BARNET

NOPALEX

Multi-tested anti-inflammatory acting on TRPV1 and reducing:

- Infrared (IR) Inflammatory Effect
- UV Irritation
- Lactic Acid Irritation
- Methyl Nicotinate Irritation

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CONCEPT

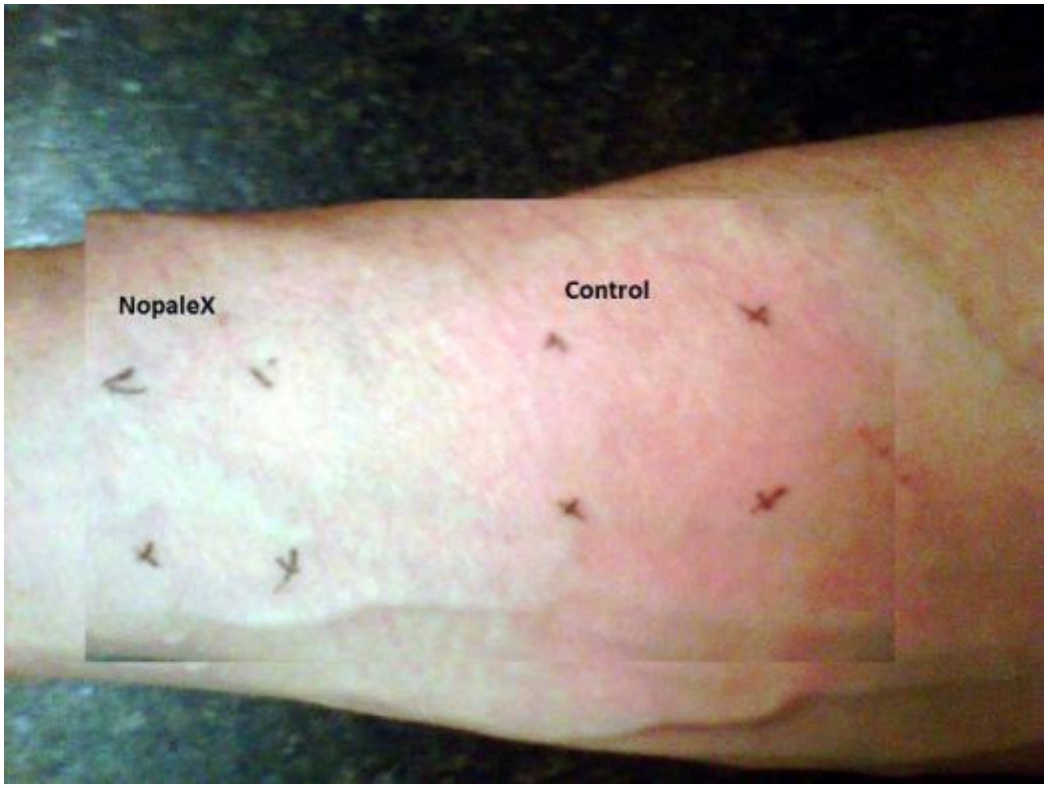
More than 30% of consumers consider themselves to have sensitive skin on either an ongoing or chronic basis. Recent research leads us to believe that a primary suspect in eliciting a sensitive skin response is the TRPV1 receptor found on skin's nerve cells and keratinocytes. This is called a neurosensory response.

Nopalex is designed to reduce neurosensory irritation caused by the application of topical products such as retinoids (slow reaction), alpha hydroxy acids (fast reaction) or preservatives which induce some type of irritation or inflammation.

Nopalex is a unique combination of cactus fermented by yeast containing low molecular weight peptides, carbohydrates and bound minerals. These bound minerals are believed to compete with the irritant to limit the activation of TRPV1.

REPEAT USE OF NOPALEX

CHALLENGE WITH METHYL NICOTINATE (MN) @ 0.1%



Protocol: Pretreatment 4 times with 3% Nopalex in water versus water: 24 hours before, 12 hours before, 6 hours before and then 30 minutes before irritation induced by methyl nicotinate (0.1%). Pictures taken about 20 minutes after MN application.

TRPV1

TRPV1: The capsaicin receptor TRPV1, a polymodal nociceptor whose expression is upregulated in a number of painful inflammatory disorders, represents a promising therapeutic target for pain relief. Potent small molecule TRPV1 antagonists are now undergoing clinical trials in patients with inflammatory or neuropathic pain.

UV exposure lowers the threshold of TRPV1 activation.

Irritants (agonists) activate the receptor and this leads to a cascade of events:

* A depolarization of nerve cells

* An activation in the keratinocyte leading to the release of Substance CGRP which will affect the nerves

NOPALEX RELEASE OF PRO-INFLAMMATORY IL-1α AS EXPRESSED IN pg

			Release of IL 1α (pg)		
Test Cell	Toxic Agent	Addition	6 hrs.	12 hrs.	24 hrs.
Control	None	None	12 pg	11.6 pg	8.4 pg
Control	5% SLS	None	45 pg	111 pg	134 pg
Experimental	5% SLS	0.1% Nopalex	23 pg	44 pg	47 pg
Experimental	5% SLS	0.5% Nopalex	17 pg	26 pg	36 pg
Experimental	5% SLS	1% Nopalex	14 pg	30 pg	33 pg

pg = picogram

CELL SURVIVAL

			Cell Survival		
Test Cell	Toxic Agent	Addition	6 hrs.	12 hrs.	24 hrs.
Control	None	None	1.04	1.05	1.02
Control	5% SLS	None	0.76	0.54	0.32
Experimental	5% SLS	0.1% Nopalex	0.98	0.94	0.95
Experimental	5% SLS	0.5% Nopalex	1.02	1.05	1.03
Experimental	5% SLS	1% Nopalex	1.02	1.09	0.98

0.1% Nopalex keeps cells alive with 5% SLS. Without Nopalex 68% of cells died in 24 hours.

NOPALEX CONTROLS THE INFLAMMATORY CHAIN INDUCED BY UV

UV

↓

PLA_2 + 133% (Phospholipase)

Arachidonic Acid

COX2 + 103% LOX2 + 140%

UV

Cytokine Release

IL8 + 76% IL 1α + 187%

UV + Nopalex

↓

PLA_2 + 45%

Arachidonic Acid

COX2 + 23% LOX2 + 67%

UV

Cytokine Release

IL8 + 15% IL 1α + 56%

With Nopalex all markers of inflammation are less expressed.

NOPALEX REDUCES INFRARED (IR) INFLAMMATORY EFFECT

Methods for evaluation of IR protection

Gene Expression

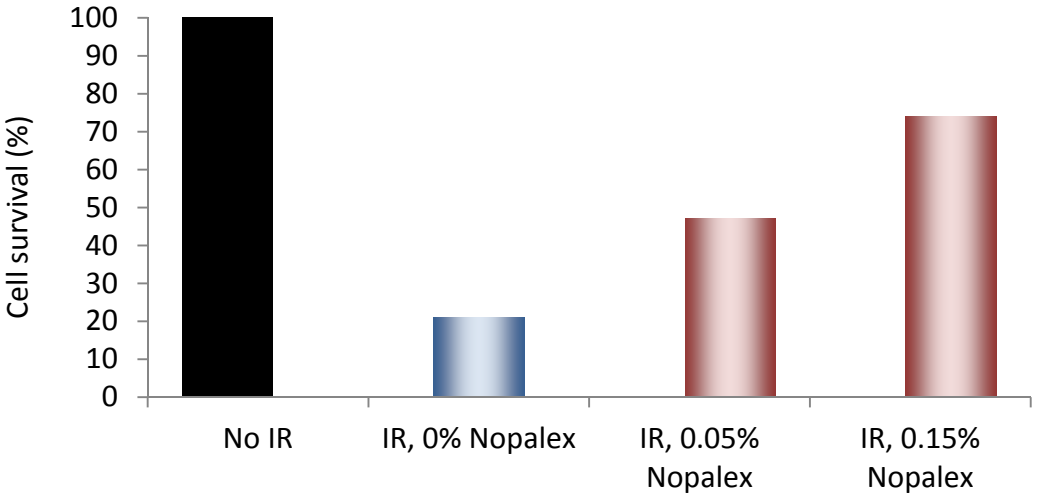
IR irradiation of cultured cells has been shown to increase the expression of certain genes in both fibroblast and keratinocyte cell cultures. It also induces long and short term changes in the skin in vivo.

To assess gene expression in cultures exposed to IR irradiation we used the Affymetrix microchip analysis system. Fibroblasts, with and without Nopalex, were subjected to increasing doses of IR exposure. We examined the gene expression via RT-PCR 24 hours later utilizing the Affymetrix test kit.

Cell Survival

Separately we examined survival via MTT assays with increasing levels of IR.

NOPALEX AND INFRARED (IR)



- IR alters gene expression
- Gene expression with IR exposure with or without Nopalex

Gene Product	Importance	Ratio Treated N / Untreated
HSP 90	Cellular Protectant	1.67
MMP1	Dermal Breakdown	0.45
IL 6	Inflammation	0.34

Conclusion:

Nopalex provided protection against IR irradiation in cell cultures examined via cell survival rates, and via the reduction of the expression of genes involved in the inflammatory process.

NOPALEX REDUCES NEUROSENSORY IRRITATION ON NASAL FOLD AREA (STINGING LACTIC ACID TEST)

Material	1 min.	5 min.	10 min.	15 min.	20 min.	30 min.	Total*	% Change
8% Lactic Acid	3.0	3.4	3.4	3.3	1.7	1.5	30.6	N/A
Plus 5% Nopalex	1.8	1.3	1.1	1.1	0.7	0.6	10.7#	66%
Plus 3% Nopalex	2.0	2.0	1.8	1.6	1.3	0.7	16.0#	48%

The total score is the summation of average reaction scores obtained at 1, 2, 3, 5, 7, 10, 12, 15, 20, 25 and 30 minutes. Results are the average of 20 subjects. # Indicates statistically significant difference from the 8% lactic acid control (Student T Test $p < 0.05$)

Lactic acid is an intense, acute stinger. The irritation is highest at 5 and 10 minutes after application. After 10 minutes the intensity of irritation was cut by 2 with 3% Nopalex.

Protocol

Twenty subjects with a history of sensitive skin washed their faces with Ivory Soap twice daily for 5 days and refrained from using moisturizers. On the day of the test they wiped the nasal fold area with alcohol 30 minutes after washing with Ivory Soap. After 30 minutes, half the subjects applied 3% or 5% Nopalex plus 8% lactic acid to one side of the nasal fold area, and only 8% lactic acid to the other side. Irritation (stinging) was scored on a 0-4 scale at defined times during the next 30 minutes. Results as shown above.

MENTHOL AND METHYL NICOTINATE TEST

We repeated the experiment on the previous page with a cosmetic formula designed to provide a "muscle soothing" effect. The gel contained low concentrations of menthol as well as methyl nicotinate as active ingredients provided a warming effect on the skin; however, it was also deemed quite irritating. For test purposes the product was modified by the addition of 5% water or 5% Nopalex.

Material	1 min.	5 min.	10 min.	15 min.	20 min.	30 min.	Total*	% Change
Muscle Soothing Gel	1.6	2.1	2.4	3.0	3.2	3.5	32.2	N/A
Gel + 5% Nopalex	1.1	1.8	1.9	2.4	2.7	2.6	23.9#	25%

The total NSI score is the summation of average reaction scores obtained at 1, 2, 3, 5, 7, 10, 12, 15, 20, 25 and 30 minutes. Results are the average of 20 subjects.

# Indicates statistically significant difference from the control (Student T Test $p < 0.05$)

Menthol effect builds up over time. Irritation is highest 30 minutes after application. Nopalex limits the development of irritation. The highest point is after 20 minutes.

NOPALEX REDUCES UV-INDUCED ERYTHEMA

Ten subjects are recruited for a 2-week study. For five days prior to the study start, subjects applied a test material to one forearm and a placebo to the other twice daily. Over the next 5 days each subject is exposed to 1MED UV on each forearm with continued product application.

After the last UV exposure the formula (with or without active) was applied to the test site by a clinician. Performance was assessed by Minolta Chroma Meter as well as by clinical assessment.

NOPALEX REDUCES UV-INDUCED ERYTHEMA

Skin Erythema after Multiple UV Exposures

(Increase in a* values, Minolta Chroma Meter)

Material	After Product Only, 5 Days Treatment	After Treatment and UV Immediate Effect	24H After UV	48H After UV
Control Gel	+ 0.8	+ 6.7	+ 14.3	+ 8.7
Gel + 5% Nopalex	- 1.3	+ 2.3#	+ 5.4#	+ 2.2#

All a* values compared to pre-treatment values. Results are the averages of ten subjects. # indicates statistically significant difference between the control and treated test sites (Student T Test $p<0.05$).

Skin Erythema after Multiple UV Exposures

(Increase in a* values, Clinical Assessment)

Material	After Product Only, 5 Days Treatment	After Treatment and UV Immediate Effect	24H After UV	48H After UV
Control Gel	+ 0.1	+ 2.4	+ 3.8	+ 3.2
Gel + 5% Nopalex	0	+ 0.7#	+ 1.8#	0 #

All a* values compared to pre-treatment values. Results are the averages of ten subjects. Results were recorded on a 5 point scale with 0 indicating no irritation and 5 indicating severe, painful redness.
# indicates statistically significant difference between the control and treated test sites (Student T Test $p<0.05$). After 48 hours no erythema can be seen by the dermatologist when Nopalex was used but it was still clearly visible without Nopalex.

SINGLE APPLICATION OF NOPALEX WITH SINGLE APPLICATION OF METHYL NICOTINATE (MN)

MN (concentration)	0.05%	% change	0.20%	% change
Nopalex 0%	1.58	0	4.48	0
Nopalex 1%	0.61	61.4%	2.81	37.3%
Nopalex 3%	0.5	68.4%	2.61	41.7%

Data is the average of 10 subjects, where $p<0.05$ paired Student T Test.

Note: Expression in % can be misleading. With MN at 0.2%, and Nopalex at 1%, the change in irritation score is changed by almost 2 units, which is an important achievement.

At both 1% and 3%, Nopalex reduced redness from MN topical application.

MN induces irritation via the release of PGE2. Nopalex stabilizes epithelial cell membranes and reduces the release of inflammatory messengers and cytokines, thus preventing the ensuing irritant response.

Protocol
Nopalex at 1% or a 3% in water is applied to the volar forearm of 10 subjects with sensitive skin 30 minutes prior to the application of Methyl Nicotinate.

Irritation is graded visually by clinicians on a 0 (no redness) to 5 (severe irritation) scale 20 minutes after the application of the Methyl Nicotinate.

CONCLUSION

Both in vitro and in vivo testing demonstrates the ability of Nopalex to reduce a number of uncomfortable reactions associated with the inflammatory response such as erythema, and stinging and burning reactions. We believe it stabilizes the trans membrane channels responsible for Ca++ transport and initiation of the inflammatory response. Nopalex also down regulates a number of different keratinocyte genes responsible for the inflammatory process.

NOPALEX

INCI Name:	Water (and) Glycerin (and) Opuntia ficus-indica Fruit Extract (and) Saccharomyces cerevisiae Extract
REACH Status:	Low volume exemption
Canada DSL:	Listed DSL / RICL (Revised In Commerce List)
China Registration:	All components are listed in the Inventory of Existing Cosmetic Ingredients in China (IECIC).
ECOCERT Status:	Compliant with the ECOCERT Standard for natural ingredients
Suggested Use Level:	1% - 5%