Skin cellular youth reprogramming as an innovative anti-ageing strategy for cosmetic ingredient

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Abstract

Background: During ageing, cells progressively lose their pluripotency, proliferative capacities, and remodelling driver role among other activities, leading to apparition of visible ageing signs such as wrinkles, eye-bags or even ageing spots.

Methods: *In vitro*, the activity of the compound Sericoside from the bark of the roots of *Terminalia sericea* was evaluated at 0.02% through a transcriptomic analysis performed on fibroblasts after 24 hours, proliferation tests on senescent fibroblasts after 72 hours. Then, a clinical study was conducted on 40 volunteers aged between 35 and 55 years' old who applied twice daily a cream containing or not Sericoside for 4 weeks. Skin elasticity and fatigue were measured with a Cutometer® with R2 and R9 paramaters respectively. Skin texture and roughness was analysed by DermaTOP-Blue method.

Results: Transcriptomic analysis evidenced that Sericoside would improve cell cycle (+85% MKI67), cell proliferation (+250% IGF1), DNA repair (+56% OGG1), pluripotency transcription factors (+36% NANOG) and stem cells maintenance (+200% SOX2). We evidenced a decrease of proliferation factor for senescent cells compared to young cells by -50% while Sericoside increased this proliferation factor by +46%, a similar rate to that of a 22 years old donor. Clinically, we evidenced Sericoside's anti-ageing effect which increased skin elasticity by +20%, reduced skin fatigue by -17% and reduced skin roughness by -10%, translating a soothing effect for Sericoside.

Conclusion: Thanks to this study, we proved that re-activating cell memory to reprogram cells pluripotency by stimulating the natural tools available in our DNA is an innovative anti-ageing strategy.

Keywords: Cell memory; Reprogrammation; Proliferation; Rejuvenation

Introduction

It is well-known that several biological mechanisms are affected by the natural ageing of the body, the large majority of them starting from a modification of gene control and ending up in visible skin ageing signs [1]. On a general point of view, characteristics of cells are different depending on age: pluripotent stem cells can turn into different tissues to replace senescent cells, and these newly differentiated and functional cells present an amazing vitality and proliferation capacity that starts decreasing throughout the natural ageing of cells [2]. As they age, stem cells number and self-renewal capabilities do not necessarily decline but they progressively lose their DNA repair capacities and their ability to produce new progenitors and differentiated effector cells is impacted [3]. As a consequence, there is a reduction of tissue regenerative potential associated to an accumulation of non-functional cells throughout life [4].

Stem cells present an important role in different skin layers: their function in the epidermis is largely described in the literature. Indeed, the epidermis is the first part of the body to be exposed to external aggressors, submitting epidermal cells to stress and damages. Thus, this multi-layered stratified squamous epithelium need a constant renewal due to its high turnover [5]. The role of stem cells in the dermis is no less important even if it is less described: stem cells are present into the dermal tissue and participate in the homeostasis maintenance and regeneration of injured skin [6]. Indeed, they have the capacity to differentiate in new functional fibroblasts able to synthesize molecules from the extracellular matrix and the elastic network, with a higher proliferation rate than cells becoming senescent.

By losing their pluripotency and functionality, skin cells show a slowing down of cells regeneration and repair function associated with a loss of their skin remodelling driver role among other activities, leading to a progressive apparition of visible ageing signs such as wrinkles, eye-bags or even ageing spots [7].

Taking in consideration this knowledge, we hypothesized that reactivating cell memory to bring them back closer to a pluripotency state appears to be a very effective and innovative strategy for cells rejuvenation.

Recent discoveries derived from ethnobotany and plants traditional use have highlighted the fact that some botanical compounds have the ability to massively stimulate the genes involved in rejuvenation mechanisms. In Tanzania, a very typical plant of the *miombo* forest environment is *Terminalia sericea*, a beautiful, majestic tree with silvery leaves, also known as a silver tree. In the bark of the roots of this tree, the plant accumulates a specific pentacyclic terpenoid: Sericoside. Sericoside is already known and largely used for its medicinal properties [8].

In this study, we evaluated the capacities of Sericoside to reprogram cells to a younger stage to define if it could be an efficient candidate to reactivate cell memory for a rejuvenation process. We analysed its effect regarding stem cells maintenance, DNA repair, cell pluripotency, cell proliferation properties and, wider, regarding global anti-ageing properties.

Materials and Methods

- **Transcriptomic study**

Normal Human Dermal Fibroblasts were stimulated in fetal calf serum (FCS)-free culture medium containing 0.02% of Sericoside. After 24h of stimulation, total RNA were extracted by Extract-all method. RNA quality was controlled and a reverse transcription was performed to obtain cDNA. RT-qPCR was made on specific plates designed to study transcriptomic expression of different genes involved in dermis biology with 10 ng of cDNA per well. The results of gene expression obtained with fibroblasts were normalized according to POLR2A (RNA polymerase II, subunit A) and HMBS (porphobiloinogen deaminase) housekeeping gene. We analysed the modulation of genes expression relative to the untreated condition.

- **Cell proliferation analysis**

Normal Human Dermal Fibroblasts from mature donors (average: 52 years old) were maintained in culture during 8 cell passaging with repeated stress by H_2O_2 at 200 µM stress to induce cell senescence. These cells were compared to fibroblasts from young donors (average: 22 years) and were seeded in a black 96-wells plates in DMEM medium

supplemented with 0.5% FCS. After cell adhesion, stimulation was performed with Sericoside at 0.02% on each cell type versus the untreated condition. At day 0, after 24h and after 72h of stimulation with Sericoside, Hoechst 33342 was added on cells for 30 minutes and pictures of total wells in Hoechst 33342 fluorescence spectrum (Ex: 350nm ; Em: 461nm) were acquired with an automate (PICO, Molecular Devices). Doubling time of each cell donor and in each condition was calculated as following:

$$T = \frac{t \times \ln(2)}{\ln(T72h) - \ln(T24h)}$$

A proliferation factor was then calculated from the obtained doubling time.

- **Protein synthesis**

Normal Human Dermal Fibroblasts were cultured in fibroblasts growth medium until confluence and were then treated with Sericoside for 3 days versus untreated condition. Treatment was renewed every day and cells were then collected and frozen at -20°C before being hydrolysed. Amino acid synthesis dosage was performed with a Beckmann 6300 analyzer using a column of ninhydrine with a detection at 440nm.

- **Clinical study**
    - **Panel description**

A placebo controlled, single blind clinical study was carried out on 40 volunteers (men and women, aged 35-55). They were randomly divided in two groups: one was required to apply an emulsion containing 5mg/ml of Sericoside while the other was required to apply a blank emulsion. Volunteers of each group had to twice daily apply their emulsion for 4 weeks. Several indicators of facial ageing have been monitored, like skin firmness, skin elasticity, dark circles, blemishes.

    - **Skin firmness and skin fatigue measurements using Cutometer®**

Skin elasticity has been evaluated by Cutometer® MPA 580 (Kourage&Khazaka) on the following parameters:

- R2 parameter (overall skin elasticity, tonicity)
- R9 parameter (anti-fatigue)

    - **Skin texture and roughness analysed by DermaTOP-Blue method**

Evaluation by Visio DermaTOP-blue (Eitech) allows contactless measures of skin profilometry and skin texture:

- 3D Skin roughness (Rz average roughness; Ra maximum roughness) -> eye contour wrinkles
- Skin texture (Sa parameter).

Eye contour benefit measured by Chromameter and DermaTOP-Blue method

Eye contour benefits in terms of eye bags and dark circles have been evaluated by Chromameter (Minolta CR 200) on the following parameters (dark circles):

- L parameter (0= black; 100 = white)
- a (green-red), b (blue-yellow) parameters (+120; -120)

Eyebag volume was evaluated by Visio DermaTOP-blue.

Statistical analysis

In vitro, data normality was first verified regarding the Gaussian law using Shapiro Wilk test. According to the results, we used unpaired parametric Student t test to compare the effect of Sericoside versus the untreated condition.

Regarding clinical data, we used a paired t student test to compare the effect of Sericoside after 4 weeks relative to D0.

Results

Gene regulation

The transcriptomic analysis was performed by RT-qPCR on dermis plates with 95 targeted genes involved in extracellular matrix, remodelling, antioxidant enzymes and stress defences, neurotrophin pathway, cell proliferation, DNA repair and stem cell markers. The used gene list is maintained confidential.

We evidenced the significant upregulation of 30 genes with Sericoside treatment, showing a strong bioactivity of the product (Table 1). Among these genes, some are involved in cell proliferation, DNA repair, extracellular matrix, dermo-epidermal junction, antioxidant defences and pluripotency transcription factor.

Pathway	Genes	Fold change	Significance	Pathway	Genes	Fold change	Significance
Antioxidant defences	**G6PD**	2.08	*	Extracellular matrix	**CD44**	1.48	**
	GPX1	1.48	*		**CYR61**	1.56	*
	GSTT1	1.40	#		**FBN1**	1.15	**
	HMOX1	1.22	#		**HAS2**	1.61	#
	MSRA	1.40	***		**TIMP1**	4.03	**
	SOD2	1.16	*		**SDC1**	1.90	**
Elastic network	**VCAN**	1.79	*	DNA repair	**GADD45A**	1.29	*
Cell cycle	**MKI67**	1.85	***		**OGG1**	1.56	*
Cell proliferation	**FGF7**	1.29	**		**XPA**	1.26	***
	IGF1	3.50	***		**XPC**	1.51	*
	IGF1R	1.40	**	Pluripotency transcription factor	**NANOG**	1.36	*
	IGFBP3	1.30	***		**POU5F1**	1.51	#
Dermoepidermal junction	**COL4A1**	1.17	#	Retinoic acid receptor	**CRABP2**	1.47	*
	COL7A1	1.45	*	Signal transduction	**CAV1**	3.86	**
Transcription factor	**MYC**	1.39	*	Stem cells maintenance	**SOX2**	3.00	#

Table 1: Genes modulation with Sericoside relative to the untreated condition.

First, we evidenced the potential benefit of Sericoside on cell rejuvenation through the upregulation of genes involved in DNA repair (XPC, XPA, OGG1, GADD45A), pluripotency transcription factors (POU5F1, NANOG), retinoic acid receptor (CRABP2), signal transduction (CAV1), stem cell maintenance (SOX2) and transcription factor (MYC)[9]–[12].

The transcriptomic analysis on fibroblasts also evidenced that Sericoside significantly upregulated several genes linked to protection and rejuvenation of the extracellular matrix, showing a potential anti-ageing activity. Indeed, the upregulation of the major genes linked to the protection & rejuvenation of the extracellular matrix (CD44, CYR61, FBN1, HAS2,

TIMP1, SDC1, VCAN) can be linked to an improvement in skin elasticity, anti-wrinkles and firmness. Regarding the genes involved in antioxidant defences (G6PD, GPX1, GSTT1, HMOX1, MSRA, SOD2), their upregulation has a relevance in the decrease of dark circles by protecting heme against oxidation. Sericoside also upregulated a variety of genes involved in dermoepidermal junction (COL7A1, COL4A1), cell proliferation (IGFBP3, IGF1R, IGF1, FGF7) and cell cycle (MKI67). Taken together, these modulated pathways present a relevance on skin texture, firmness and anti-sagging.

- **Improvement of senescent cells proliferation**

Following the transcriptomic study which evidenced various anti-ageing benefits of Sericoside, among them cells rejuvenation, we went further by studying the effect of this active ingredient on cell proliferation. Indeed, it is well described in the literature that the capacity of cells to proliferate is an important marker of youth and is impacted during ageing. The proliferation study we performed confirmed this knowledge since we evidenced that senescent cells presented a significant decrease of cell proliferation factor by -50% (Figure 1). Interestingly, the stimulation of senescent cells with Sericoside significantly increased this proliferation factor by +46%, close to the young state.

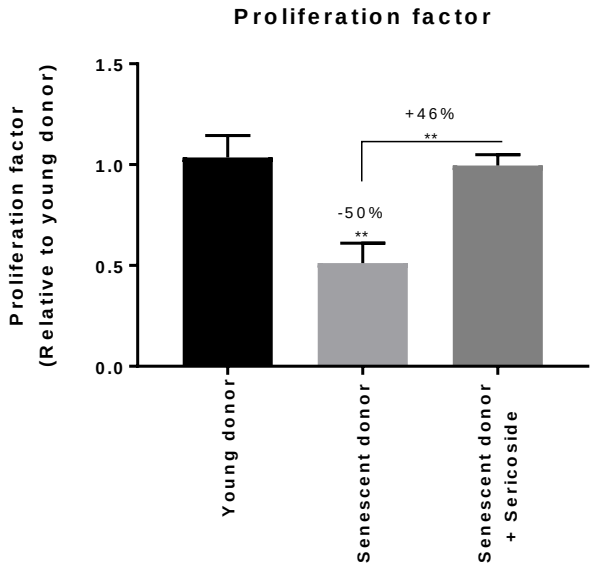

Figure 1: Impact of senescence and restoration of cell proliferation factor with Sericoside

We transposed the treated senescent cells' doubling time to a 22 years-old state by calculating the age corresponding to the obtained doubling time thanks to the trend line equation (Figure 2).

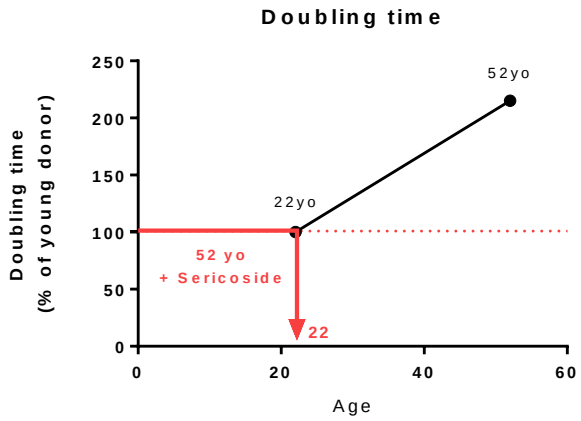

Figure 2: Estimation of age recovery for senescent cells treated with Sericoside

- **Stimulation of protein synthesis**

Collagen is the predominant matrix skin protein and it is known to impart textile strength to skin. However, collagen decreases with ageing (and UV exposure and other external challenges).

To evaluate the impact of Sericoside on collagen synthesis, and more specifically on total proteins and proline production (one of the most relevant amino acids in collagen and elastin production, the one providing spatial structure to collagen), human fibroblasts have been treated with Sericoside to evaluate the potential increase in their synthesis compared to a control medium.

We evidenced that Sericoside increased total protein synthesis by +54% with a specific reference to proline content which was boosted by +10% (Figure 3). These data complete the transcriptomic study regarding the boosting effect of Sericoside on collagen synthesis.

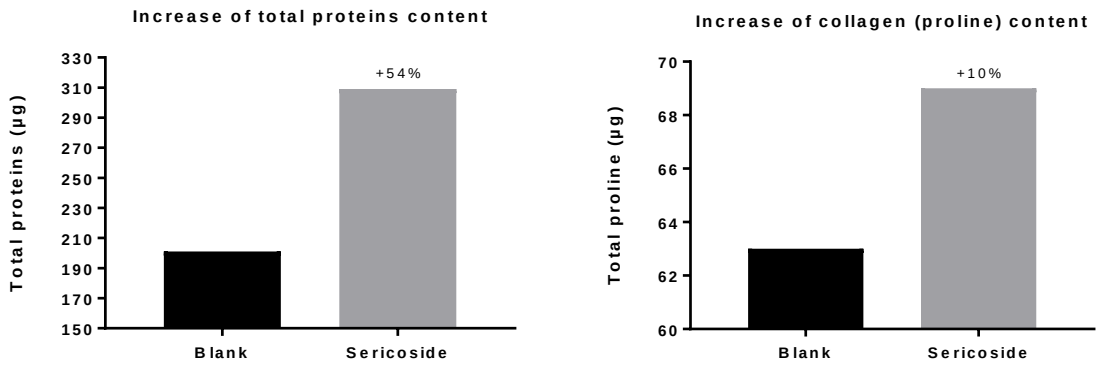

Figure 3: Impact of Sericoside on total proteins synthesis (left panel) and collagen synthesis (right panel) by fibroblasts.

- **Clinical evaluation**
    - **Reversing the clock of ageing**

First, we evaluated the efficacy of our product on skin elasticity and skin fatigue after 1 month of application. Overall skin elasticity increased by +20%* (R2 parameter) and skin tiredness (R9 parameter) decreased by -16.5%* (Figure 4). These data showed a significant increase in skin elasticity and a reduction of skin fatigue after only 30 days of usage.

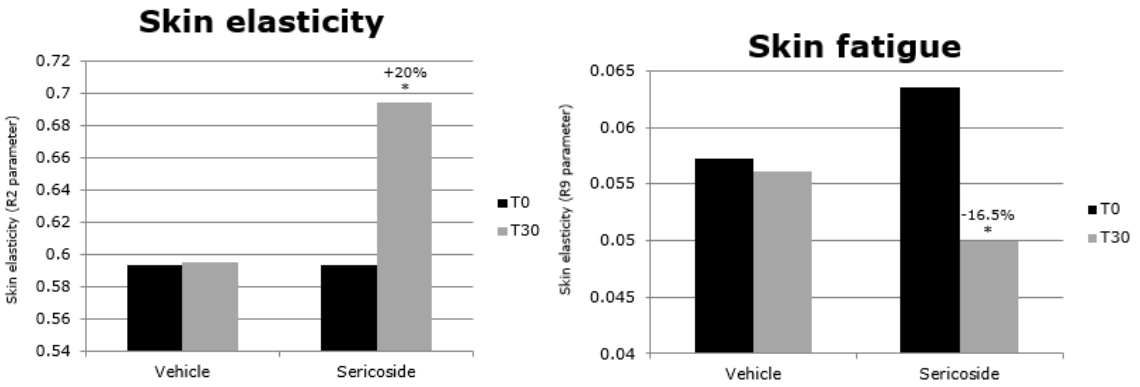

Figure 4: Impact of Sericoside application on skin elasticity and skin fatigue measured by Cutometer in comparison with placebo control.

We continued to explore the anti-ageing property of Sericoside by analysing the skin roughness on periorbital area as a way to see visible reduction of ageing signs on face. We measured Rz parameter which represents the arithmetic average of the different roughness

segments calculated from 5 succeeding segments of the same length. It is defined as mean depth of roughness.

Our data showed that the Skin roughness (Rz) as per mean maximum height profile decreased by -9.5%*. The skin texture amelioration was perceivable with touch. This effect was confirmed by the visualization of skin texture analysed by skin surface topography (parameter Sa) which indicates the roughest points (difference between peak and hollowness) – red points. After the treatment red points are remarkably less present (Figure 5).

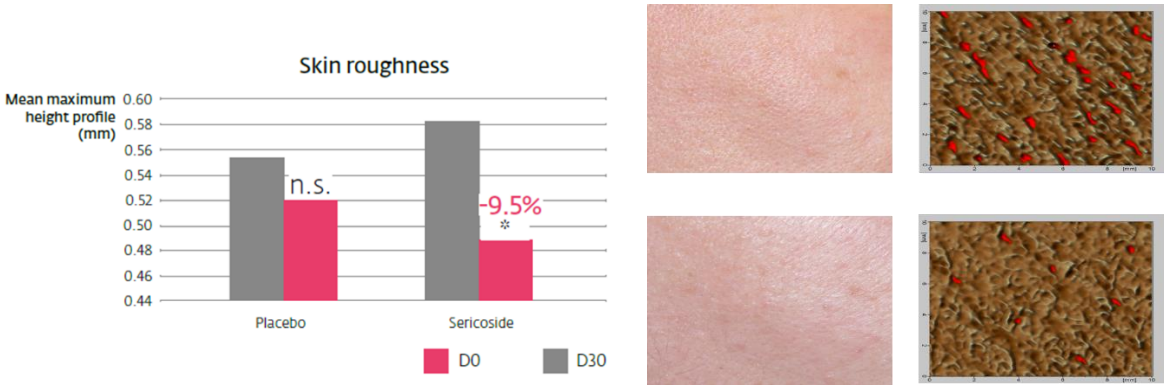

Figure 5: Impact of Sericoside application on skin roughness (left panel) and skin texture visualization (right panel) analysed by DermaTOP-blue method in comparison with placebo control.

As a consequence of control of clock of ageing, we can observe a strong reduction of wrinkles on the periorbital area after 30 days of application. Skin wrinkles are represented by 3D imaging and high resolution pictures. Skin wrinkles were visibly less deep after a month of treatment with Sericoside (Figure 6).

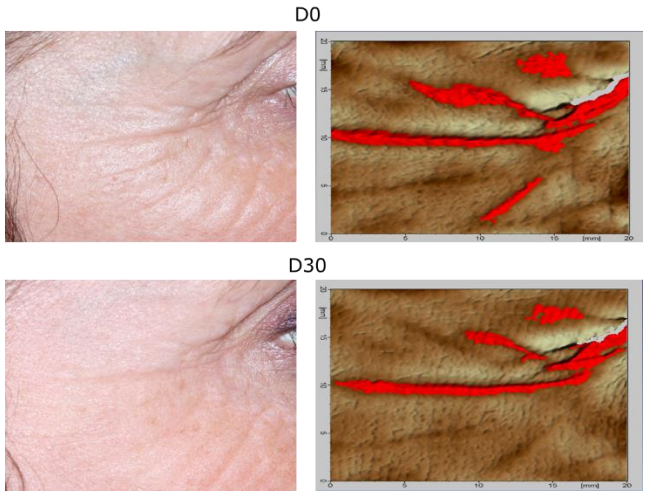

Figure 6: Impact of Sericoside application on skin wrinkles focus on periorbital area visualized d by DermaTOP-blue before and after application (D0 versus D30)

- **Impact on Dark Circles**

Secondly, we analysed the impact of Sericoside on dark circles improvement after 30 min of application using the same panel previously described.

Sericoside has been able to induce a significant amelioration in dark circles in three times more volunteers compared to the placebo group (Figure 7). The percentage of volunteers showing a perceivable improvement in dark circles appearance reached 45%.

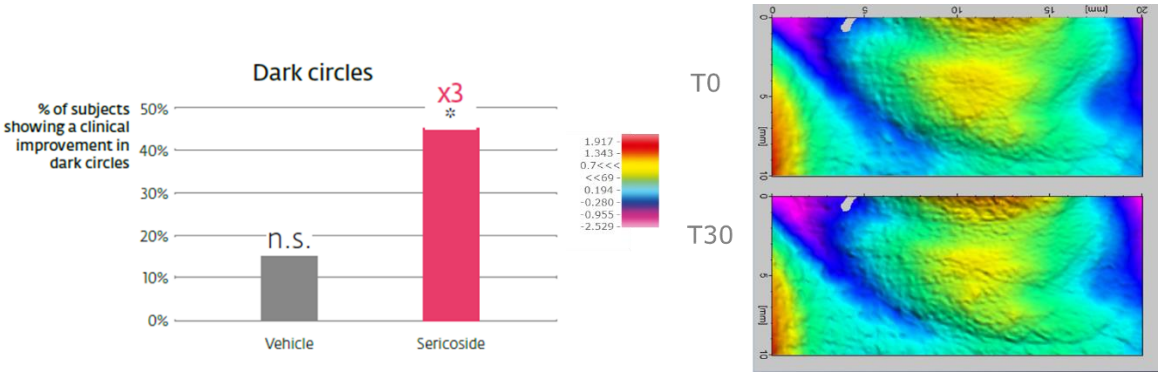

Figure 7: Impact of Sericoside application on dark circles focus on periorbital area visualized d by Chromameter (left panel) and visualized by DermaTOP-blue (right panel) in comparison with Placebo control

Regarding eye bags, the mean positive volume of eye bags decreased by -20 mm^3, whereas no amelioration was observed in the placebo group (Figure 8).

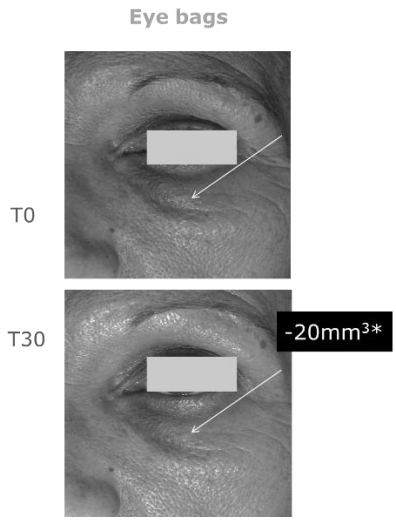

Figure 8: Impact of Sericoside application on dark circles focus on periorbital area showing a reduction of volume by -20 mm^3.

Discussion

To resume, we first evidenced that Sericoside is able rejuvenate cells by stimulating cell memory leading to the reprogrammation of cell pluripotency. Indeed, our active stimulated the expression of genes such as SOX2, NANOG or C-MYC which is an efficient cocktail for a natural iPS cells reprogrammation [13]. Indeed, SOX2 has been evidenced by Narayan *et al* to work with POU5F1 as activators during the reprogrammation of human fibroblasts to iPS cells [14]. NANOG gene is an embryonic stem cell marker that is involved in the maintenance of undifferentiated state of pluripotent stem cells [15]. In the context of cellular reprogramming, C-MYC enhances the conversion of somatic cells into induced pluripotent stem cells [16]. Sericoside also stimulated the expression of XPC, XPA, OGG1 and GADD45A, genes involved in DNA repair, which is a function from stem cells that can be negatively impacted during ageing. These transcriptomic results highlighted Sericoside as a good candidate to reactivate cell memory to bring back them to pluripotency state.

This rejuvenation process induced by Sericoside lead to a reactivation of cells functions. Indeed, the active also stimulated the expression of genes involved in various pathways: reactivation of antioxidant defences with G6PD, GPX1, GSTT1, HMOX1, MSRA and SOD2; extracellular matrix composition with CD44, CYR61, FBN1, HAS2, TIMP1, SDC1 and VCAN; cell proliferation with IGFBP3, IGF1R, IGF1, FGF7 and MKI67; and even dermo-epidermal junction integrity with COL7A1 and COL4A1.

Cell rejuvenation was also confirmed by the significant increase of senescent fibroblasts' proliferation factor, allowing us estimating a rejuvenation of cells presenting a doubling time equivalent to a 22 years old donor. This huge rejuvenation of fibroblasts' metabolism was confirmed with an increase of proteins synthesis by +54% and especially the collagen as observed by the increase of proline content with +10%, reinforcing the set of data showing a rejuvenation of cells with Sericoside.

With clinical studies, we showed that cells rejuvenation led to a wide anti-ageing efficacy with an improvement of skin elasticity, firmness and reduction of skin fatigue after 28 days of application. This anti-ageing effect can be linked with the stimulated expression of genes involved in the protection and production of the extracellular matrix. We also demonstrated a reduction of wrinkles and an improvement of skin texture by reducing of skin roughness

on the periorbital area. These clinical benefits also made the link with the transcriptomic study which showed an improvement of extracellular matrix composition, cell proliferation and dermo-epidermal junction. Finally, Sericoside reduced dark circles and of eye bags, confirming the antioxidant properties of the product.

Conclusion

Thanks to this study, we proved that reprogramming cells by stimulating the natural tools available in our DNA is an innovative way to rejuvenate the skin. Sericoside proved its strong efficacy on this innovative strategy.

Conflict of Interest Statement: NONE.

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