind, randomized, *ex vivo* study. Subjects consumed hops powder extract, hops resin extract, or ibuprofen, and provided blood samples before and at intervals for 9 h following the first dose. Plasma was extracted and analyzed in a validated COX-1 and COX-2 inhibition assay.

Results: There were no differences between active treatments or ibuprofen control in COX-2 inhibitory action, as indicated by 9-hour COX-2 Area over the Inhibition Curve (AUC); however, hops extract produced a 9-hour COX-2/COX-1 AUC ratio of about 0.4 (i.e. some degree of COX-1 sparing), compared to 1.5 for ibuprofen (i.e. no COX-1 sparing).

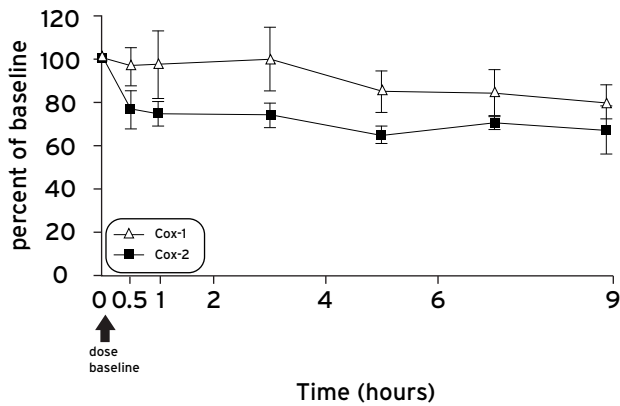
Conclusion: Hops extract exhibited COX-2 inhibition over 9 h equivalent to ibuprofen 400 mg but had significant COX-1 sparing activity relative to ibuprofen. Hops extracts may represent a safe alternative to ibuprofen for non-prescription anti-inflammation.

Hops Extract vs. Ibuprofen in Cyclooxygenase Models

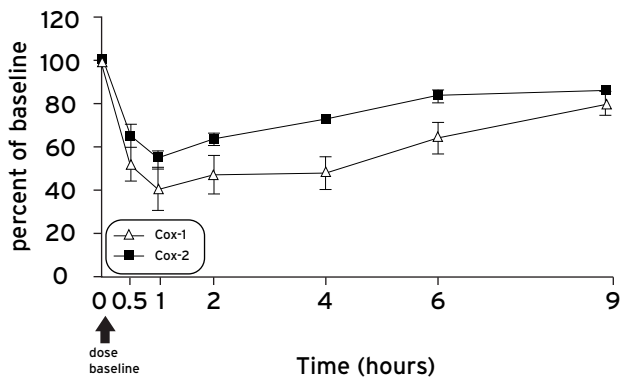
Hops powder 300mg x 4



Hops resin 450mg



Ibuprofen 400mg x 1

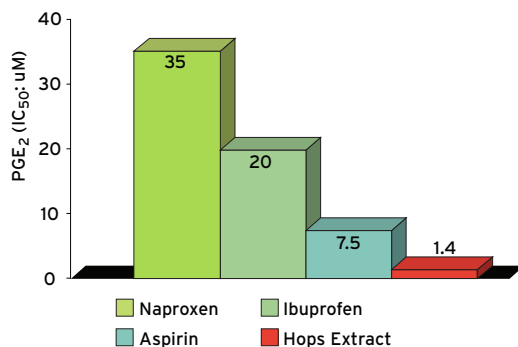


Ile. Selective COX-2 Inhibition

Source: Lidbury et al.: *The effects of AH88 on the activity of COX-1 and COX-2 using human whole blood and A549 cells. Pharmachem internal file.*

By using the "Human Whole Blood Assay" (WBA) and the "William Harvey Modified Whole Blood Assay" (WHMA), the gold standard for assessing COX inhibition and selectivity, COX-1 was measured in WBA as TXA₂ (thromboxane) and COX-2 measured in WHMA as PGE₂ (prostaglandin E₂). Hops extract was found to inhibit human COX-1 and COX-2 with 10-100 fold selectivity towards the inhibition of COX-2 at the IC₅₀ and IC₈₀ levels. As an inhibitor of COX-2 it was, in comparison to earlier published studies (Warner et al. 1999), similar or more potent than ibuprofen, and more potent than aspirin, ibuprofen or naproxen. Hops extract was more COX-2 selective in this assay than any traditional NSAID, such as aspirin, diclofenac, ibuprofen or naproxen and more selective than newer compounds such as celecoxib, etodolac, meloxicam, and rofecoxib. There have been a number of previous reports of relative selectivity of NSAIDs as inhibitors of human COX-1 and COX-2, particularly using the human whole blood assay (Patrighiani et al., 1994; Brideau et al., 1996; Young et al., 1996; Patrighiani et al., 1997). In addition, this assay was used to determine the activities of a wide range of NSAIDs (Warner et al., 1999). Analysis of data from these assays shows a good correlation between selectivity towards COX-1 and gastrointestinal toxicity (Warner et al., 1999; Mitchell & Warner, 1999). The data from the assays presented here suggest that the hops extract might not cause much gastrointestinal toxicity through inhibition of COX-1 at does sufficient to produce significant inhibition of COX-2.

COX-2 Selectivity vs. OTC NSAIDs



IIf. COX-2 Selectivity in Head-to-Head Comparison to Ibuprofen (*in vitro*)

Source: M. Lemay, M.A. Murray, A. Davies, H. Roh-Schmidt, K. Randolph: Cyclooxygenase inhibiting activity of anti-inflammatory dietary supplements. Information provided by Nutrilite Health Institute. Access Business Group LLC, 5600 Beach Boulevard, Buena Park, CA, 90622 USA.

In a CACO-2 cell line a supercritical hops extract, showed a COX-2/COX-1 IC₅₀ selectivity ratio of 0.06, compared to 4.2 for active control ibuprofen. In a pilot *ex vivo* study, 24 subjects consumed either 4 doses of hops extract, willow-hops extract, or Product X (a commercially available putative Cox-2 inhibitor), over 6 h , or 1 dose of ibuprofen 400 mg. Plasma samples obtained before and for 9 h after dosing were evaluated in a validated *ex vivo* COX assay. *Ex vivo*, the mean maximum COX-2 inhibition was $48.6 \pm 4.3\%$ from hops and $46.9 \pm 3.9\%$ from the active control [t(10) = 0.29, p = 0.77, 2-tailed]. The selectivity ratio over 9h was 0.45 ± 0.09 for hops and 1.56 ± 0.41 for ibuprofen [t(10) = 2.6. p = 0.03, 2-tailed]. The hops extract inhibited COX-2 to a degree comparable to ibuprofen, but with more favorable COX-2 selectivity, suggesting that the hops extract may be useful in reducing inflammatory prostanoids while providing long-term safety advantages.

See charts on page 9.

IIg. COX-2 Selectivity in Head-to-Head Comparison to Ibuprofen (*ex vivo*)

Source: M. Lemay, M.A. Murray, A. Davies, H. Roh-Schmidt, K. Randolph: A dietary supplement is a selective COX-2 inhibitor both in vitro and ex vivo in healthy human volunteers. Information provided by Nutrilite Health Institute. Access Business Group LLC, 5600 Beach Boulevard, Buena Park, CA, 90622 USA.

The aim of the study was to evaluate botanical extracts identified as possessing cyclooxygenase-1 (COX-1) sparing activity *in vitro* in an *ex vivo* clinical intervention and then in a tolerability study. Seventeen putative botanical anti-inflammatories or pain-relievers were evaluated *in vitro* for COX-1 and COX-2 inhibitory potency and selectivity. Two different formulations of a standardized hops extract (resin and powder) were compared with an ibuprofen control in a double-blind, randomized, human *ex vivo* study. Subjects provided blood samples before and at timed intervals for 9 h after the first dose. Plasma was analyzed in a COX-1 and COX-2 inhibition assay. There were no differences between treatments or control in COX-2 inhibition, as indicated by 9 h COX-2 Area over the Inhibition Curve (AUC). Hops powder or hops resin extract produced a 9-hour COX-1/COX-2 AUC ratio of about 0.4, compared to 1.5 for ibuprofen. The hops extracts exhibited COX-2 inhibition over 9 h equivalent to ibuprofen 400 mg but had significant COX-1 sparing activity. In the tolerability study, one formulation caused stomach upset in most subjects, but the other formulation was as well tolerated as placebo. Hops extracts may thus represent a safe alternative to ibuprofen for non-prescription anti-inflammation.

See charts on page 9.

IIIh. COX-2 Inhibition and Safe Use in Joint Health

Source: S. Hougee, J. Faber, A. Sanders, W.B. Berg, J. Garssen, H.F. Smit, M.A. Hoijer: Selective inhibition of COX-2 by a standardized CO₂ extract of *Humulus lupulus* in vitro and its activity in a mouse model of zymosan-induced arthritis. *Planta Med* 2006, 72(3):228-233.

A standardized CO₂ extract from *Humulus lupulus* L. (hops extract) was investigated for its selective COX-1/2 inhibitory properties. An *in vitro* model of inflammation using lipopolysaccharide (LPS)-stimulated human peripheral blood mononuclear cells (PBMC) was used as a model to investigate the effect of hop extract on PGE₂ production. COX-1/2 selective inhibition by the hop extract was investigated in a COX-1 whole blood assay (WBA) and a COX-2 WBA. To evaluate the *in vivo* activity of hop extract, it was administered orally to C57BL/6 mice in which inflammation of the right joint was induced by injecting zymosan intra-articularly. *Ex vivo* PGE₂ production of LPS-stimulated blood cells was determined. Also, the effect of hop extract on healthy and arthritic cartilage was investigated as well as effects on inflammatory joint swelling. Hops extract inhibited PGE₂ production by LPS-stimulated PBMC without compromising the metabolic activity of these cells. Furthermore, hop extract showed a decline in PGE₂ production in the COX-2 whole blood assay (WBA) with an IC₅₀ of 20.4 microg/mL, while in the COX-1 WBA no inhibition of PGE₂ production was observed. This indicates a COX-2 selective inhibition. The COX-1 inhibitor SC-560 inhibited PGE₂ production in the COX-1 WBA but not in the COX-2 WBA. At 2 microM, celecoxib inhibited PGE₂ production in the COX-2 WBA by 92% and in the COX-1 WBA by 50%. When hop extract was administered orally to C57BL/6 mice in which joint inflammation was induced with zymosan, PGE₂ production in *ex vivo* LPS-stimulated whole blood was significantly decreased by 24%, suggesting that hops extract becomes bioavailable. Furthermore, oral administration of hops extract showed no negative or positive effects on healthy cartilage proteoglycan synthesis, or on zymosan-induced arthritic cartilage proteoglycan synthesis. However, no effect of oral administration of 1.25 mg hops extract daily was observed on joint swelling. In conclusion, this standardized CO₂ extract of *Humulus lupulus* could be a useful agent for intervention strategies targeting inflammatory disorders and/or inflammatory pain.

III. Inhibition Lipid Peroxidation and Radical Scavenging Activity

Source: M. Tagashira, M. Watanabe, N. Uemitsu: *Antioxidative activity of hop bitter acids and their analogues. Biosci Biotechnol Biochem* 1995, 59(4):740-2.

Hop bitter acids, humulones and lupulones, were shown to have potent DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity (RSA) and lipid peroxidation inhibitory activity (LIA). Furthermore, 5-acetyl lupulones and 4-methyl lupulones had more potent LIA than native lupulones but no RSA. This result indicates that the beta, beta'-triketone moiety of the lupulones has LIA.

IIj. Antioxidative Capacity

Source: M. Ono: *Antioxidant activity of various hop fractions. JIH internal file, 2008.*

	ORAC Total (umol VtE/g) Hydro + Lypo	DPPH (umol TE/g)	HORAC (umol/CAE/g)	NORAC (umol TE/g)	SOD (KunitSODeq/g)	SOAC (umolTE/g)	FRAP (umol TE/g)
Alpha Acids	4894 (2488 + 2406)	3942	218	839	2	1748	412
Iso-Alpha acids	1837 (1623 + 214)	803	77	29	2	937	451
Vitamin C	5500						
Vitamin E	3000						

III. Human Clinical Trial

Source: R. Jäger, M. Purpura: Efficacy of oral Perluxan® intake in subjects with knee osteoarthritis: a randomized, double-blind study. Pharmachem internal file, Summary of Clinical Trial Results, 2007.

Perluxan® has been successfully tested in a human clinical trial, significantly reducing pain as early as 2 hours after administration.

Background: A randomized, double-blind, pilot study was performed to evaluate the effects of 14-days oral supplementation of the anti-inflammatory herbal extract Perluxan®, a standardized supercritical carbon dioxide hops extract containing 30% alpha acids, on individuals with osteoarthritis of the knee.

Method: A Western Ontario McMasters Osteoarthritis Index (WOMAC) symptom assessment questionnaire was used to measure improvements of pain relief before (pretest) and after (posttest) 14 days of Perluxan® (1 g x d⁻¹, n = 12 or 2 g x d⁻¹, n = 12) or placebo (n = 12) intake.

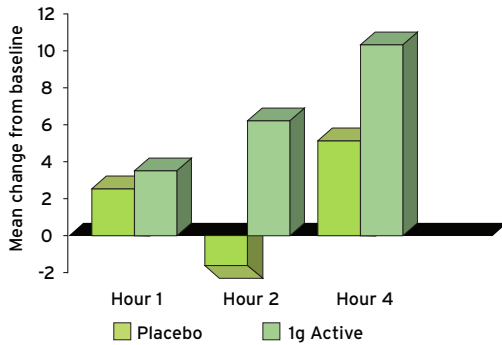
Results: *Walking on a Flat Surface:* significant improvement in pain relief with 2 g x d⁻¹ Perluxan® supplementation at hour 2 post-dosing on day 1, day 2 and 4 and with 1 g x d⁻¹ Perluxan® intake at day 2 and 6 in comparison to placebo (mean change from baseline, p < 0.05). *While in Bed:* significant improvement in mean pain relief with 2 g x d⁻¹ Perluxan® intake at day 3 and with 1 g x d⁻¹ Perluxan® supplementation at day 3, day 12 and day 13 in comparison to placebo (mean change from baseline, p < 0.05). *Sitting or Lying:* low-dose intake (1 g x d⁻¹ Perluxan® significantly improved mean pain relief (p < 0.05) in comparison to high-dose supplementation (2 g x d⁻¹ Perluxan®).

Conclusion: It is concluded that 14 days of either 1 or 2 g of oral Perluxan® supplementation significantly improved parameters of Osteoarthritis pain.

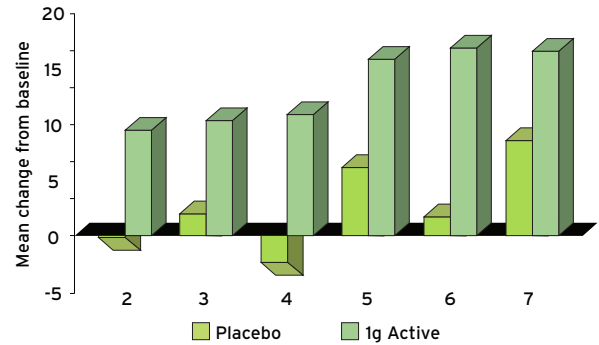
Human Clinical Trial Tables

A sampling of human clinical data derived from *Efficacy of oral Perluxan® intake in subjects with knee osteoarthritis: a randomized, double-blind study.*

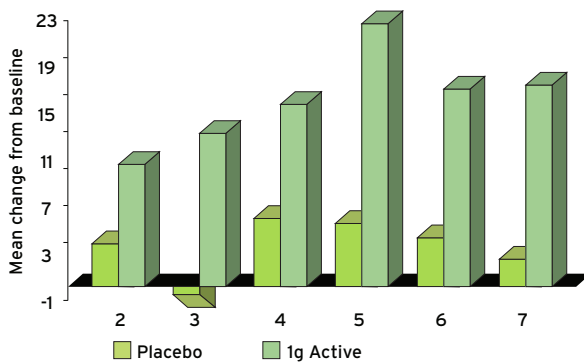
Walking on flat surface
Mean change from baseline pain scores at hours 1, 2, and 4



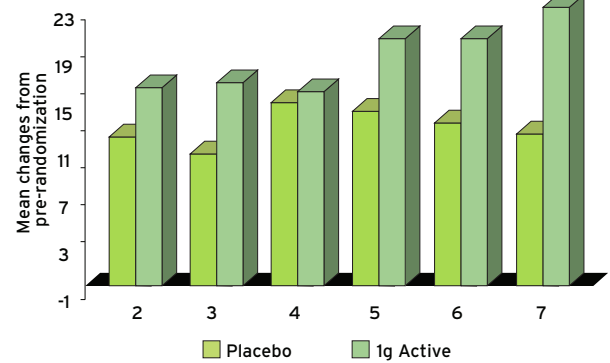
Walking on flat surface
Mean change from baseline pain scores at days 2 to 7



At night while in bed
Mean change from pre-randomization pain scores at days 2 to 7



While sitting or lying
Mean change from pre-randomization pain scores at days 2 to 7



The final report of this study is available upon request.

Product Data Sheet

Product	Perluxan® SD	
Botanical Name	<i>Humulus lupulus</i> L.	
Plant Part Used	Cones	
Extraction	Supercritical carbon dioxide	
Carriers	Maltodextrin, food starch, silica	
Content (HPLC)	NLT 30% alpha and iso-alpha acids (humulone, cohumulone, adhumulone, iso-cohumulone and iso-adhumulone)	
	NMT 10% beta acids (lupulone, colupulone)	
Appearance	Free-flowing powder	
Color	Light green to yellow	
Aroma	Sweet	
Taste	Bitter	
Delivery	Tablets, capsules	
Total Plate Count	LT 10,000 CFU/g	
Yeast & Mold	LT 100 CFU/g	
Salmonella	negative	
E. coli	negative	
Staphylococcus aureus	negative	
Pseudomonas aeruginosa	negative	
Heavy Metals	As	<0.1ppm
	Cd	<0.1ppm
	Cs	<0.1 ppm
	Pb	<0.5ppm
	Hg	<0.1ppm

Non-GMO / Free of milk, egg, fish, shellfish, tree nuts, peanuts, wheat/gluten and soy
Hops (*Humulus lupulus* L.) is GRAS as a food ingredient (21 CFR 182.20)
U.S. Patent Nos. 7,144,590, 7,279,186, 5,604,263 and patents pending
Grown and made in the USA



Perluxan®
t (866) 963-2007
www.perluxan.com



Gregory Bonfilio
t (707) 766-7060
gbonfilio@pharmachemlabs.com

Proprietary Nutritionals, Inc.
a subsidiary of Pharmachem Laboratories, Inc.
265 Harrison Avenue
Kearny, NJ 07032 USA
t (519) 647-2071
www.pnibrands.com

© 2009 Pharmachem Laboratories, Inc.
Perluxan® is a registered trademark of Pharmachem Laboratories.
The statements in this document have not been evaluated by the FDA. This product is not intended to diagnose, treat, cure or prevent any disease.

perluxan®

A UNIQUE BOTANICAL ANTI-INFLAMMATORY AGENT

CLINICAL STUDY OVERVIEW



Efficacy of oral Perluxan® intake
in subjects with knee osteoarthritis:
a randomized, double-blind
placebo-controlled study

Contact Info

Perluxean®
t (866) 963-2007
www.perluxan.com

Gregory Bonfilio
t (707) 766-7060
gbonfilio@pharmachemlabs.com

Proprietary Nutritionals, Inc.
a subsidiary of Pharmachem Laboratories, Inc.
265 Harrison Avenue
Kearny, NJ 07032 USA
t (519) 647-2071
f (201) 622-1415
www.pnibrands.com



© 2009 Pharmachem Laboratories, Inc.
Perluxean® is a registered trademark of Pharmachem Laboratories.

The statements in this document have not been evaluated by the FDA. This product is not intended to diagnose, treat, cure or prevent any disease.



A UNIQUE BOTANICAL ANTI-INFLAMMATORY AGENT

TABLE OF CONTENTS

Executive Summary	3
Background	4
Methods	4
Results	
Pain Relief Walking on a Flat Surface	6
Pain Relief While in Bed	8
Pain Relief Sitting or Lying	9
Pain Relief Standing Upright	10
Pain Relief Up and Down Stairs.....	11
Additional	12
Discussion	13
Conclusion	14

Executive Summary

Background

A randomized, double-blind, pilot study was performed to evaluate the effects of 14-days oral supplementation of the anti-inflammatory herbal extract Perluxan®, a standardized supercritical carbon dioxide hops extract containing 30% alpha acids, on individuals with osteoarthritis of the knee.

Method

A Western Ontario McMasters Osteoarthritis Index (WOMAC) 5-item symptom assessment questionnaire was used to measure improvements of pain relief before (pretest) and after (posttest) 14 days of Perluxan® (1 g × d⁻¹, n = 12 or 2 g × d⁻¹, n = 12) or placebo (n = 12) intake.

Results

Walking on a Flat Surface: significant improvement in pain relief with 2 g × d⁻¹ Perluxan® supplementation at hour 2 post-dosing on day 1, day 2 and 4 and with 1 g × d⁻¹ Perluxan® intake at day 2 and 6 in comparison to placebo (mean change from baseline, p<0.05). *While in Bed:* significant improvement in mean pain relief with 2 g × d⁻¹ Perluxan® intake at day 3 and with 1 g × d⁻¹ Perluxan® supplementation at day 3, day 12 and day 13 in comparison to placebo (mean change from baseline, p<0.05). *Sitting or Lying:* low-dose intake (1 g × d⁻¹ Perluxan®) significantly improved mean pain relief (p<0.05) in comparison to high-dose supplementation (2 g × d⁻¹ Perluxan®).

Conclusion

It is concluded that 14-days of either 1 or 2 g of oral Perluxan® supplementation significantly improved parameters of Osteoarthritis pain.

Background

The Western Ontario MacMaster Osteoarthritis Index (WOMAC) is a validated instrument designed specifically for the assessment of lower extremity pain and function in Osteoarthritis (OA) of the knee or hip. The WOMAC is a reliable and sensitive instrument for the detection of clinically important changes in health status following a variety of interventions (pharmacologic, surgical, physiotherapy, etc.). It probes clinically-important symptoms in the areas of pain, stiffness and physical function in patients with osteoarthritis of the hip and/or knee. The index consists of 24 questions (5 pain, 2 stiffness and 17 physical function).

This study investigated the pain relieving effects of short-term Perluxan® intake, a standardized supercritical carbon dioxide hops extract containing 30% alpha acids, on individuals with osteoarthritis of the knee, using the WOMAC 5-item Symptom Assessment investigating OA pain severity scores during *Walking on a Flat Surface, Up and Down Stairs, While in Bed, Sitting or Lying and Standing Upright*.

Methods

Subjects

Thirty-six subjects meeting the criteria of osteoarthritis of the knee according to the American College of Rheumatology [ACR], Class I, II or III, have been included in the study. Inclusion criteria were a VAS pain score of ≥ 30 to ≤ 80 on question 1A of the 5-item WOMAC assessment questionnaire and a Body-Mass-Index (BMI) of < 39.9 .

Study Protocol

A randomized, double-blind placebo-controlled study was performed over a period of 15 days. Subjects were required to report twice to the study site. On day one (pre-test), a physical assessment of the knees was conducted, at which the investigator confirmed the target knee selected. A WOMAC 5-item symptom assessment questionnaire (24-hour pain assessment) was completed and the time to perform a 20-meter walk on a flat surface was recorded. Height, weight, BMI and vital signs (blood pressure, heart rate, and body temperature) were measured. A fasting blood sample was collected to analyze blood works.

Subjects, who currently used NSAIDs or any other anti-inflammatory or pain medication, were instructed to discontinue taking any such products during the remainder of the study. Subjects were instructed that, if necessary, up to 2,000 mg of acetaminophen, was allowed to be taken for arthritis and other pain, but for no more than two days per week (rescue medication). The use of acetaminophen had to be discontinued 48 hours prior to visit 2, and within two hours after intake of the study product.

Subjects were instructed to record any use of acetaminophen, the amount taken, and the reason it was taken in a self-assessment and product use diary. Subjects were instructed to bring all unused study product, the study product containers and the diaries with them to the post-test and were remained to fast at least 10 hours before the post-test and not to take any study product on that day.

Methods (cont.)

The subjects reported back to the study site on day 15 (post-test). Time to perform the 20-meter walk on the flat surface was recorded and vital signs measured. A fasting blood sample was collected to analyze blood works. Study product was collected, checked for compliance, and diaries were reviewed and collected.

Demographic

The subjects age ranged from 39 to 74 years, the average age was 57 years. The majority of subjects were Caucasian (92%) and female (78%). There were no significant differences among groups on these demographics.

Experimental Conditions

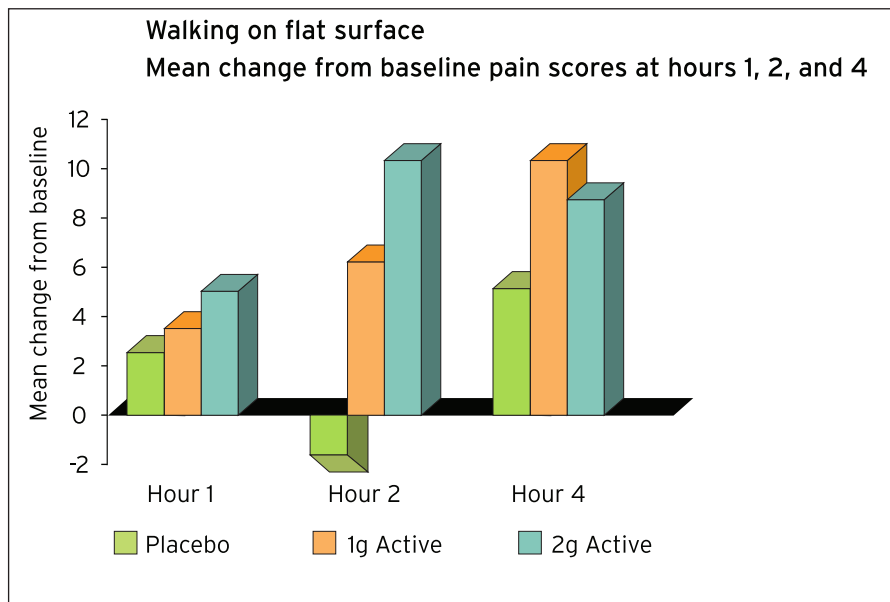
Qualified subjects were assigned in random order to either the 1 g × d⁻¹ Perluxan® (n=12), the 2 g × d⁻¹ Perluxan® (n=12) or the placebo (n=12) group. The 14-day supplementation period was started immediately after the pre-test to assess current pain (baseline) and was continued until the day before the post-test. Subjects from the Perluxan® groups received a standardized supercritical carbon dioxide hops extract containing 30% alpha acids at a rate of 1 or 2 g per day, while the others received corresponding placebo supplements.

Statistics

Between-group comparisons for all efficacy variables were conducted using t-tests. If the between group comparison showed statistic significance, an ANOVA multiple comparison, using Tukey's method was performed.

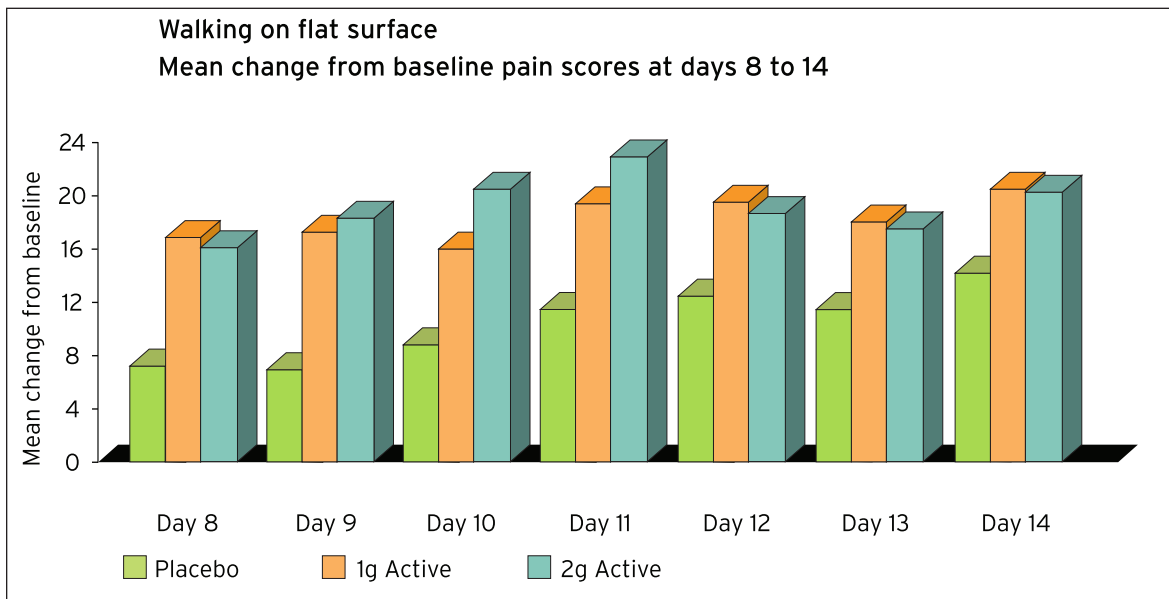
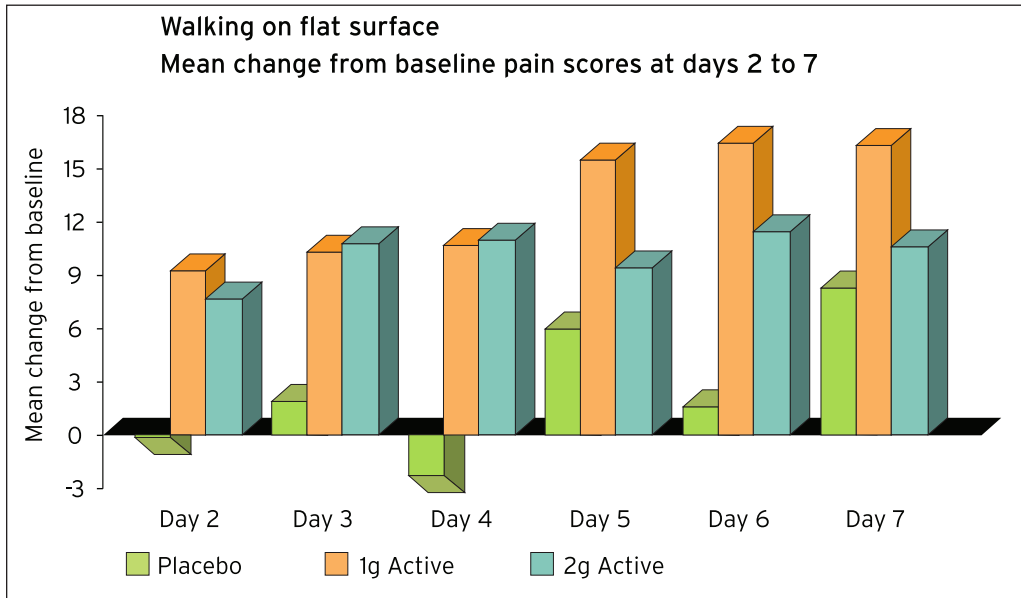
Results: Pain Relief Walking on a Flat Surface

2 g × d⁻¹ Perluxan® intake resulted in a significant (p<0.05) improvement of mean pain relief after 2 hours on the first day of supplementation. 1 and 2 g × d⁻¹ Perluxan® supplementation showed an improvement of pain relief after hours 1 and 4 in comparison to placebo; however, the effect was not statistically significant.



1 and 2 g × d⁻¹ Perluxan® intake significantly (p<0.05) improved mean pain relief at days 2 and 4 in comparison to placebo. In addition, 1 g × d⁻¹ Perluxan® supplementation resulted in a significant (p<0.05) reduction of pain relief on day 6. Both Perluxan® groups continuously showed greater pain relief than placebo.

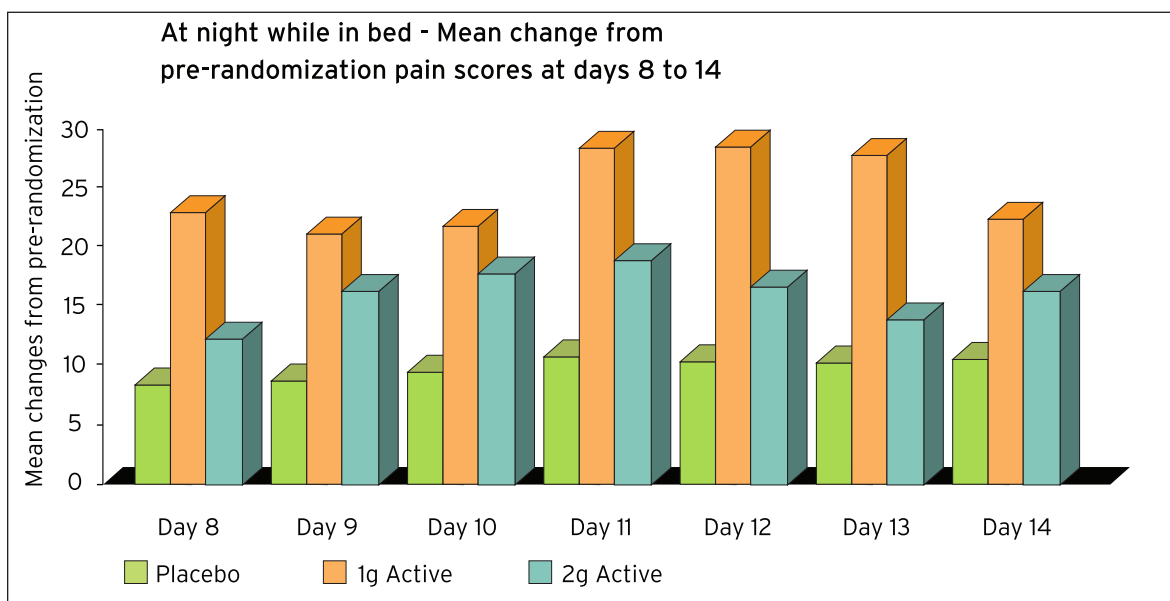
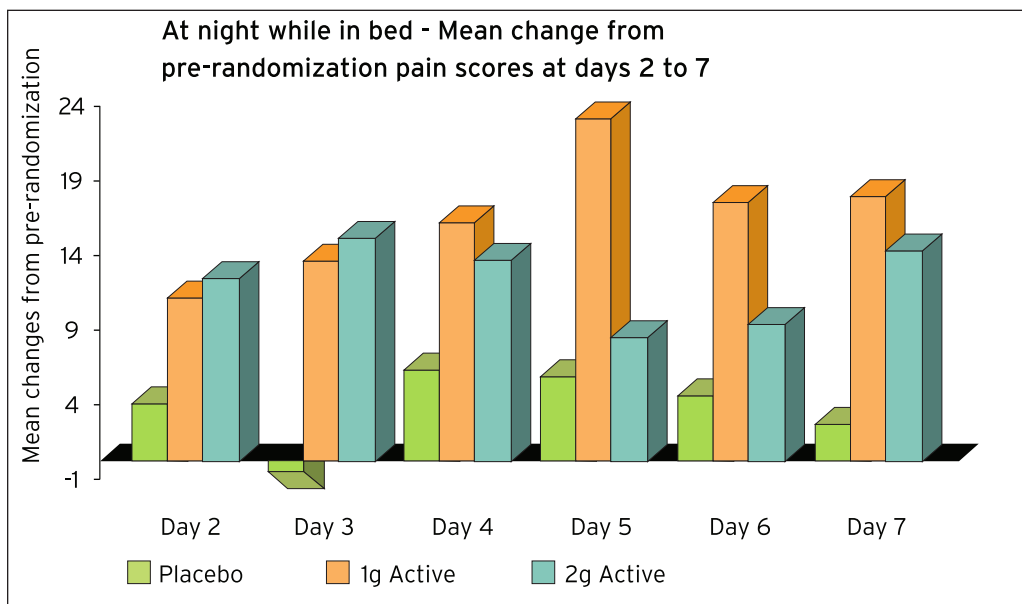
Results: Pain Relief Walking on a Flat Surface (cont.)



Results: Pain Relief While in Bed

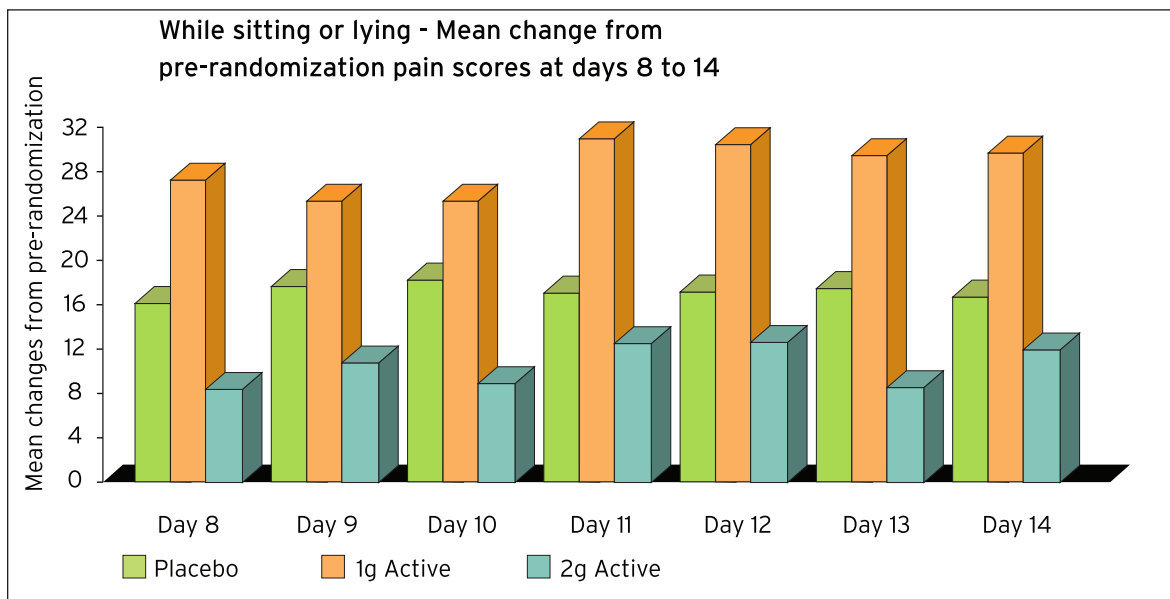
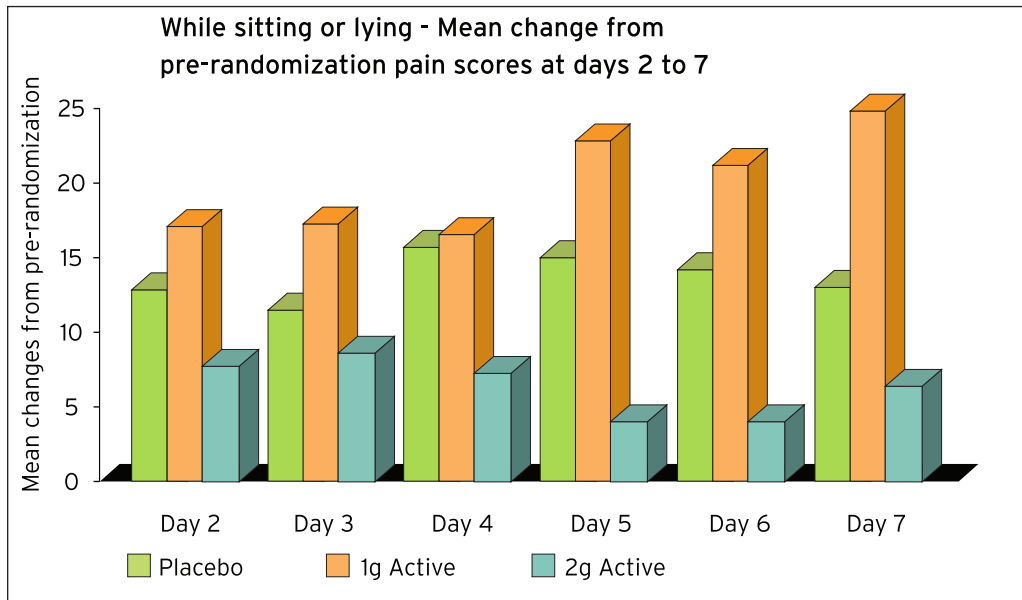
1 g × d⁻¹ Perluxan® supplementation exhibited a quick onset and continued improvement of mean pain relief while in bed in comparison to placebo. The improvements in mean pain relief were significant (p<0.05) on days 3, 12 and 13 and a sub analysis of subjects with percent improvement of ≥25% showed significant improvements (p<0.05) over placebo on days 4 and 5.

2 g × d⁻¹ Perluxan® intake seemed to have a more immediate effect on mean pain relief while in bed; however, improvement over placebo was not significant. Comparison to 1 g × d⁻¹ Perluxan® showed no additional effect.



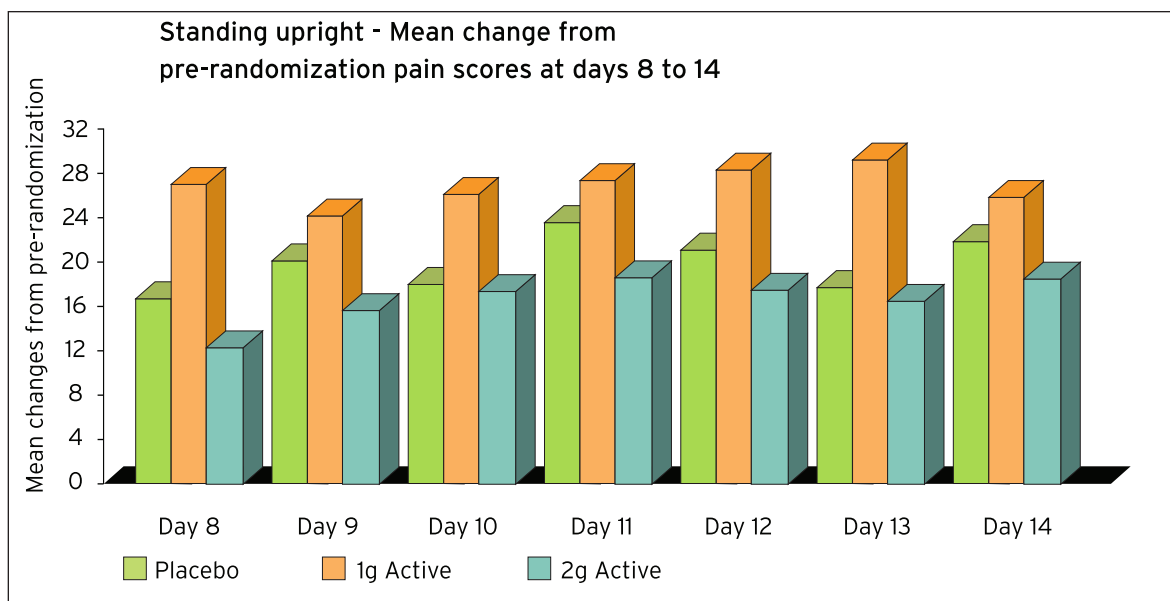
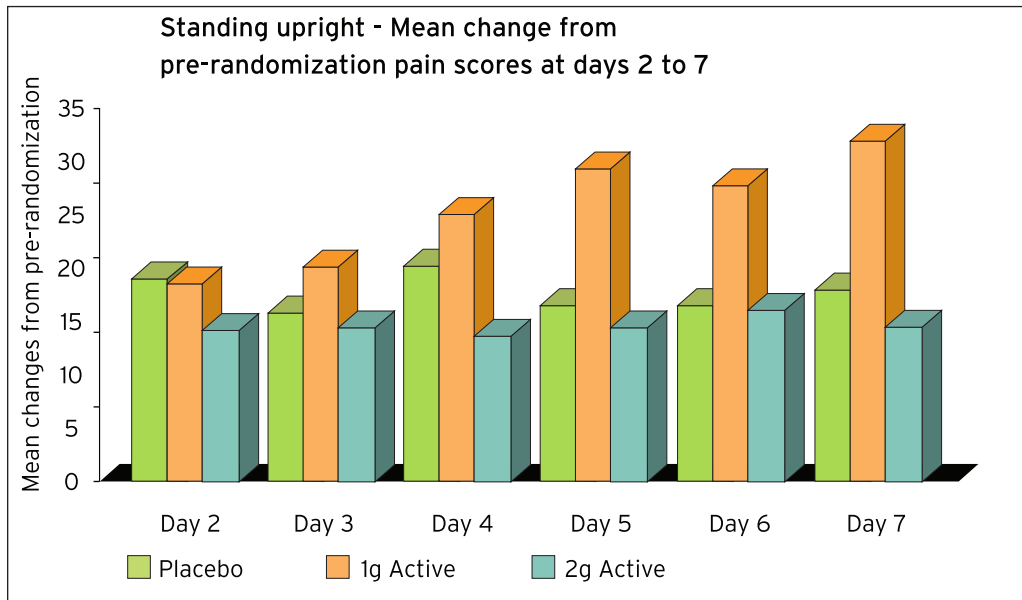
Results: Pain Relief Sitting or Lying

1 g × d¹ Perluxan® intake showed improvements of mean pain relief compared to placebo during all test days. The improvements in the 1 g × d¹ Perluxan® group were significant on days 6, 7, 8, 10 and 12 in comparison to the 2 g × d¹ Perluxan® group.



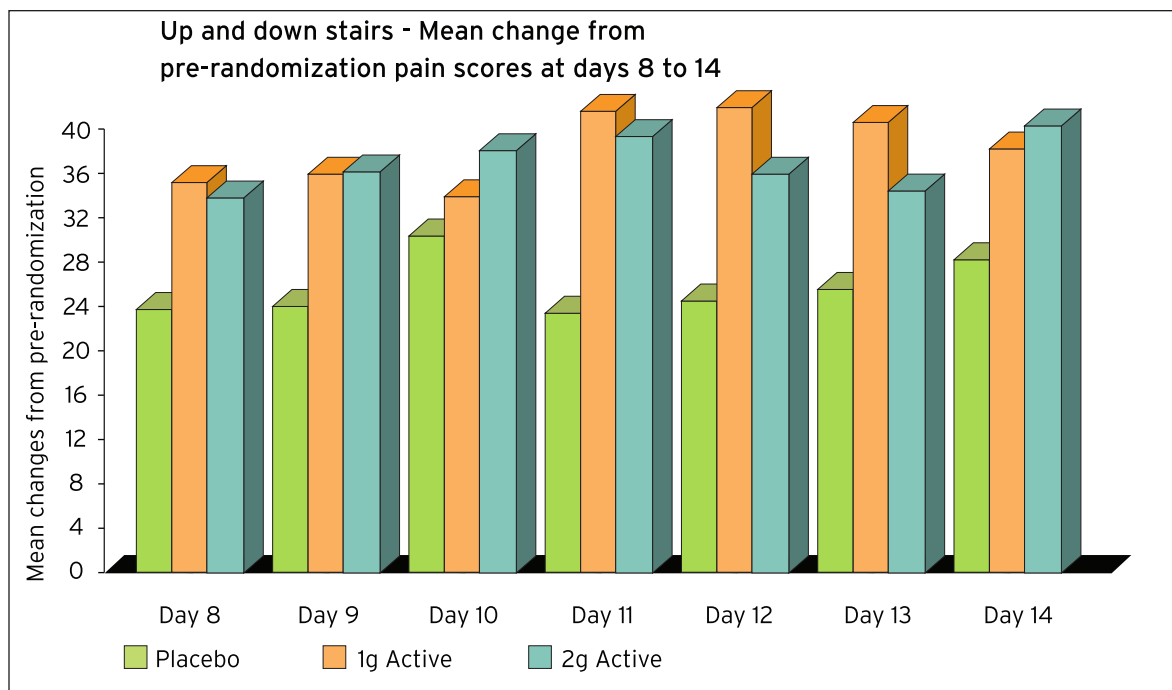
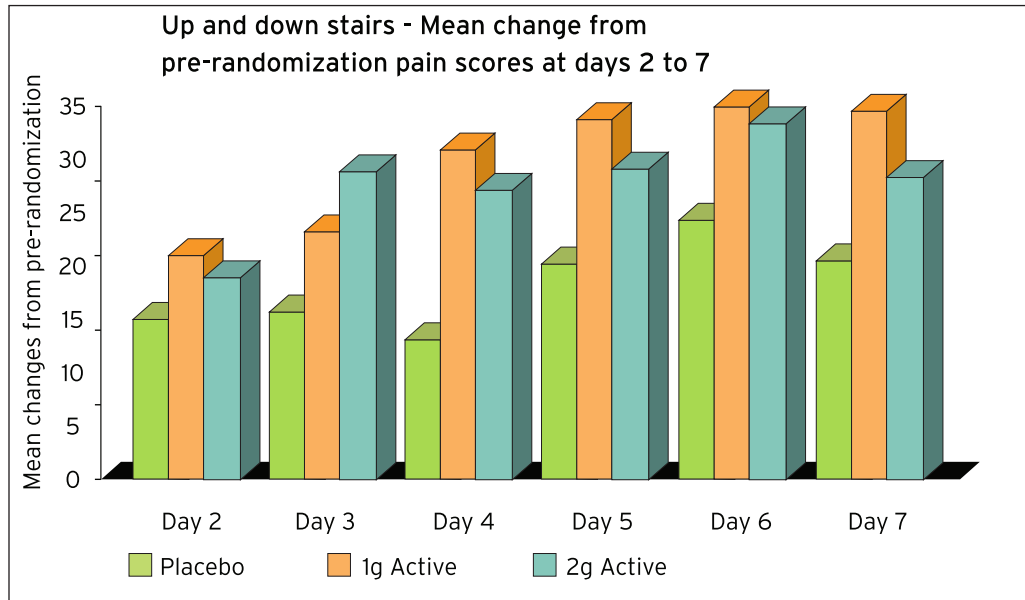
Results: Pain Relief Standing Upright

1 g × d¹ Perluxan® intake showed improvements of mean pain relief compared to 2 g × d¹ Perluxan® and placebo during all test days, however, the effects were not statistically significant.



Results: Pain Relief Up and Down Stairs

1 g × d⁻¹ Perluxan® intake showed improvements of mean pain relief compared to 2 g × d⁻¹ Perluxan® and placebo during all test days, however, the effects were not statistically significant.



Results: Additional

Twenty-meter walk

No significant difference could be measured between the placebo (21.33 seconds pre-test, 20.00 seconds post-test), the 1 g × d⁻¹ Perluxan[®] (19.00 second pre-test, 17.80 seconds post-test) and the 2 g × d⁻¹ Perluxan[®] group (19.64 seconds pre-test, 19.73 seconds post-test) in the twenty-meter walk test.

Blood works

The analysis of blood works (Na, K, Ca, Cl, CO₂, Anion Gap, Glucose, Bun, Creatinine, T Protein, Albumin, T Bilibubin, AST, ALT and ALK Phos) before and after supplementation did not show any differences between groups.

Rescue Medication

Four subjects used rescue medication (acetaminophen). Three subjects in the placebo group (on days 1, 6, and 14, respectively) and one subject in the 2 g × d⁻¹ Perluxan[®] group (on days 7 and 11).

Adverse Events

Four subjects (two in the placebo group and two in the 2 g × d⁻¹ Perluxan[®] group) experienced eight adverse events during the study. Seven of these adverse events were deemed to have no relationship to the treatment. One adverse event in the 2 g × d⁻¹ Perluxan[®] group (mild, intermittent belching) was deemed to have a possible relationship to the treatment. All adverse events were resolved by the end of the study.

Discussion

Standardized carbon dioxide extract of hops (*Humulus lupulus* L.) has been proven to be an effective strategy targeting inflammatory disorders and/or inflammatory pain in mice by selective COX-2 inhibition (Hougee et al., *Planta Med.* 2006, 72(3), 228-233). This result is consistent with Lemay et al.'s study, showing that standardized hops extract exhibited equivalent COX-2 inhibition but significant COX-1 sparing activity relative to ibuprofen (*Asia Pac J Clin Nutr.* 2004, 13(Suppl), S110) in a randomized, double-blind *ex vivo* design.

The aim of this study was to investigate the efficacy and safety of 1 and 2 g × d⁻¹ Perluxan[®] supplementation on individuals with osteoarthritis of the knee in a randomized, double-blind, placebo-controlled design.

The critical success factor for treatment of pain is a self-determined fast acting pain relieving effect of the product to motivate the individual to continuously take the supplement. Perluxan[®] intake showed a fast acting effect on mean pain relief and significant improvement over placebo could be measured after only 2-hours after the first intake.

1 g × d⁻¹ Perluxan[®] has been established as an effective dose to improve pain relief in individuals with OA of the knee, significantly improving mean pain relief while in bed, sitting or lying and walking on a flat surface. High-dose Perluxan[®] intake (2 g × d⁻¹) seemed to have no additional long-term benefit.

The effectiveness of Perluxan[®] supplementation is supported by the limited use of rescue medication in the treatment groups in comparison to placebo (three in placebo group, none in 1 g × d⁻¹ Perluxan[®] group, one in 2 g × d⁻¹ Perluxan[®] group).

The comparison of pre- and post-supplementation blood work and the adverse side effect monitoring showed that Perluxan[®] seemed to be well-tolerated.

Perluxan[®] improved pain relief with the ultimate outcome of increased function and better quality of life.

Further studies are needed to establish the lowest efficacious dose of Perluxan[®] supplementation to improve pain relief in individuals with OA and to investigate the effects of Perluxan[®] intake on non-pain related OA parameters (24-question WOMAC).

Conclusion

It is concluded that 14-days of either 1 or 2 g of oral Perluxan® supplementation significantly improved parameters of OA pain.

Patients are seeking natural alternatives to non-steroidal anti-inflammatory drugs (NSAIDs) after the realization in recent years that extreme complications, including gastric ulcers, bleeding, heart complications and even deaths have been associated with the use of NSAIDs.

Combining Perluxan® with dietary supplements (e.g. Glucosamine and Chondroitin) that may bring nutrients to the joints, might stop or even repair the damage to the joint. None of the standard pharmaceutical agents can accomplish this goal. In addition, natural COX-2 alternatives for pain and arthritis relief might offer cost advantages over drugs.

Perluxan® has been proven to be an effective alternative for pain relief in individuals with osteoarthritis of the knee.

**Perluxan®**

t (866) 963-2007
www.perluxan.com

**Gregory Bonfilio**

t (707) 766-7060
gbonfilio@pharmachemlabs.com

Proprietary Nutritionals, Inc.

a subsidiary of Pharmachem Laboratories, Inc.

265 Harrison Avenue
Kearny, NJ 07032 USA
t (519) 647-2071
www.pnibrands.com

© 2009 Pharmachem Laboratories, Inc.

Perluxan® is a registered trademark of Pharmachem Laboratories.

The statements in this document have not been evaluated by the FDA. This product is not intended to diagnose, treat, cure or prevent any disease.

perluxan®

A UNIQUE BOTANICAL ANTI-INFLAMMATORY AGENT

WHITE PAPER



Contact Info



Perluxan®

t (866) 963-2007
www.perluxan.com

Gregory Bonfilio

t (707) 766-7060
gbonfilio@pharmachemlabs.com

Proprietary Nutritionals, Inc.

a subsidiary of Pharmachem Laboratories, Inc.

265 Harrison Avenue
Kearny, NJ 07032 USA
t (519) 647-2071
f (201) 622-1415
www.pnibrands.com

© 2009 Pharmachem Laboratories, Inc.
Perluxan® is a registered trademark of Pharmachem Laboratories.

The statements in this document have not been evaluated by the FDA. This product is not intended to diagnose, treat, cure or prevent any disease.



A UNIQUE BOTANICAL ANTI-INFLAMMATORY AGENT

TABLE OF CONTENTS

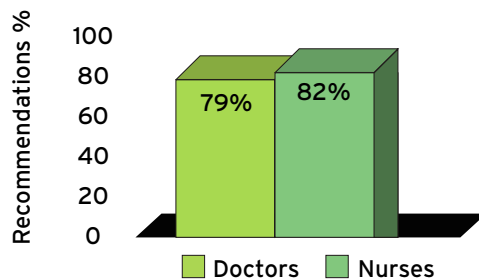
Dietary Supplement Industry Overview	3
Herbal Supplements Gain in Popularity and Use	4
Inflammation and It's Relationship to Disease	5
A Brief History of Hops	8
Perluxan®: A Leap Forward in the Evolution of Natural Medicine	9
Perluxan®: Clinically Confirmed for Safety & Efficacy	11
Education and Support	12
Perluxan®: A Unique Solo or Combo Ingredient	13

Dietary Supplement Industry Overview

Sales in the global nutraceutical industry are projected to reach \$187 billion by 2010, buoyed by increasing sales in traditional markets like the U.S. and the European Union (EU), and by emerging markets such as China and India. The growth of the top performing products in the estimated \$22 billion U.S. dietary supplement market is rising at double-digit rates thanks to growing consumer focus on health, better industry regulation and trends geared towards greater globalization and financing.

The percentage of Americans using dietary supplements is up several percentage points, according to an annual survey conducted by Ipsos-Public Affairs for the Council for Responsible Nutrition (CRN), Washington, D.C.. "Consumers' use of dietary supplements remained fairly consistent in 2007, with 68% of American adults saying that they take dietary supplements, compared to 66% the year before," the report indicates. "Interestingly, this year's survey showed that more adults than last year consider themselves to be 'regular' users of dietary supplement products, with 52% of Americans identifying themselves in that category, up from 46% in 2006." Consumers also have more reinforcement of their interest in dietary supplements. CRN's recent Healthcare Professionals Impact Study found that "more than three-quarters of U.S. physicians (79%) and nurses (82%) recommend dietary supplements to their patients." (Source: Nutraceuticals World).

Physicians Recommending Supplements (%)



Overall growth during 2007-2008 is estimated in the 4.5% to 6% range, with some variations. A-to-Z nutrients, like multivitamins, will stay in the big box stores as low-growth-rate commodities, and some botanicals are down in sales if not negative. But there is tremendous consumer interest in probiotics, certified organic cosmetics, home care and pet care; some of these are easily showing double-digit growth, and some are growing in the 20% to 40% range.

According to Natural Marketing Institute's (NMI) 2007 Health & Wellness Trends Survey, the top health categories for supplements are weight loss, heart health, digestion, pain relief (arthritis or joint pain), seasonal allergies, diabetes, vision and eye health. According to the NMI survey, the top supplements used by consumers in 2007 were, in order of frequency, multivitamins, calcium, vitamin C, fish oil, vitamin E, antioxidants, vitamin B/B complex and omega-3s. The largest increases in 2007 consumer use came from fish oil and omega-3s. Similarly, the largest use decrease during the same period was for soy supplements.

Herbal Supplements Gain in Popularity and Use



Herbal dietary supplement sales in the United States demonstrated growth in multiple market channels during both 2006 and 2007. A report published in the latest issue of the nonprofit American Botanical Council's (ABC) quarterly journal *HerbalGram*, indicates that total herb supplement sales rose over 4% each year within the U.S. market. The report features herb supplement sales statistics from Information Resources Inc. (IRI) and supplement industry market research firm Nutrition Business Journal (NBJ).

According to the article, total sales of herbal dietary supplements within all sales channels of the US market reached approximately \$4.8 billion in 2007. This figure represents a 4.4% increase in total herb supplement sales from 2006 and indicates a continued trend of steady growth over the past few years. NBJ made this estimate from data derived from surveys of supplement companies, interviews with major retailers and industry experts, and various published and unpublished secondary material. The sales data for supplements does not include sales for herbal teas.

IRI has calculated herb supplement sales in FDM (i.e., various food, drug, and mass market retailers) to be \$268 million for 2007. The IRI data is considered by many industry experts as some of the most reliable econometric sales data available on herbal supplements for this specific market channel, though it does not include sales from Wal-Mart, Sam's Club, other large warehouse buying clubs or convenience stores. IRI's 2007 herb supplement sales statistic represents an increase of 7.6% over its 2006 figure for herb supplement sales and approximately 5.6% of the total estimated market. (If Wal-Mart and other sales were included, the total for the FDM channel would be at least 15% of total herb supplement sales.)

"These statistics reflect increased consumer interest in taking more responsibility for their health," said Mark Blumenthal, founder and executive director of ABC, and a co-author of the market report. "Many Americans take herbs, other dietary supplements, herbal teas and related products as part of their daily dietary and health routines. These products are becoming more mainstream and less 'alternative' for millions of people."



Inflammation and its Relationship to Disease

Inflammation is a process by which the body's white blood cells and chemicals protect us from infection and foreign substances such as bacteria and viruses. In some diseases, however, the body's defense system (immune system) inappropriately triggers an inflammatory response when there are no foreign substances to fight off. In these diseases, called autoimmune diseases, the body's normally protective immune system causes damage to its own tissues. The body responds as if normal tissues are infected or somehow abnormal.

Diseases associated with the misdirected inflammation include varying forms of arthritis, cardiovascular disease, cancer, osteoporosis, Alzheimer's disease, diabetes and obesity.

Arthritis, or painful inflammation and stiffness of the joints is highly prevalent throughout our aging population. The following are some key statistics:

- 58 million American adults with arthritis (CDC, 2006, based on 2004 data).
- 46.5 million (22%) have doctor-diagnosed arthritis (28 million women & 18.5 million men) (CDC, 2006, based on 2004 data).
- 11.5 million (according to CDC) or 23.2 million (according to Arthritis Foundation) live with chronic pain but have not been diagnosed by a doctor.
- 51% of adults 75 years and above reported having an arthritis diagnosis (Vital Health Stat. 2004; 10(222); based on 2002 data).
- 66% of adults with doctor diagnosed arthritis are overweight or obese (unpublished CDC data, based on 2002 data).
- Doctor diagnosed arthritis is expected to reach 67 million by 2030 (Arthr Rheum 2006, based on NHIS 2003 data).
- Costs related to Arthritis are \$86.2 billion dollars annually (Arthritis Foundation, MMWR 2004, based on 1997 data).
- Arthritis is the leading disability for Americans (more than 39 million medical visits each year and second only to heart disease as a cause of work disability (Arthritis Foundation).
- Individuals with arthritis have significantly worse health-related quality of life (HRQOL) (J Rheum 2003).
- Women, particularly menopausal women, have the most severe arthritis (hormone related).



Inflammation is characterized by redness, swollen joints warm and sore to the touch, joint pain, stiffness and loss of joint function. Inflammation may also be associated with “flu-like” symptoms including fever, chills, fatigue and loss of energy, loss of appetite and muscle stiffness.



When inflammation occurs, chemicals from the body’s white blood cells are released into the blood or affected tissues in an attempt to rid the body of foreign substances. This release of chemicals increases the blood flow to the area and may result in redness and warmth. Some of the chemicals cause leakage of fluid into the tissues, resulting in swelling and pain.

Additionally, the increased number of cells and inflammatory substances within the joint cause irritation, wearing down of cartilage (cushions at the end of bones) and swelling of the joint lining.

Inflammation can also affect internal organs. Inflammation of the heart (myocarditis) may cause shortness of breath or leg swelling.

Inflammation of the small tubes that transport air to the lungs may cause an asthma attack. Inflammation of the kidneys (nephritis) may cause high blood pressure or kidney failure, and inflammation of the large intestine (colitis) may cause cramps and diarrhea. There are a number of treatment options commonly used for inflammatory diseases including medications, rest and exercise, and surgery to correct joint damage.

Conventional treatment options include non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, ibuprofen or naproxen and corticosteroids (such as prednisone). NSAIDs are inhibitors of the enzymes Cox-1 & Cox-2. Cox-1 is known to both protect the GI tract and control the production of prostaglandins primarily linked to inflammation and pain.

NSAID’s are available in either prescription or over-the-counter (OTC) forms and fall into three basic categories: traditional NSAIDs, selective COX-2 inhibitors and salicylates.

- Traditional NSAIDs: With 20 prescription medications in the group—three of which are available in lower-strength, non prescription doses—traditional NSAIDs are the largest subset of the NSAID class.
- COX-2 Inhibitors: Like traditional NSAIDs, COX-2 inhibitors help reduce pain and inflammation but are designed to be safer for the stomach. Digestive tract studies have shown less stomach damage from COX-2 inhibitors compared to traditional NSAIDs; however, COX-2 drugs do not have as much history of use.
- Salicylates: The original category of NSAIDs, the salicylates (suh-LIS-uh-lates), includes aspirin and is still preferred by many patients and doctors. Frequent large doses, which can cause some serious side effects, usually are needed to control arthritis pain and inflammation. (Source: Arthritis Foundation)

The widespread use of NSAIDs has meant that the adverse effects of these drugs have become increasingly prevalent, because both the negative and positive effects are dose-dependent. An estimated 10-20% of NSAID patients experience GI adverse events estimated to result in 103,000 hospitalizations and 16,500 deaths per year in the U.S. and represent 43% of drug-related emergency visits. (Source: Dr. Gary A. Green, MD).

The following is a more comprehensive listing of known NSAID side-effects: (Source: U.S. Food and Drug Administration)

NSAID's Known Side Effects	
Serious side effects	Other side effects
Heart attack	Stomach problems
Stroke	Constipation
High blood pressure	Diarrhea
Heart failure from fluid retention	Gas
Kidney problems, kidney failure	Heartburn
Bleeding in stomach and intestines	Nausea
Low red blood cells, anemia	Vomiting
Life threatening skin reactions	Dizziness
Liver problems, liver failure	
Asthma attacks	

A growing number of those afflicted with pain and inflammation associated with arthritis, and other common ailments, seek natural treatment alternatives that do not carry the dangerous side-effects experienced with prescription or OTC medications. One botanical or 'phyto' medicine becoming increasingly popular as a safe and effective alternative for the treatment of inflammatory conditions and related pain and discomfort is a specialized extract of a traditional medicinal herb: *Humulus lupulus* or Hops.

A Brief History of Hops

The female flower cone of the hops plant (*Humulus lupulus* L.), a climbing vine native to North America, Europe and Asia, is primarily used to add flavor to beer. Numerous cultivators of hops are selectively utilized by brewers to balance the sweetness of malt with bitterness, adding flowery, fruity and herbal aromas. The earliest references to hop cultivation are during the 8th and 9th century AD from the Hallertau district in Germany. Although it is not clear whether these hops were used in brewing, by the 14th century the Dutch had developed a taste for hopped German beer.

Medieval brewers were initially reluctant to use hops in beer, claiming it caused “melancholy and tormenting disease.” Hops should be avoided during depression. Hop tea is a nerve tonic, a mild sedative and a muscle relaxant. The estrogen content increases lactation and is an aphrodisiac for men. Exported by the Flemings, hopped beer found its way across the English Channel in the early 1400s. Although hops were brought to England by the Romans for use as a vegetable, the English brewers were appalled at its use in beer. It was many decades before the merits of hopped beer were accepted and hops incorporated into mainstream brewing practices in England.

Hops were brought during the early 17th century to the colonies in North America. American colonists brewed with imported hops, spruce bark and sassafras root, until 1629 when the Massachusetts Bay Co. ordered hop seeds from England. The colonists employed hops in many ways. They extracted wax for use as a reddish-brown dye, used the fibers for textiles and ate the young shoots. By 1859 nearly 90 percent of the hops used in the United States were grown in New York. But as land



began to fill with farmers and cities, hop farms were pushed West. California's Central Valley became a hop-growing region, and from there, hop farms quickly spread north to Oregon and Washington where they are still commercially grown today.

The unpollinated female hop is higher in the humulone fractions which give beer its bitter flavoring. The cone of the female hop is made of many overlapping petals. At the base of each petal is the seed where sticky yellow glands produce the resins and oils that provide aroma and bitterness. The petals also contain tannin which aids in clarifying the beer. Today one can find numerous strains of New World and Old World hops. The United States is second in hop production only to Germany. With a bit of practice many around the world have begun to recognize the distinctive tastes of various available hop strains.

Perluxan®: A Leap Forward in the Evolution of Botanical Medicine

Hops has numerous traditional therapeutic applications, including the treatment of anxiety and insomnia due its mild sedative and relaxing effects, and is used during menopause for its high phytoestrogen content. However, it was not until recently that the anti-inflammatory activity of certain hops fractions, primarily derived from resinous extracts, was discovered. The so called alpha acids, isolated and enriched by state-of-the-art supercritical carbon dioxide extraction technology, have shown great potential in helping support the body's natural response to inflammation and pain. Numerous early laboratory studies discovered what was later confirmed by human clinical research: hops is a potent and well-tolerated anti-inflammatory agent.

Perluxan® is a stable, free-flowing aromatic powder standardized to contain a minimum 30% alpha and iso-alpha acids (humulone, co-humulone). At low doses, the compounds may inhibit bone resorption by reducing the transition of osteoblasts to osteoclasts; at higher doses, the humulone fractions reduce inflammation and associated pain by isolating the actions of prostaglandin E₂ and other inflammatory enzymes. In theory, Perluxan® offers both a non-estrogenic solution to bone loss while also providing relatively fast pain relief due to its anti-inflammatory mechanisms.

To help better understand the evolution of Perluxan® it is important to note that, during the late 1990's, a handful of research companies began looking for natural ways to specifically improve joint health and ease minor pain. Extensive literature reviews of 230 botanicals in combination with a state-of-the-art screening assay, determined the overall effectiveness of anti-inflammatory agents extracted from plants. The early botanical cell assays yielded about 20 candidates, and these herbal finalists participated in a series of proof-of-principle studies alongside OTC and prescription drugs to investigate their potential of eventually becoming a natural, efficacious alternative to many of the drugs and supplements used to control the body's inflammation process and reduce minor pain.

Of those 20 prospects, one ingredient was identified as having the highest chance of success. This ingredient, a standardized extract of hops previously known for its antioxidant and sedative properties, was subsequently tested at the respected William Harvey Institute in the U.K. using the gold-standard William Harvey Whole Blood Assay.

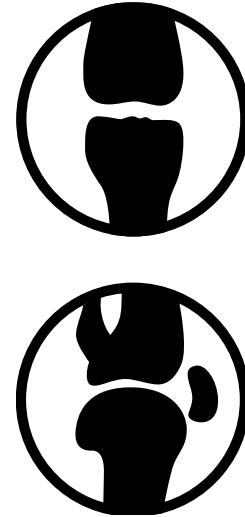
The powdered extract used in the research, known today as Perluxan®, was specifically designed to contain high amounts of naturally occurring alpha acids and was characterized by a good solubility and bioavailability when compared to the other botanicals. The extract was also made to be virtually free of both phytoestrogens and sleep-inducing compounds, to maximize its safety and efficacy in joint health.

The chart below helps to better understand Perluxan's key features, benefits and proven mechanisms of action.

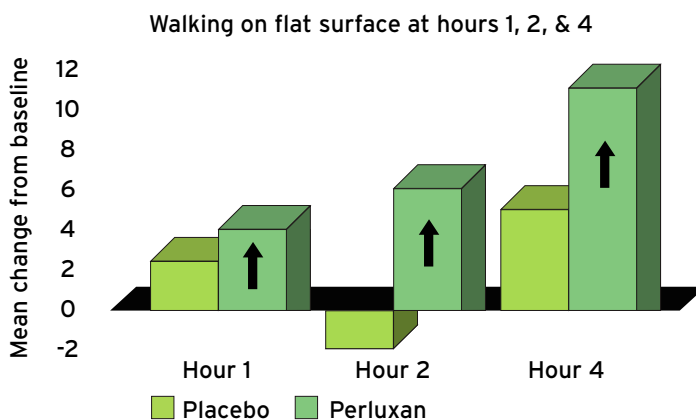
Perluxan®	
Standardized	<ul style="list-style-type: none"> • A proprietary extract of hops cones with a specific content of alpha acids • Designed to be virtually free of phytoestrogenic and sleep inducing compounds
Researched	<ul style="list-style-type: none"> • Documented multiple mechanisms of action in human <i>in vitro</i> and <i>in vivo</i> studies • The subject of extensive ongoing research and human clinical studies • Worldwide patents and patents pending
Safe & Effective	<ul style="list-style-type: none"> • A GI friendly alternative for the temporary relief of minor everyday aches and pains • Supports joint health and mobility • Well-tolerated and easily absorbed • Fast-acting
Versatile	<ul style="list-style-type: none"> • Anti-inflammatory • Anti-oxidative • Bone resorption inhibitive
Key Mechanisms of Action	<ul style="list-style-type: none"> • Moderately selective COX-2 inhibitor • Potent inhibitor of PGE₂ • Mildly lowers TXA₂ • Positive effects on gene expression • Supports joint and bone health • Bioavailable and well tolerated in tablet and capsule form
Dosage	<ul style="list-style-type: none"> • Loading phase - 500/mg 2 to 3 times per day • Maintenance - 500/mg once per day • No maximum

Perluxan®: Clinically Confirmed for Safety & Efficacy

The effect of a standardized supercritical carbon dioxide hops extract containing 30% alpha acids (Perluxan®) on individuals with osteoarthritis of the knee was investigated in a randomized, double-blind, placebo-controlled study. Thirty-six (36) individuals ingested either placebo or Perluxan® in capsule form, and the effect of the ingredient to improve pain relief after 14 days of supplementation (post test) was measured by WOMAC (symptom assessment questionnaire) and compared to starting values (pre test). The WOMAC is a validated instrument designed specifically for the assessment of lower extremity pain and function in Osteoarthritis (OA) of the knee or hip and is a reliable and sensitive instrument for the detection of clinically important changes in health status. This study investigated pain severity scores during everyday activities such as *Walking on a Flat Surface, Up and Down Stairs, While in Bed, Sitting or Lying and Standing Upright*.



The subjects' ages ranged from 39 to 74 years; the average age was 57 years. The majority of subjects were Caucasian (92%) and female (78%). The critical success factor for treatment of pain was a self determined fast acting pain relieving effect of the product to motivate the individual to continuously take the supplement. Perluxan® intake showed a fast acting effect on mean pain relief and significant improvement over placebo could be measured after only two (2) hours following the first dose. By the end of the second week, it was clear that Perluxan® helped to relieve minor pain during normal daily activities and may have improved joint mobility in the active group. It was concluded that 14 days of oral Perluxan® supplementation significantly improved parameters of osteoarthritis pain. The effectiveness of Perluxan® was also supported by the extremely limited use of rescue medication in the treatment groups in comparison to placebo. The comparison of pre-and post-supplementation blood work and the adverse side effect monitoring also showed that Perluxan® was well-tolerated and led to no gastrointestinal discomfort. The chart below serves to spotlight Perluxan's fast acting effect on pain relief at 1, 2, and 4 hours during the everyday activity of walking. (Clinical Study Overview available upon request.)



Education and Support

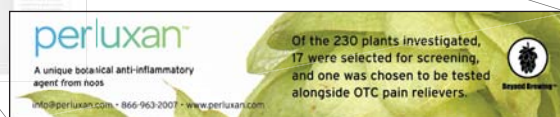
Proprietary Nutritionals Inc. (PNI) understands the importance of building brand awareness for specialty supplement products and is committed to ongoing marketing support for the long-term. Work is currently underway to develop an effective media strategy for Perluxan® with the goal of making both the trade and the consumer highly familiar with this unique nutraceutical brand. Planning is currently underway to initiate the following programs:

- **National Consumer Education and Branding Programs** These programs will demonstrate a strong initiative with considerable muscle to move product off retail shelves. The aim is to educate the consumer on the significant differences between Perluxan® and all other competitive brands on the U.S. market. PNI has chosen a consumer communication organization they believe will be most effective in disseminating the Perluxan story and to gain optimum awareness for the brand. The overall program will include national TV, radio & print vehicles.
- **Pharmacist Education Program** PNI also plans to educate pharmacists and pharmacy technicians via educational CE programs that allow these health care professionals to learn in detail about the anti-inflammatory/pain category and the unique features and benefits related to Perluxan.
- **Mainstream Executive Education Program** In the U.S., the plan is to align with top FDM trade journals to serve as an educational conduit as well as a national source of advertising. The program will be designed for Executives, Buyers, Pharmacists and Technicians at Retail Chain Pharmacy across Food Drug/Mass & Wholesaler channels. Reaching executives, Pharmacists and Techs in combination with online exposure to store level-executives at retail pharmacy is an all encompassing winning combination.

Initial trade exposure for Perluxan® will be implemented in key natural product health related trade journals. These will include:



- Nutritional Outlook
- Nutraceuticals World
- Functional Ingredients
- Natural Products Insider



Throughout 2009 and in the future, these trade journals and others will carry the Perluxan® message via full-page, full-color print advertisements. The Perluxan® brand will also have an active presence on industry websites and blogs, with distinctive, dynamic and identifiable advertising linking visitors to technical and marketing materials.

Perluxan®: An Effective Solo or Combo Ingredient

Perluxan® is a unique all-natural anti-inflammatory supplement primarily targeted for the treatment of joint discomfort and has been shown clinically to act as a safe and efficacious natural medicine alongside conventional OTC drug therapies.

It is interesting to note that the proven healing benefits inherent within a growing number of botanical, alternative remedies are now a primary mode of treatment for arthritis versus many long-standing pharmaceutical/OTC drug therapies. Millions of consumers now realize the overwhelming benefits of going the natural route when it comes to maintaining optimum health and well-being, asking the question, "Why medicate with conventional therapies that compromise the body's innate healing processes when you can reach a positive treatment result naturally?"

Perluxan® can be utilized as a stand-alone remedy or integrated as part of a more comprehensive formulation. There are a multitude of such options that exist, but one example that stands out as special in terms of product efficacy and overall sales potential is the union of Perluxan® with glucosamine.

Glucosamine is a naturally derived form of amino sugar that is believed to play a role in cartilage formation and repair. Glucosamine is extracted from from crab, lobster or shrimp shells; a vegetarian form can also be fermented using a patented process from corn biomass.

Studies have shown that some people with mild to moderate osteoarthritis (OA) taking glucosamine reported pain relief at a level similar to that of NSAIDs such as aspirin and ibuprofen. Some research indicates that glucosamine supplements might also slow cartilage damage in people with OA (Source: Arthritis Foundation). And yet, the main problem with glucosamine supplementation is that the ingredient takes weeks to work in humans via oral dosing and is often discarded by the consumer before the effects can be experienced.

Those who suffer from arthritis have both short-term and long-term health concerns; each sufferer desires a speedy respite from the pain and discomfort associated with this disease condition as well as bodily repair in the long term. However, because the proven health benefits associated with glucosamine take time and do not manifest for several weeks once the course of treatment has been initiated, in the short-term the user is left in a state of physical discomfort that often initiates the drive towards OTC or pharmaceutical pain relievers and the termination of any natural course of treatment.

A formulation inclusive of Perluxan® and glucosamine is highly complimentary and exemplary of a unique natural medicine with broad scale “experiential” efficacy. Perluxan® in synergy with glucosamine provides a clear solution by delivering the key “dual action” benefits of short-term pain relief in combination with the long-term reparation of joint cartilage. This specific combo of natural substances is exciting and represents a significant opportunity for both the nutraceutical trade and the consumer.

As background, sales within the glucosamine supplements category are trending steadily upwards. In the U.S., category sales reached \$872 million in 2008, a 16% increase on sales of \$753 recorded in 2003. In comparison, global sales stood at almost \$2 billion last year, which represents a massive 62% increase since 2003, when sales came in at \$1.2 billion. The US market is expected to reach \$891 million in consumer purchases by 2013, while the global market is projected to reach sales of \$2.3 billion for the same period. (Source: Nutra-Ingredients-USA.com)

The timing is right for nutraceutical manufacturers participating in the joint health category to begin developing a new generation of joint care products. Formulators who are interested in Perluxan® should consider riding the coattails of this positive and consistent glucosamine sales trend and offer their customers a product of unprecedented broad-scale efficacy.

To summarize, whether formulated as a stand-alone phyto-active ingredient or in combination with glucosamine or aother botanical alternatives, Perluxan® has clearly raised the bar by providing the trade and consumer with a one-of-a-kind natural anti-inflammatory to help elevate the quality of life for millions of arthritis sufferers worldwide.

Proprietary Nutritionals welcomes your comments and questions. For further information, including a full technical file, please contact us or visit www.perluxan.com.



Perluxan®
t (866) 963-2007
www.perluxan.com



Gregory Bonfilio
t (707) 766-7060
gbonfilio@pharmachemlabs.com

Proprietary Nutritionals, Inc.

a subsidiary of Pharmachem Laboratories, Inc.
265 Harrison Avenue
Kearny, NJ 07032 USA
t (519) 647-2071
www.pnibrands.com

© 2009 Pharmachem Laboratories, Inc.
Perluxan® is a registered trademark of Pharmachem Laboratories.

The statements in this document have not been evaluated by the FDA. This product is not intended to diagnose, treat, cure or prevent any disease.

When Waiting for Glucosamine Starts to Hurt

Perluxan® is a unique botanical extract derived from the climbing hops vine, clinically shown in human studies to reduce pain-causing enzymes and relieve joint discomfort.

In combination with glucosamine, Perluxan® offers a solution by delivering the dual-action benefits of short-term minor pain relief and long-term joint repair.

A clinical study of patients with knee pain showed Perluxan® had a fast-acting effect and significant improvement over placebo after only two hours following the first dose.



perluxan®
RELIEF IN REAL TIME™

