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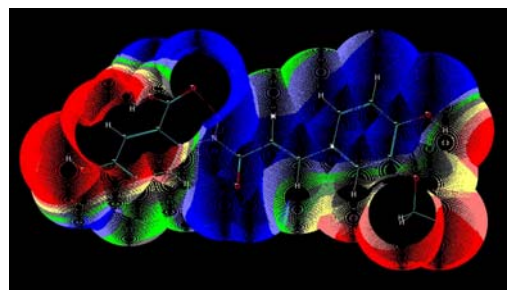
Drago-Calm

Natural Anti-irritant
with Anti-histaminic Properties



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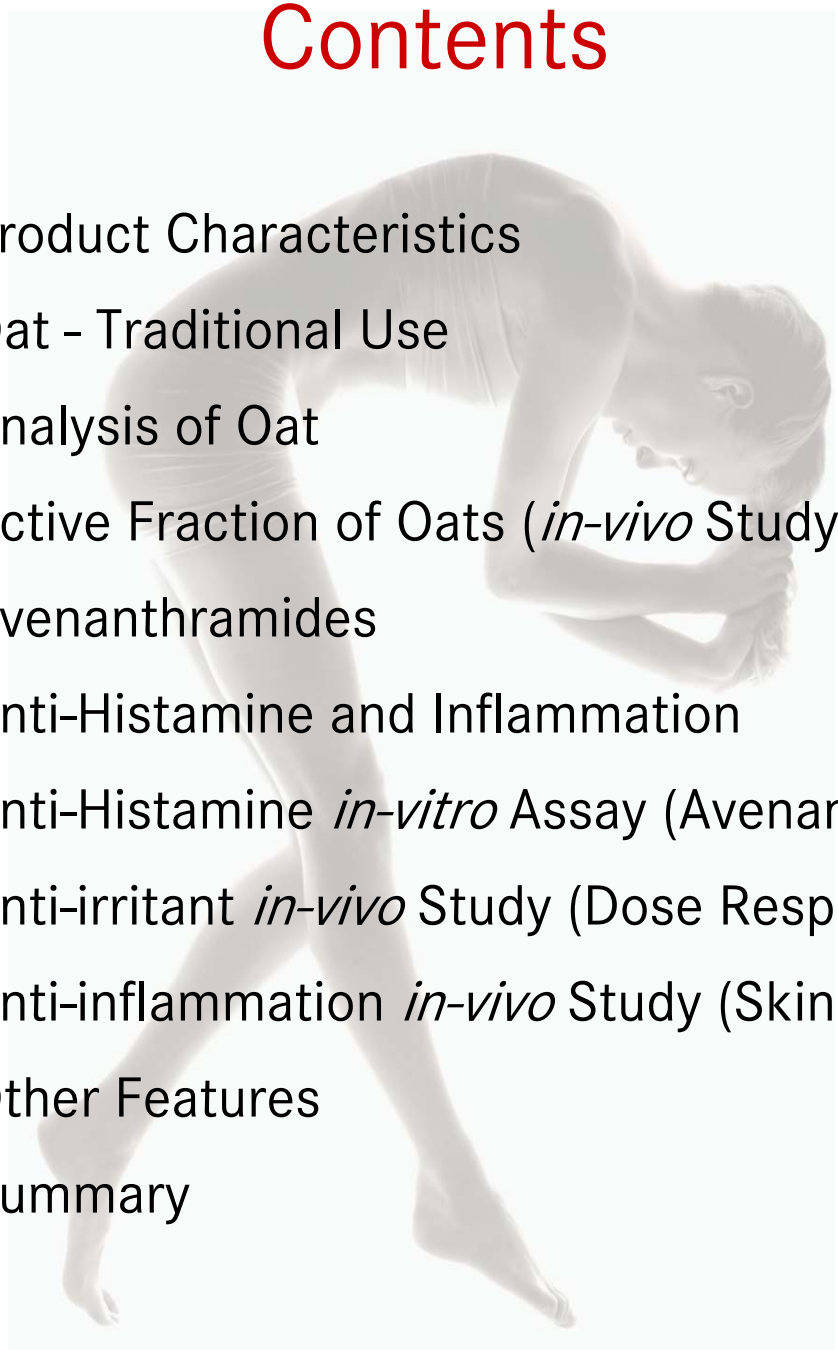

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- Analysis of Oat
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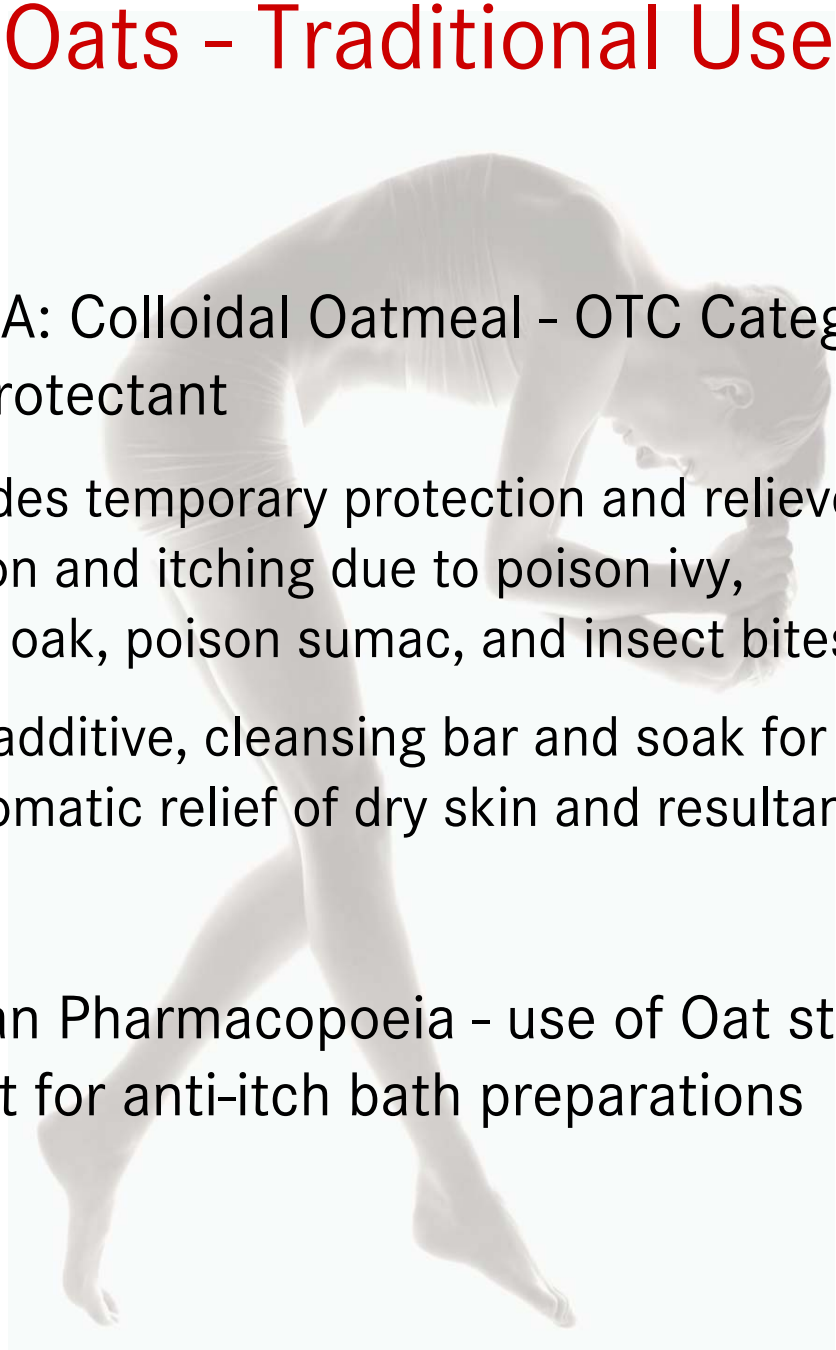
Product Characteristics

- Clear liquid with low odor and light yellow color
- Standardized to 100ppm Avenanthramides from Oat
- Anti-irritant with anti-histaminic properties
- Stable at low pH
- Recommended use level: 0.5 - 5%
- INCI: Water, Glycerin, Avena Sativa (Oat) Kernel Extract
- Product # 674463

Oats - Traditional Use



- US FDA: Colloidal Oatmeal - OTC Category I skin protectant

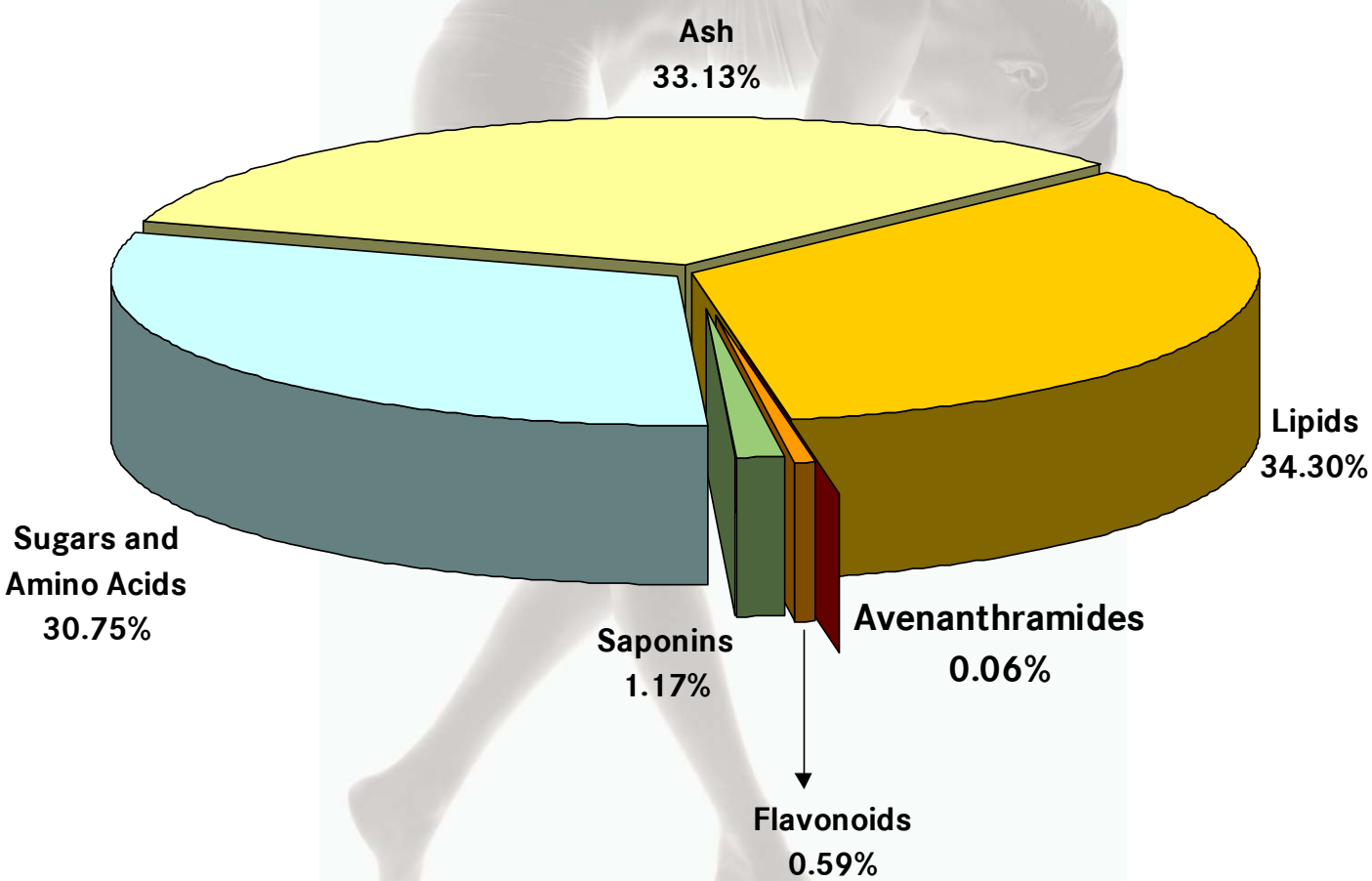
  - provides temporary protection and relieves minor irritation and itching due to poison ivy, poison oak, poison sumac, and insect bites

  - bath additive, cleansing bar and soak for the symptomatic relief of dry skin and resultant itching

- German Pharmacopoeia - use of Oat straw extract for anti-itch bath preparations

Analysis of Oat

Mass Distribution of Separated Oat Fractions



Active Fraction of Oats

Test to Evaluate the Oat Fractions for Efficacy *in-vivo*

<u>Test design:</u>

- 9 human subjects in the test panel
- 7 Oat fractions diluted with 50% Ethanol to a constant volume
- minimal erythemal dose (MED) of the subjects determined by UV light exposure
- Skin irritation induced by 1.5 times of MED
- Base line redness evaluation with Minolta Chromameter 24h after UV irradiation

Active Fraction of Oats

Test to Evaluate the Oat Fractions for Efficacy *in-vivo*

<u>Test design (contd.):</u>

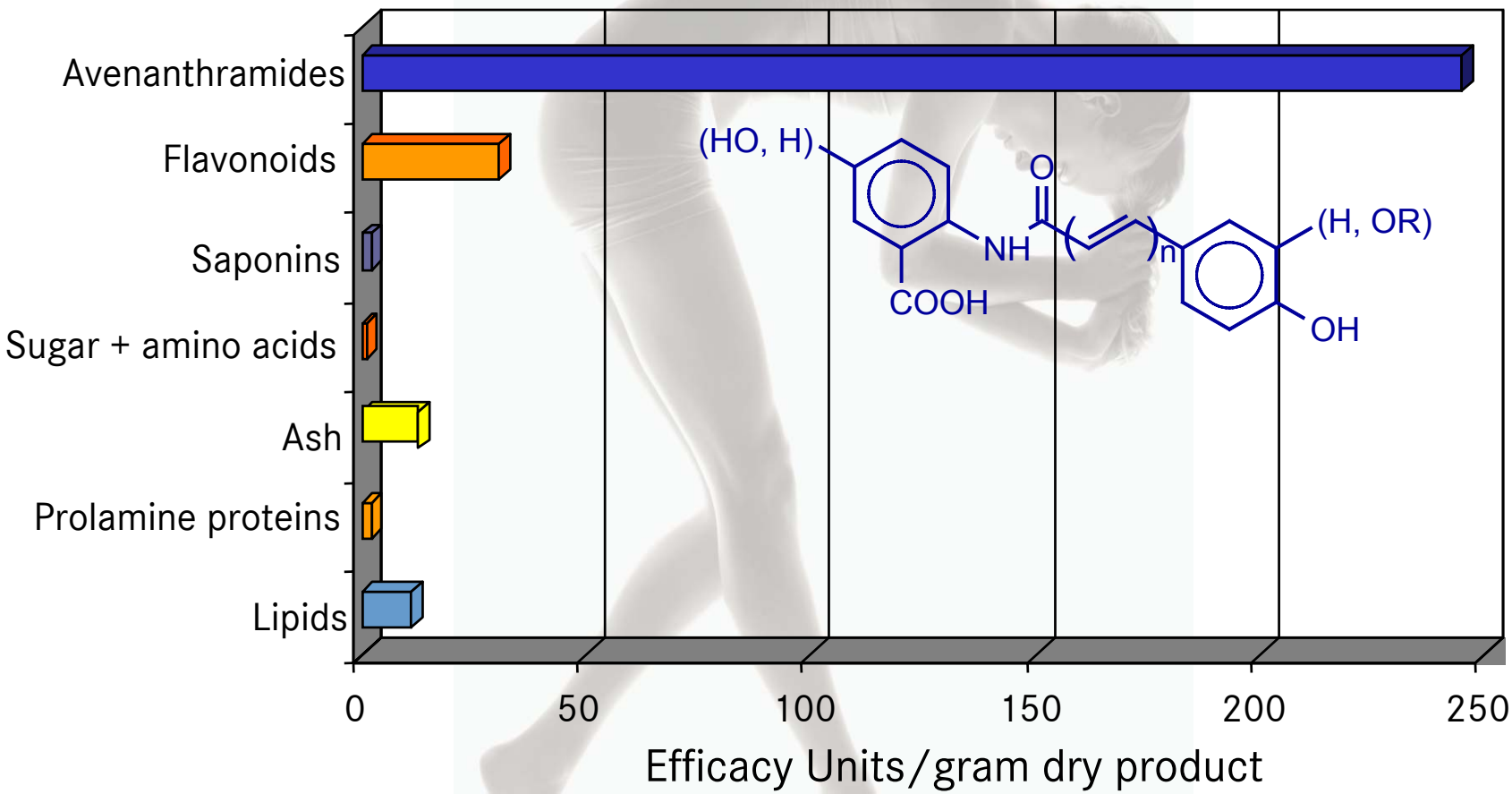
- Treatment of test areas with the products
- Chromameter reading 24h after treatment
- Calculation of redness reduction relative to baseline and relative to the dry matter content of the Oat fraction
- Results given in efficacy units per gram

<u>Results:</u>

- ✔ Avenanthramides - the active components of Oat

Active Fraction of Oats

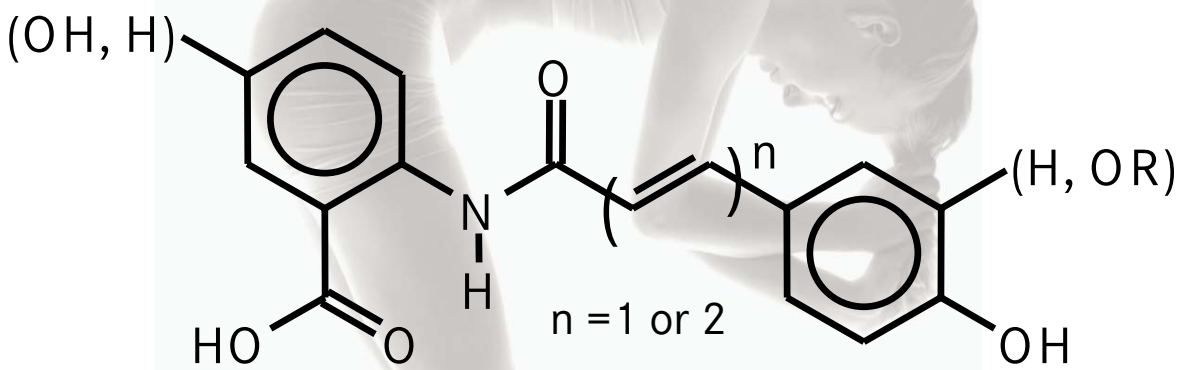
Test to Evaluate the Oat Fractions for Efficacy *in-vivo*



Avenanthramides are the Active Constituent of Oats

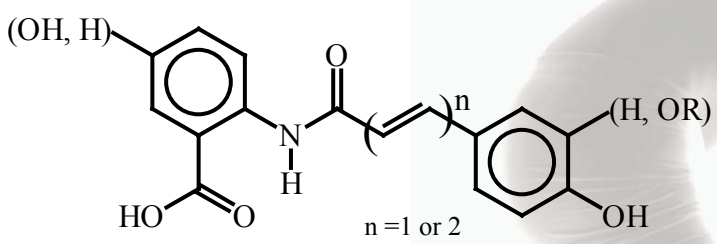
Avenanthramides

What are they?

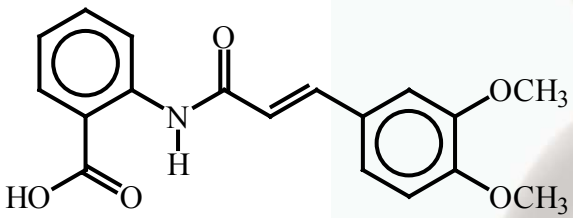


- Complex mixture of phenolic compounds
- Only found in oats in nature *(and hence the name)*
- Avenanthramides A, B & C occur in Oats
- High temperature and low pH stable

Avenanthramides



(Avenanthramide)

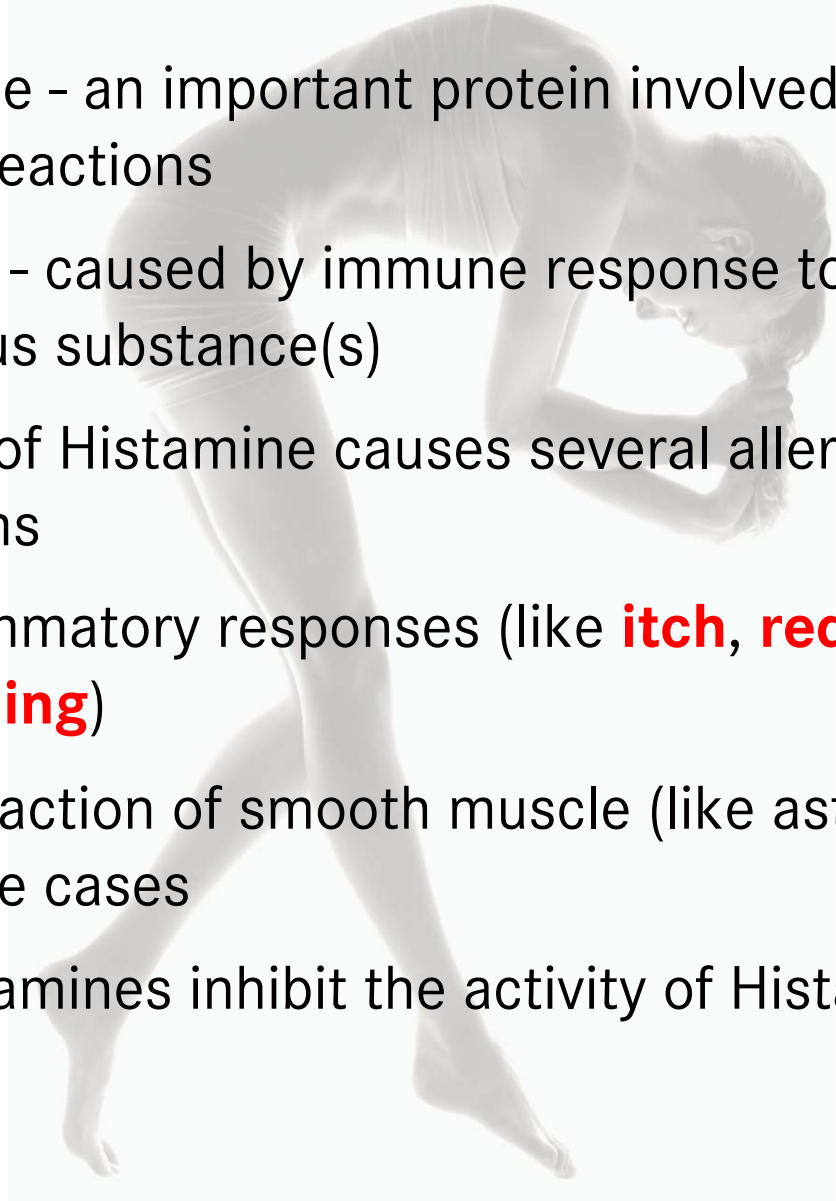


(Tranilast)

Avenanthramides are analogues to Tranilast, a topical drug

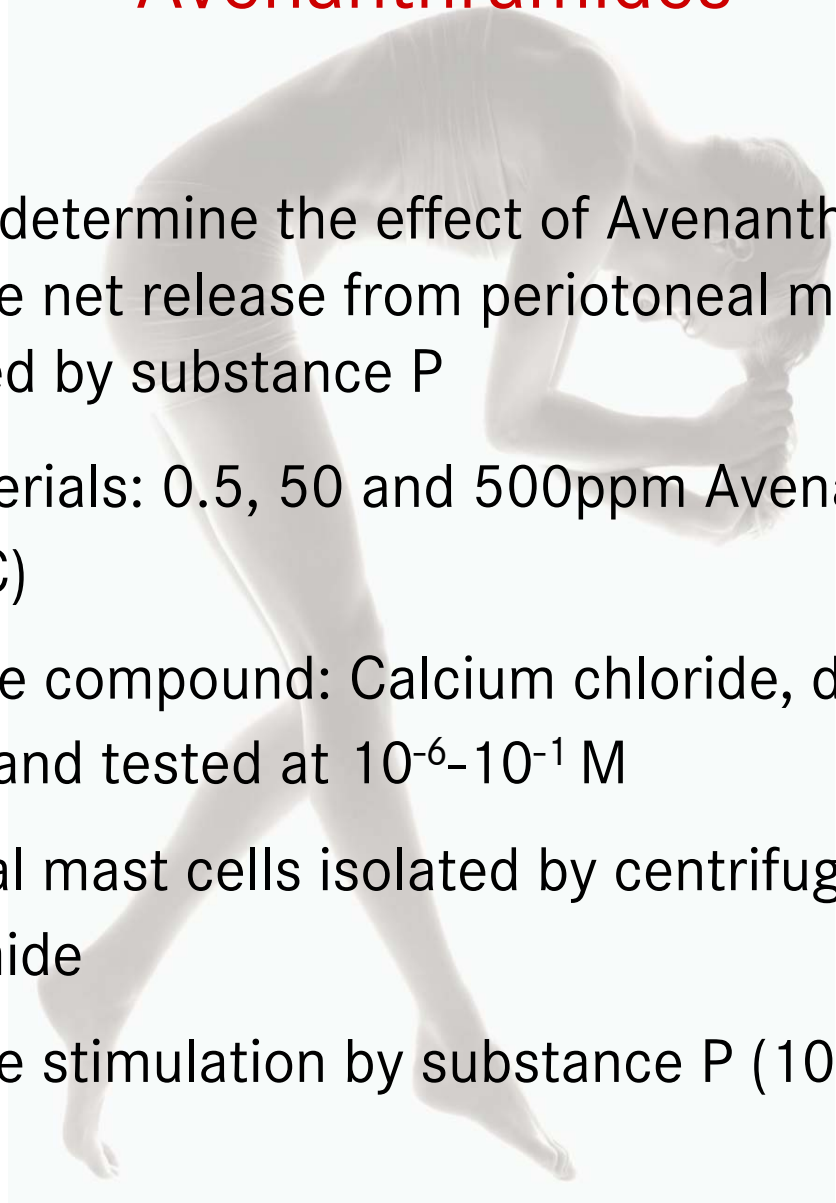
- anti-Histamine
- anti-inflammatory
- anti-oxidant
- lipoxygenase inhibitor
- cutaneous anaphylaxis inhibitor

Histamine and Inflammation



- Histamine - an important protein involved in many allergic reactions
- Allergies - caused by immune response to normally innocuous substance(s)
- Release of Histamine causes several allergic symptoms
  - inflammatory responses (like **itch**, **redness**, **swelling**)
  - contraction of smooth muscle (like asthma), in severe cases
- Anti-histamines inhibit the activity of Histamine

Anti-histamine *in-vitro* Assay
Avenanthramides



Protocol

- Study to determine the effect of Avenanthramides on Histamine net release from periotoneal mast cells, stimulated by substance P
- Test materials: 0.5, 50 and 500ppm Avenanthramides (Σ A, B, C)
- Reference compound: Calcium chloride, diluted in buffer medium and tested at 10^{-6}-10^{-1} M
- Peritoneal mast cells isolated by centrifugation on metrizamide
- Histamine stimulation by substance P (10µM)

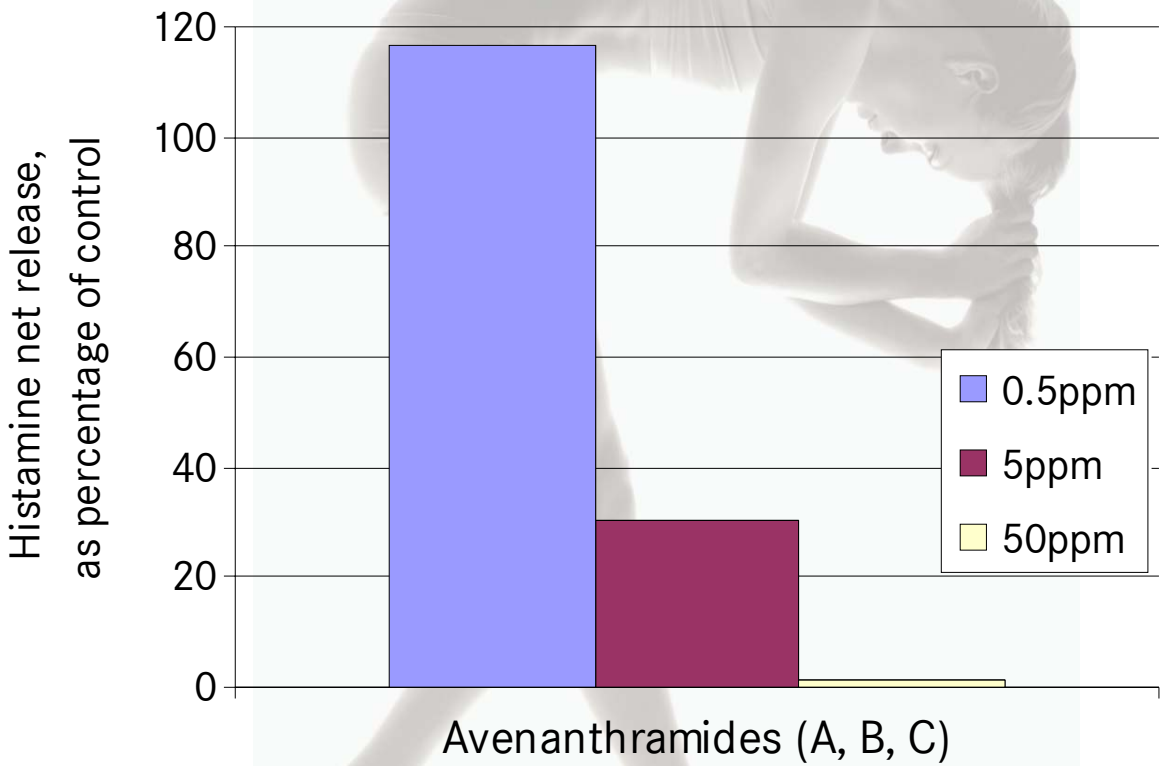
Anti-histamine *in-vitro* Assay
Avenanthramides

Protocol (contd.)

- Mast cells incubated in the presence of reference or assay products and substance P for 2min. (2 replicates)
- Basal release control: cells incubated in the absence of substance P and the assay and reference products
- Supernatants harvested by centrifugation
- Histamine contained in supernatants derivatized by OPT (o-Phthalaldehyde)
- Secreted histamine quantified by measuring the fluorescence intensity using spectrofluorometer

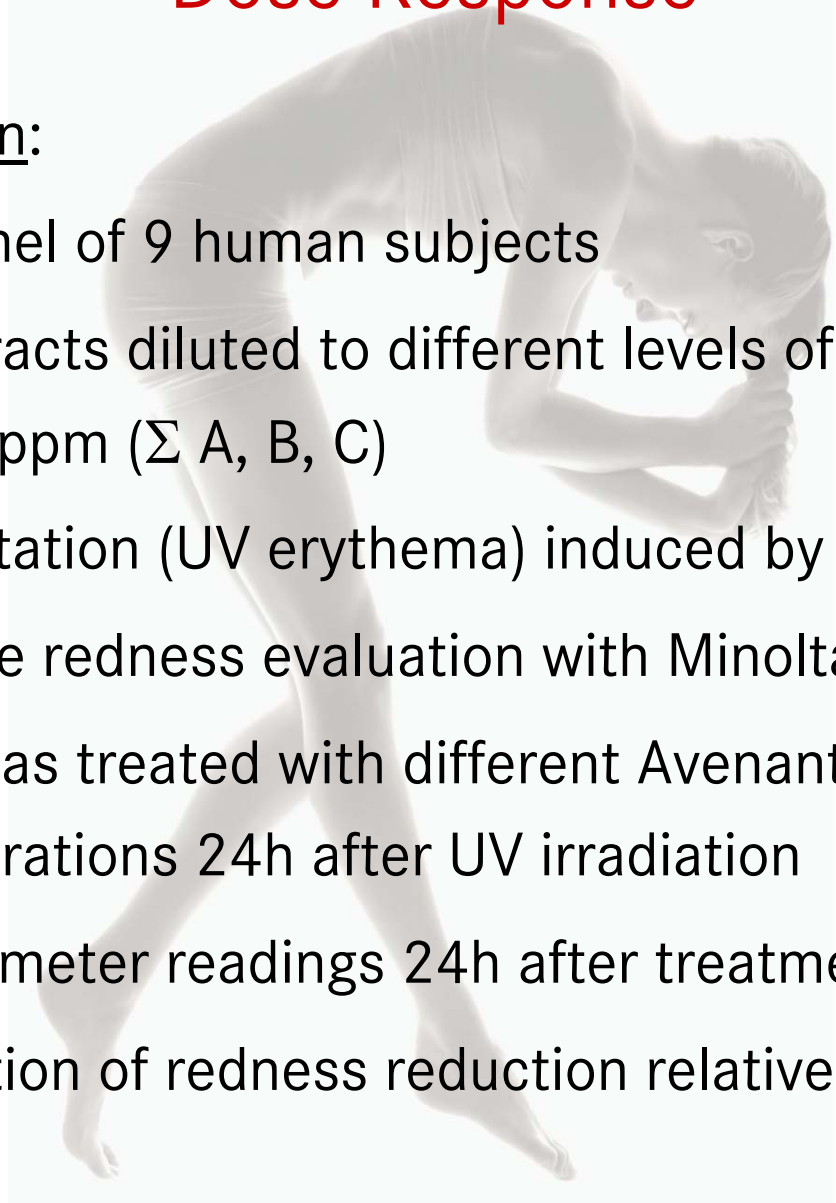
Anti-histamine *in-vitro* Assay

Avenanthramides



Avenanthramides inhibit Histamine net release from peritoneal mast cells in a dose dependent manner

Anti-irritant *in-vivo* Study
Dose Response



Test design:

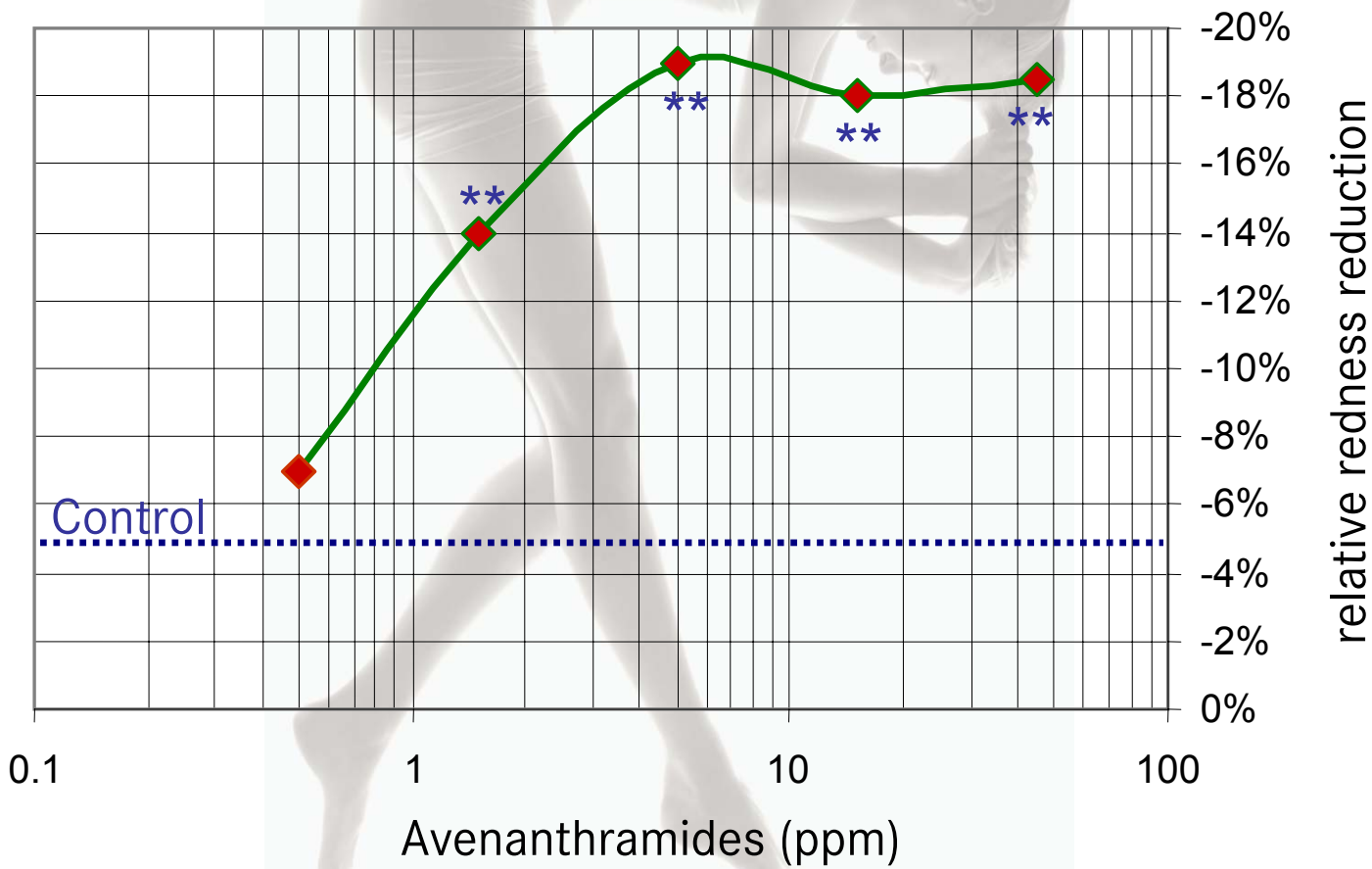
- Test panel of 9 human subjects
- Oat extracts diluted to different levels of Avenanthramides 0.5 - 45ppm (Σ A, B, C)
- Skin irritation (UV erythema) induced by 1.5 times of MED
- Base line redness evaluation with Minolta Chromameter
- Test areas treated with different Avenanthramide concentrations 24h after UV irradiation
- Chromameter readings 24h after treatment
- Calculation of redness reduction relative to baseline

Anti-irritant *in-vivo* Study

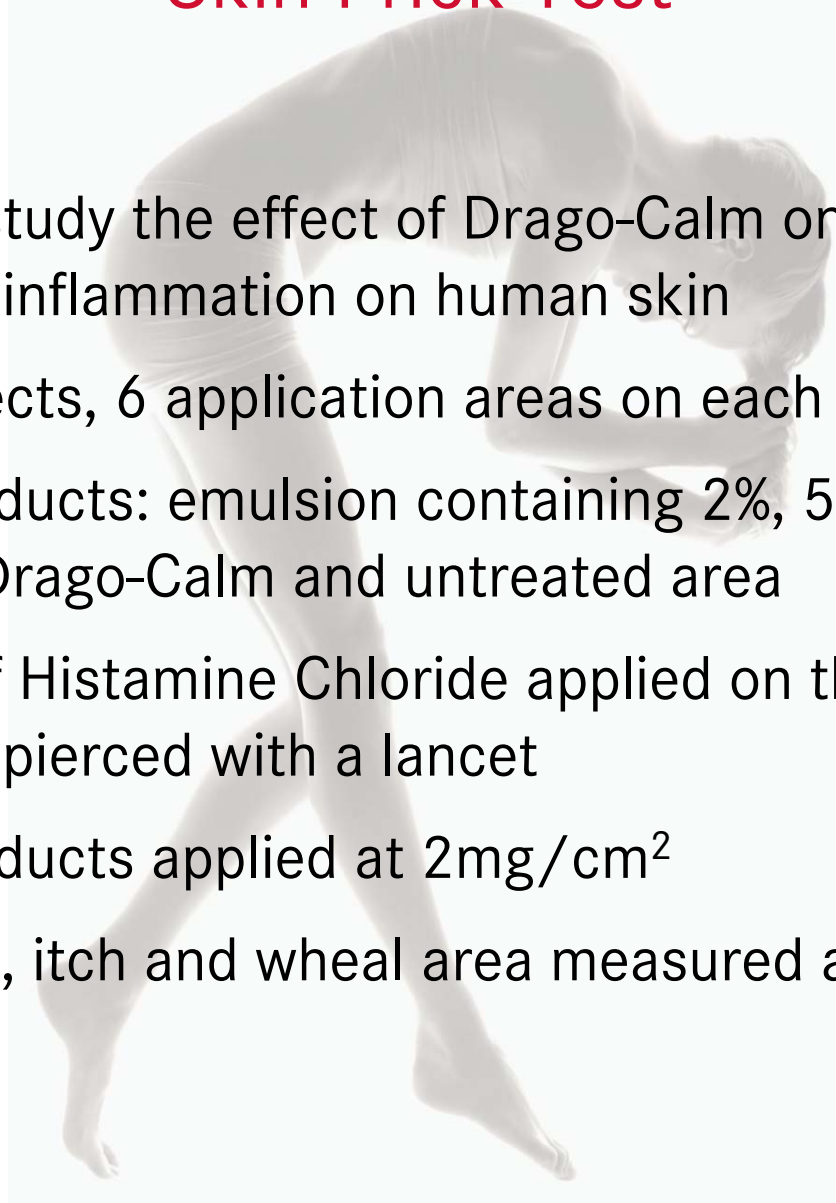
Dose Response

Results

Dose response curve with Avenanthramides



Anti-inflammation *in-vivo* Study
Skin Prick Test



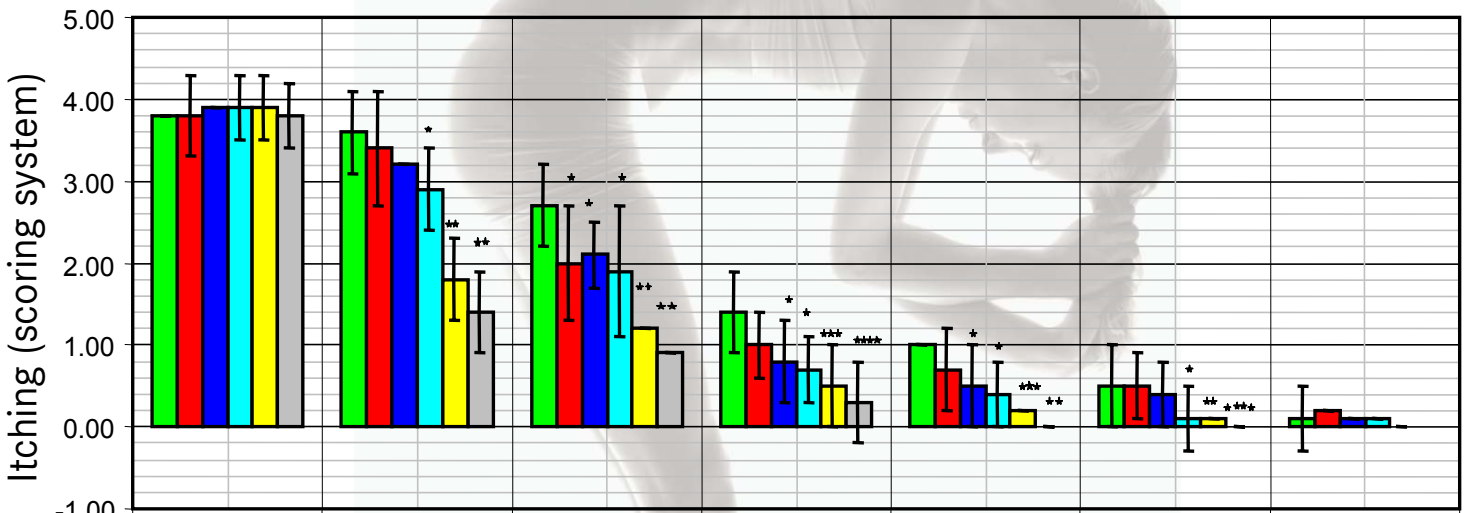
Protocol

- Test to study the effect of Drago-Calm on Histamine induced inflammation on human skin
- 10 subjects, 6 application areas on each subject
- Test products: emulsion containing 2%, 5%, 10%, 25% or 50% Drago-Calm and untreated area
- Drops of Histamine Chloride applied on the skin and the skin pierced with a lancet
- Test products applied at 2mg/cm^2
- Redness, itch and wheal area measured at specific intervals

Anti-inflammation *in-vivo* Study
Skin Prick Test

Results

Itch



	0	0.5	1	1.5	2	2.5	3
untreated	3.8	3.6	2.7	1.4	1	0.5	0.1
2	3.8	3.4	2	1	0.7	0.5	0.2
3	3.9	3.2	2.1	0.8	0.5	0.4	0.1
4	3.9	2.9	1.9	0.7	0.4	0.1	0.1
5	3.9	1.8	1.2	0.5	0.2	0.1	0
6	3.8	1.4	0.9	0.3	0	0	0

*p<0,05 versus untreated;
***p<0,05 versus untreated, 2

hours

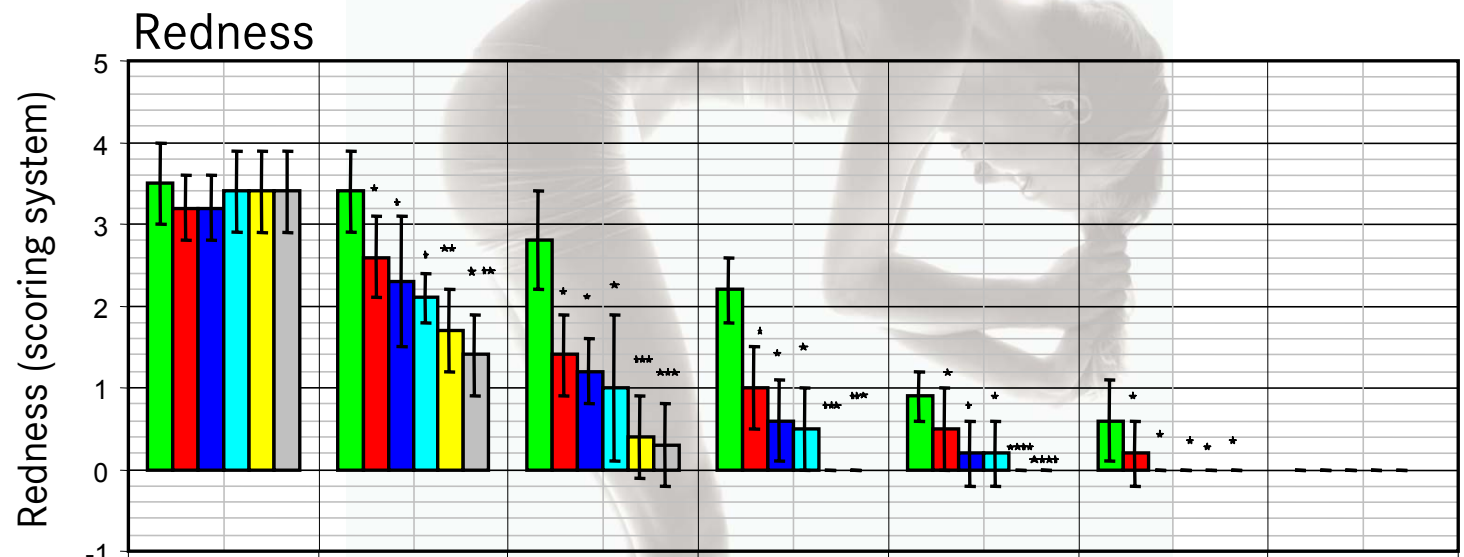
**p<0,05 versus untreated,2, 3, 4;
****p<0,05 versus untreated, 2, 3

1. Untreated
2. 2ppm Avenanthramides (Σ A, B, C)
3. 5ppm Avenanthramides (Σ A, B, C)
4. 10ppm Avenanthramides (Σ A, B, C)
5. 25ppm Avenanthramides (Σ A, B, C)
6. 50ppm Avenanthramides (Σ A, B, C)

Anti-inflammation *in-vivo* Study

Skin Prick Test

Results



	0	0.5	1	1.5	2	2.5	3
untreated	3.5	3.4	2.8	2.2	0.9	0.6	0
2	3.2	2.6	1.4	1	0.5	0.2	0
3	3.2	2.3	1.2	0.6	0.2	0	0
4	3.4	2.1	1	0.5	0.2	0	0
5	3.4	1.7	0.4	0	0	0	0
6	3.4	1.4	0.3	0	0	0	0

*p<0,05 versus untreated;
**p<0,05 versus 2, 3

***p<0,05 versus 2, 3, 4, untreated;
****p<0,05 versus 2, untreated

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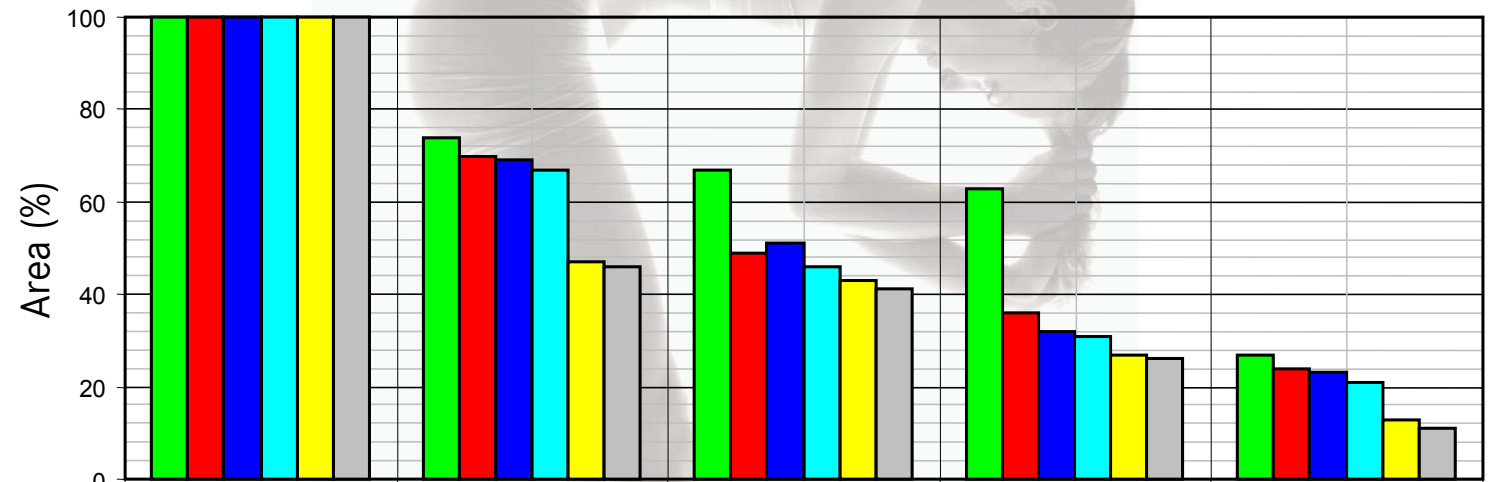
Anti-inflammation *in-vivo* Study
Skin Prick Test

Results

Area of wheal (relative to initial condition)



	0	0.5	1	1.5	2
untreated	100	74	67	63	27
2	100	70	49	36	24
3	100	69	51	32	23
4	100	67	46	31	21
5	100	47	43	27	13
6	100	46	41	26	11

hours

1. Untreated
2. 2ppm Avenanthramides (Σ A, B, C)
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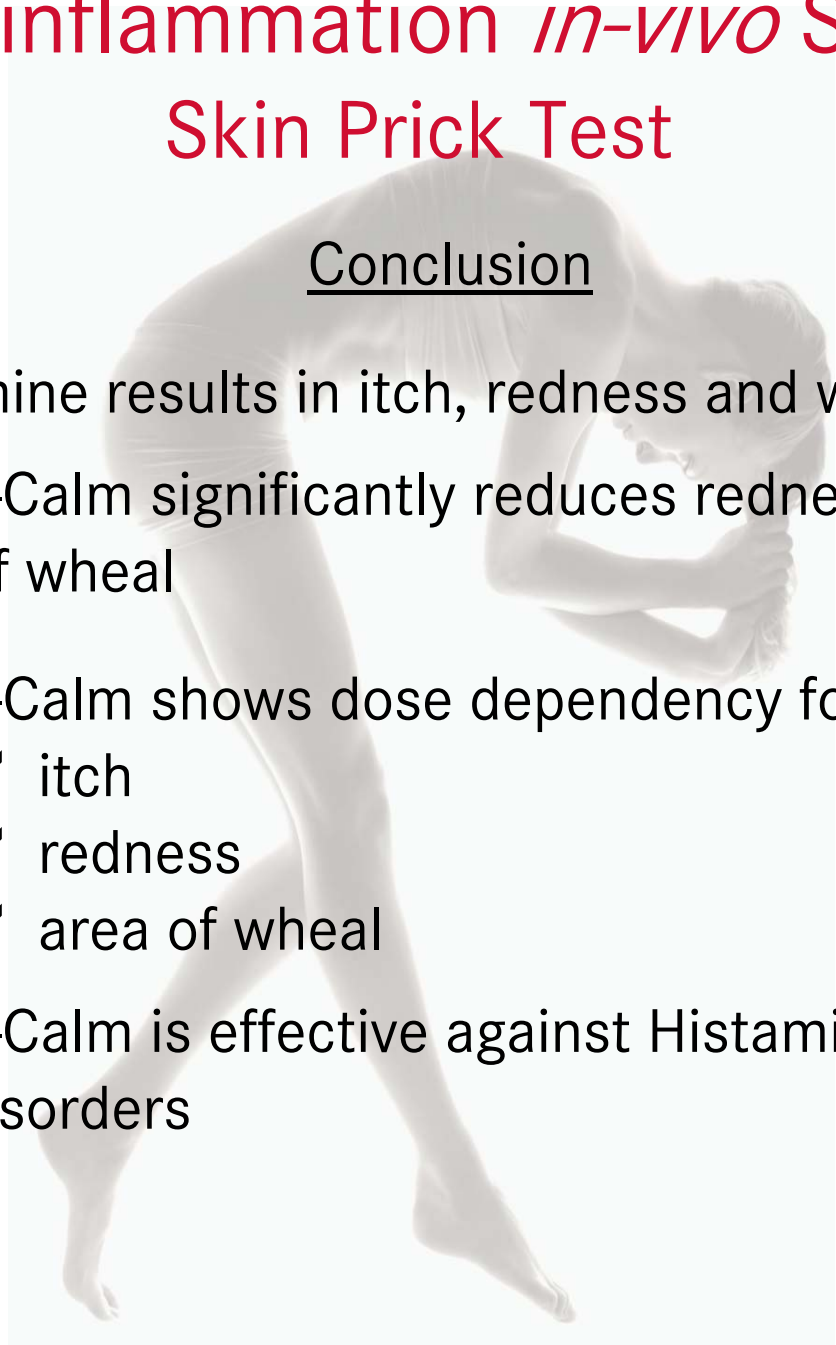
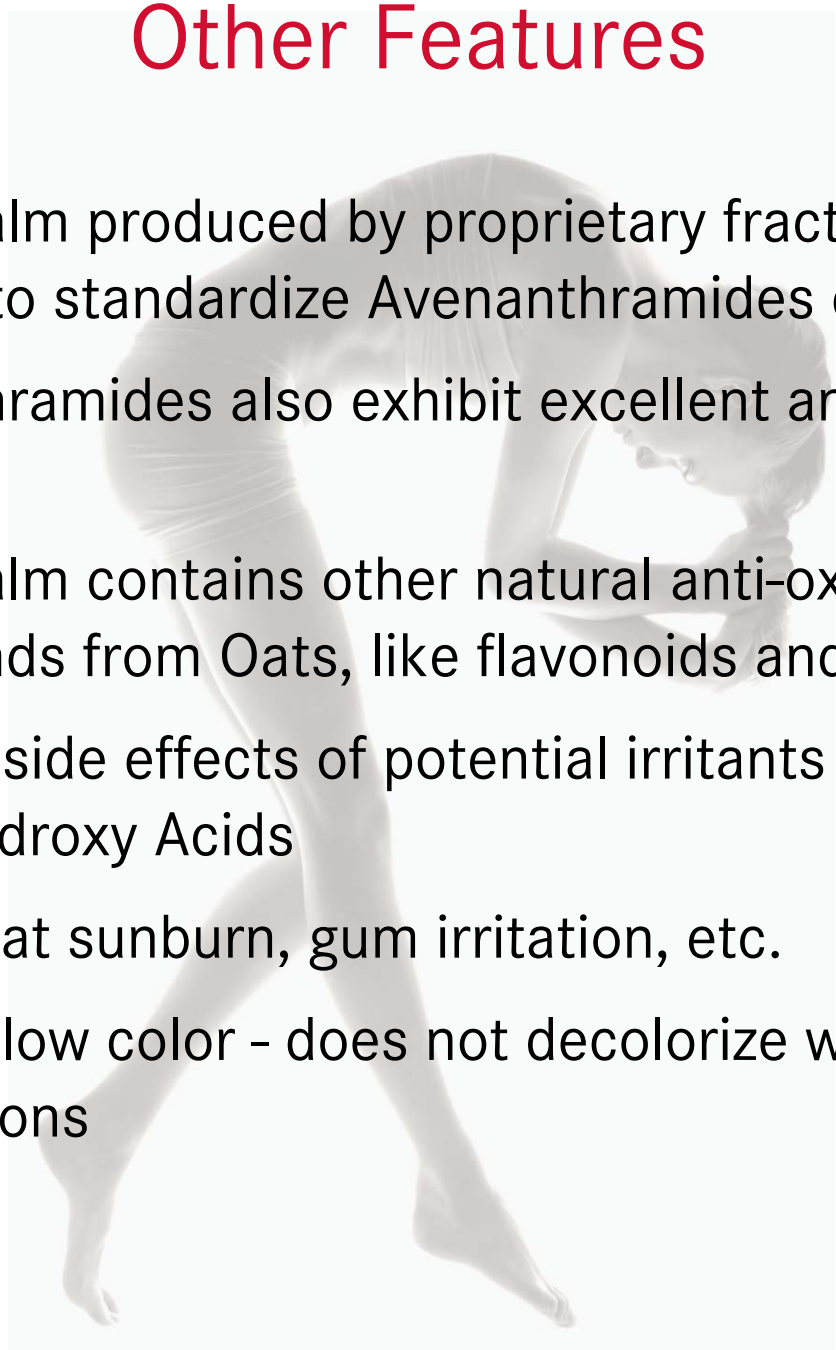
Anti-inflammation *in-vivo* Study
Skin Prick Test



Conclusion

- Histamine results in itch, redness and wheal
- Drago-Calm significantly reduces redness, itch and area of wheal
- Drago-Calm shows dose dependency for reduction of:
  - ✔ itch
  - ✔ redness
  - ✔ area of wheal
- Drago-Calm is effective against Histamine related skin disorders

Other Features



- Drago-Calm produced by proprietary fractionation process to standardize Avenanthramides content
- Avenanthramides also exhibit excellent anti-oxidant activity
- Drago-Calm contains other natural anti-oxidant compounds from Oats, like flavonoids and polyphenols
- Reduces side effects of potential irritants like Alpha Hydroxy Acids
- Helps treat sunburn, gum irritation, etc.
- Slight yellow color - does not decolorize white formulations

Summary
Drago-Calm
Natural Anti-irritant
with Anti-histaminic Properties

- Avenanthramides - main active constituents of Oat
- Drago-Calm - standardized to 100ppm Avenanthramides
- Natural anti-irritant, long history of safe use
- Topical anti-histamine
- Reduces itch, redness and wheal due to irritation
- Reduces the irritation of other actives like AHAs