

Regulation of muscular contractions by aromatic seashore plant extracts

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Objective:

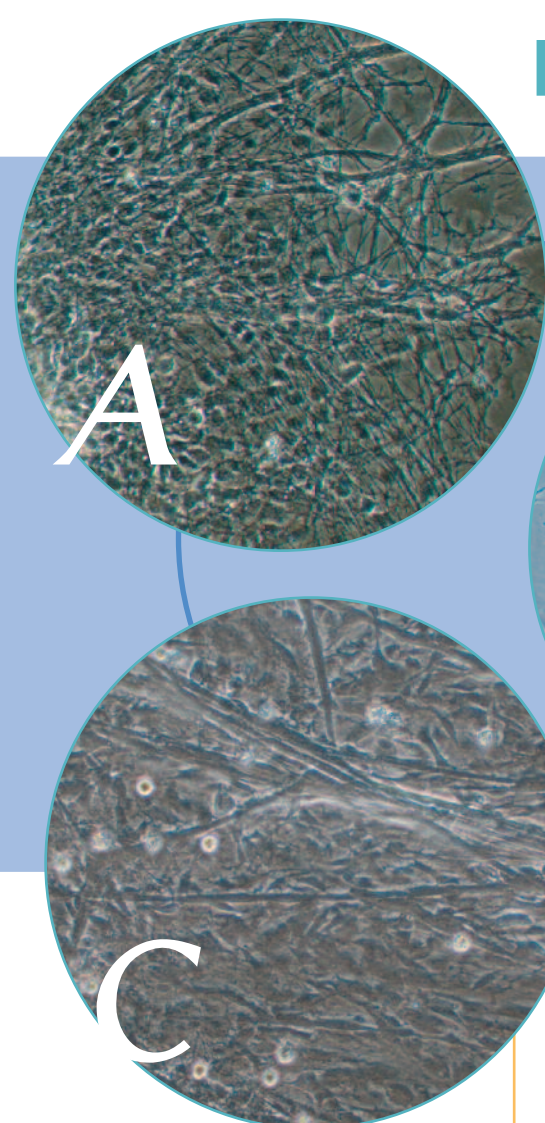
Selection of safe active ingredient for cosmetic applications to reduce expressive face wrinkles.

Introduction:

Face wrinkles are due to repeated muscular contractions. Treatments by botulinum toxin A (Botox) are effective to reduce them by blocking transmission between neurons and muscles, but not without side effects. Today, a more cosmetic approach is to find safer ingredients that can reduce the muscular contraction. It was shown that beta-endorphin, neuropeptide produced by the skin with relaxing activity, is stimulated by the application of plant extracts containing essential oils.

We have tested the effect of 3 aromatic plants extracts (*Helichrysum italicum*, *Crithmum maritimum* and *Lavandula stoechas*), obtained by a high-tech vacuum microwave hydro-distillation process (VMHD), and the effect of beta-endorphin on the modulation (inhibition) and the recovery of the contractile activity using an *in vitro* nerve-muscle model.

Material and methods



Nerve-muscle coculture

- ▶ Motoneurons (rat spinal cord explants) isolated from rat embryo loaded onto the muscle cell culture (Photograph A).
- ▶ Human muscular cells (primary human striated muscle cells) cultivated as mono layers differentiated into myofibers structure (Photograph B).
- ▶ After 3 weeks, nerve-muscle junctions fully develop as motor plates, which exhibit the same properties and features as end plates (motor plates) in vivo (Photograph C).

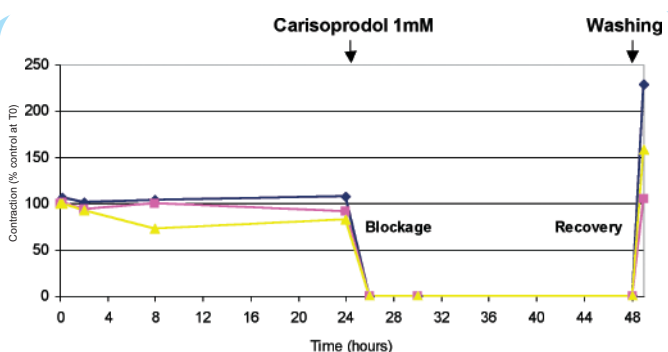
Photographs : Motoneurons isolated from rat embryo (A) and human muscle cells (B) were cocultured. After 3 weeks, nerve-muscle junctions were developed (C).

Tested products and experimental conditions

- ▶ Carisoprodol at 1mM used as a positive control for blocking muscular contraction frequency.
- ▶ 3 aromatic plants extracts tested at 3 concentrations on nerves-muscle co-cultures
 - Evaluation of the frequency of contractions after 10min, 2h, 6h and 24h of incubation.
 - Elimination of the product by washing and observation of the recovery of the contraction during 1h, 4h and 24h.
- ▶ Beta-endorphin tested at 1 pg/ml.

Results:

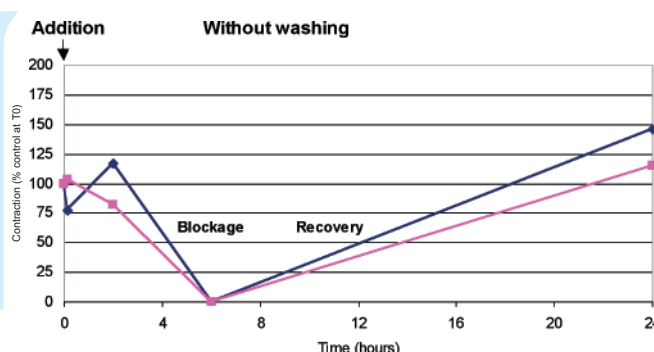
Validation of cellular model



- ▶ After 3 weeks of culture, the contraction frequency was stable until 24h.
- ▶ Carisoprodol at 1mM blocked the contraction after 10min of contact
- ▶ Without washing, the frequency remained very low even after 24h (this effect was not reversible)
- ▶ After elimination of Carisoprodol by washing, it was normal again after 1h.

Measure of contraction frequency until 24h and then effect on Carisoprodol at 1mM on the contraction of 3 fibers

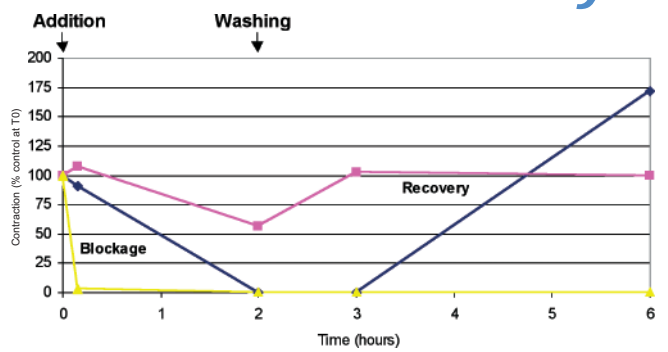
Effect of beta-endorphin



- ▶ Contractions totally blocked after 6h
- ▶ Frequencies of contractions returned to normal after 24h
- ▶ Without washing, inhibition is transitory and totally reversible.

Effect of beta-endorphin at 1pg/ml on the contraction of 2 independent fibers

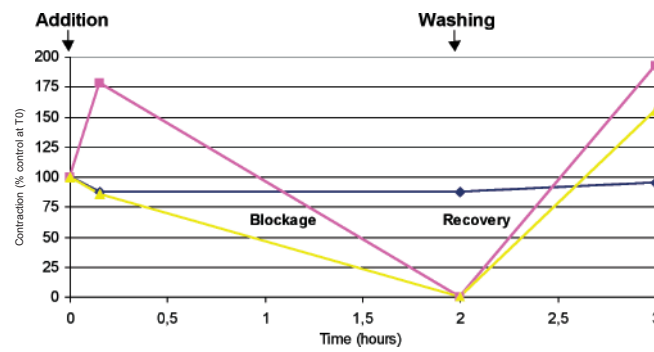
Effect of *Helichrysum italicum* extract



- ▶ At 0.2%, the muscular contractions blocked after 2h of contact.
- ▶ At 1% and 5%, the contractions of 2 fibers out of 3 blocked after 2h and 10min respectively.
- ▶ After elimination of the extract at 1% and 5%, the frequencies returned to normal after 4h and 1h respectively.

Kinetic of the effect of Helichrysum italicum extract at 0.2% on the contraction of 3 fibers

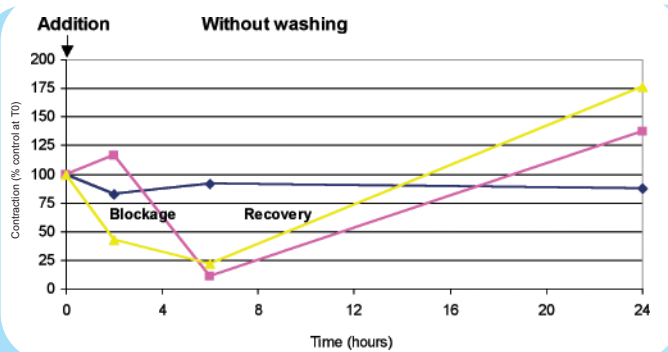
Effect of *Crithmum maritimum* extract



- ▶ At 5%, the muscular contractions partially blocked after 2h of contact.
- ▶ After elimination of the extract, the frequencies of contraction returned to normal after 1h.

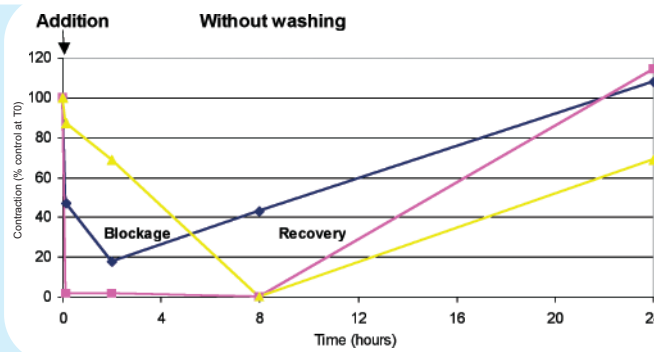
Kinetic of the effect of Crithmum maritimum extract at 5% on the contraction of 3 fibers

Effect of *Lavandula stoechas* extract



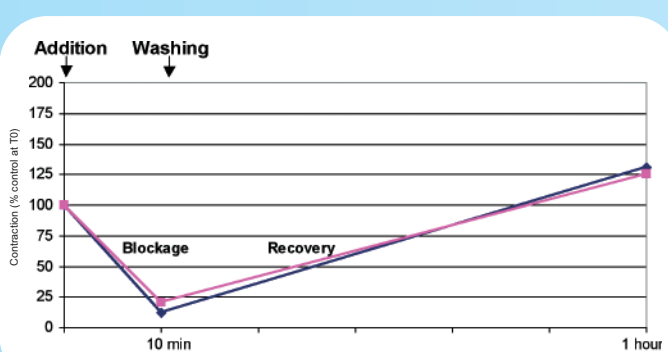
- ▶ The kinetic of the effect of *Lavandula stoechas* extract at 2.5% showed that 2 fibers out of 3 were blocked until 6h and then this effect was reversible without washing.

Kinetic of the effect of Lavandula stoechas extract at 2.5% on the contraction of 3 fibers



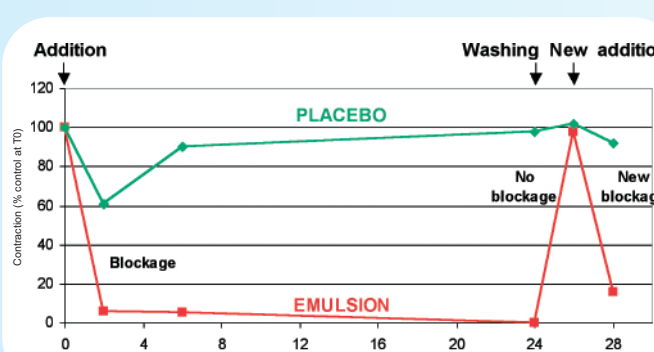
- ▶ A study of the kinetic of the effect of a more concentrated *Lavandula stoechas* extract at 5% showed an inhibition of 1 fiber out of 3 and a blockage of the 2 others after 10min of contact until 8h. Whatever the concentration, a reversible effect was obtained with the 3 fibers without washing.

Kinetic of the effect of 11x concentrated Lavandula stoechas extract tested at 5% on the contraction of 3 fibers



- ▶ The more concentrated extract, tested at 0.2%, 1% and 5% blocked the muscular contractions of 3 fibers out of 3 after 10min of contact.
- ▶ After elimination of the extract, the frequencies returned to normal after 1h at 0.2% and 1%, and only 1 fiber out of 3 stayed blocked at 5%.

Kinetic of the effect of Lavandula stoechas extract at 0.2% on the contraction of 2 fibers



- ▶ A microemulsion containing 20% of *Lavandula stoechas* oil (0.2% of *Lavandula stoechas* oil at final concentration), obtained by CO2 supercritical extraction in the presence of a co-solvent, was tested at 1% : it blocked the fibers after 2h until 24h, but the blockage was reversible with the elimination of the product and the fibers were blocked again after a new addition of the product.

Reversible and repetitive effect of a microemulsion at 1% containing 20% of Lavandula stoechas oil (0.2% at final concentration) on the contraction on 1 representative fiber (red) compared with the effect of the co-solvent of extraction (green)

Conclusion:

- Interest of a new model for cosmetology
- Description of the effects of 3 aromatic plants extracts
- Selection of the most active plant extract: *Lavandula stoechas* extract
- Active ingredients usable into cosmetic for anti-age applications