Mirabilis Jalapa Plant Active Mediates the Malate-aspartate Shuttle Regulation and the Cellular Oxidation Prevention

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Introduction:

***A*ging** is a complex process, characterized by a gradual decrease in physiological functions that is often associated with numerous disorders. Mitochondria are considered to have a significant impact on aging due to their critical role in the regulation of bioenergetics, oxidative stress and cell death. Mitochondrial reducing equivalent shuttles play an important role in most cells in regulating the balance between NAD+ and NADH levels in the cytoplasm and mitochondria.

Recent research suggests that the malate–aspartate NADH shuttle is a novel metabolic longevity regulator. Several studies have shown that mitochondrial bioenergetic deterioration is an important factor in aging and age-related illnesses. One of the causes of aging is the cumulative damage from reactive oxygen species (ROS) to mitochondrial DNA which reduces maintenance of the energy supply required during aging for cellular repair, homeostasis mechanisms and mitochondrial biogenesis.

The Mirabilis Jalapa active plant cell is a new industrialized advanced product made from dedifferentiated leaf cells and elicited to produce large quantity of phytoalexins. The aim of this study was to investigate the extent of Mirabilis Jalapa's effect. We tested it on older (with slower mitochondrial activity) and young cells (with optimal activity). Our data show that our dedifferentiated and elicited Naolys Mirabilis Jalapa active plant cells display strong anti-aging properties through an increase of the cellular metabolism processes.

Materials & Methods:

Malate/Aspartate Shuttle assay

Different cellular subcultures at P2, P4, P6, P8 were used to evaluate malate-aspartate shuttle (MAS) activity. MAS activity was monitored after cells were treated with different concentrations (0.5%, 1% and 2.5%) of Naolys Mirabilis Jalapa active plant cells encapsulate caffein's and mitochondria isolation. After treatment of the cells with the product under study, the mitochondria were isolated and the activity of the malate-aspartate shuttle was determined. Briefly, 50 µl of mitochondrial suspension was mixed with 2ml (final concentration in mM) 300 mannitol, 10 potassium phosphate, 10 Tris, 10 potassium chloride, 5 magnesium chloride, 2aspartate, 2ADP and 0.14NADH with 3U/ml malate dehydrogenase (MDH) and 2U/ml Aspartate aminotransferase mitochondrial at pH 7.4. The oxidation of NADH was monitored at 340 nm at a constant temperature of 37° C for 4 minutes. Malate-aspartate shuttle activity was initiated by the addition of 4mM malate and 4mM glutamate (the final concentration). The dosage of the malate/aspartate shuttle activity is measured at 340nm for 4 minutes.

Lipoperoxidation assessment by malondialdehyde (MDA)

24hours after the treatment of reconstituted epidermises; the cellular suspension was put in a buffer containing reagents [250 l of Tris buffer (50 mM, pH 8) containing NaCl (0.1M) and EDTA (20 mM), 25 l of SDS (7%), 300 l of HCl (0.1 N), 38 l of phosphotungstic acid at 1% in water, 300 l of thiobarbituric acid at 0.67% in water]. After 1 hour of incubation of the samples in dark at 50° C and 10 min in an ice bath (0° C), 300 µl of n-butanol were added and tubes were shaken vigorously. After centrifugation for 10 min at 10000 g at 0° c, the n-butanol phase from each sample containing MDA-TBA adduct was separated and analysed by HPLC and fluorometric detection (excitation at 515nm; emission at 535 nm). The assessment of proteins was performed at 595 nm according to BRADFORD method using a spectrophotometer.

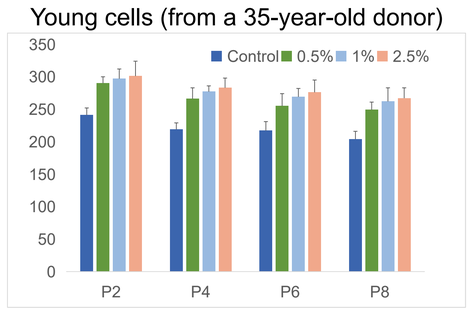
Clinical scoring

Assessment of the effect on the cutaneous state by self-scoring: an improvement of the cutaneous state by self-scoring according to the items "Radiance of the complexion "and "Luminosity of the complexion" after 28 days of applications in conditions of normal use is studied on female of Phototype (Fitzpatrick, from I to III) with Caucasian skin aged from 25 to 65 years old and of all skin types on the face presenting with a dull complexion. The self-scoring consists in a visual assessment of the cutaneous state before then after application of the investigational product to the experimental area, in order to determine its cosmetic efficacy. For this purpose, the subject sits in front of the table Evalux bench® (Orion concept) with a long-life and calibrated lighting (leds - 6000° K). The self-scoring was performed using a scale scored in 10 points (from 0 to 9) according to the following items: Radiance of the complexion (0= not radiant, 9= very radiant] and Luminosity of the complexion (0= not luminous, 9= very luminous).

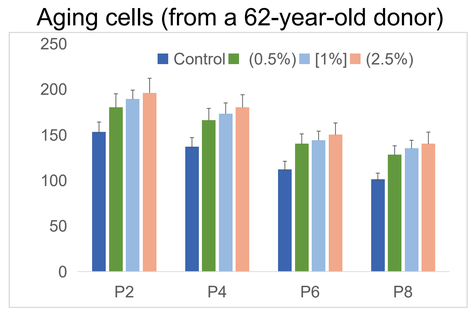
Results & Discussion:

Malate/Aspartate Shuttle assay conducted in young and aging cells

Young cells (from a 35-year-old donor)



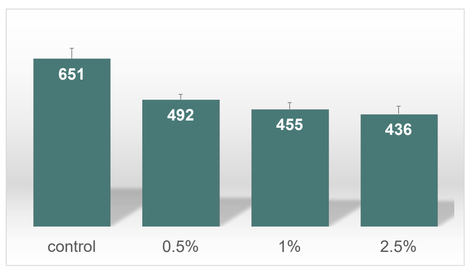
Aging cells (from a 62-year-old donor)



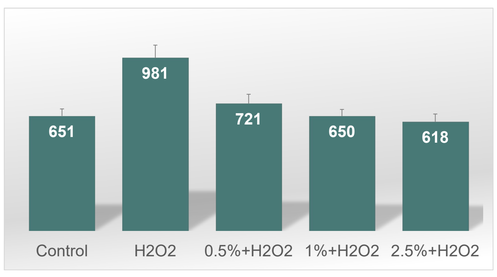
The results show that shuttle activity is higher in normal physiological cells (donor 35 years) compared to normal senescent cells (donor 62 years). This indicates that malate/aspartate activity decreases with age.The treatment of the cells with Mirabilis Jalapa active plant cell resulted in a restoration of the activity of the malate/aspartate shuttles under normal conditions of senescence (donor 62 years): aging of the donor and the conditions of culture.

Lipoperoxidation assessment by MDA analysis

In physiological conditions

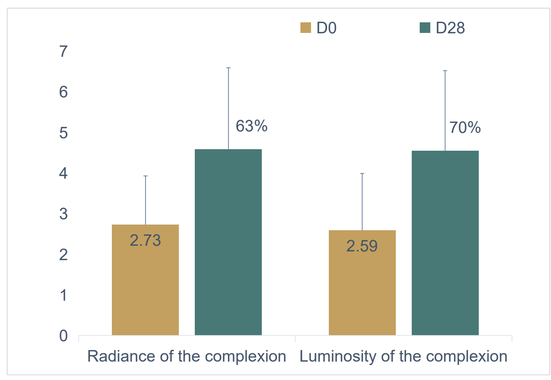


In induced conditions by hydrogen peroxide



The assessments of MDA demonstrate that Mirabilis Jalapa active plant cell at the concentrations of 0.5%, 1% and 2.5% reduces significantly the physiological MDA production and protects significantly the cells against the lipoperoxidation induced by hydrogen peroxide.

Assessment of the effect on the cutaneous state by self-scoring



Mirabilis Jalapa active plant cell enhances the complexion's radiance and the general well-being of the skin. The results obtained by clinical scoring showed an increase in the complexion's radiance and brightness by 63% and 70% respectively after 28 days of application in older women.

Conclusions:

The use of plant cell culture of Mirabilis Jalapa encapsulating caffein's is an innovative active and a sustainable source of bioactive compounds that Naolys has developed for the cosmetics industry by combining the efficacy of the product with the protection of biodiversity. Our study clearly indicates that the dedifferentiated and elicited Mirabilis Jalapa bioactive plant cells display strong anti-aging properties by strengthening the cellular metabolism processes necessary for healthy cell functions and for more relaxed, radiant skin. It also helps to restore resistance to external threats.

References:

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