

The Anti-Aging and Health Promoting Effect on Skin by MitoBeauty™

Abstract:

The study shows a decrease in wrinkle based on a purely natural product from berries.

Introduction

The stratum corneum forms a water impermeable barrier in the uppermost layer of the epidermis (Denda, Fuziwara, & Inoue, 2004). Aging has a huge impact on the skin and its ability to regenerate. Furthermore, diseases such as atopic dermatitis will also impact the quality of the skin (Denda et al., 2004). Especially, parameters such as hydration and pH are changed in the aging skin (Trojahn, Dobos, Lichterfeld, Blume-Peytavi, & Kottner, 2015). MitoBeauty™ is an extract of black- and redcurrants high in polyphenols and particularly high in the patented active compound Ribetril A®, a polyphenol-alkaloid present in certain cultivars of black- and redcurrant (Weidner & Weidner, 2015). MitoBeauty™ may have an effect on the intracellular level of metabolites through the inhibition of enzymes. *In vitro* studies showed Ribetril A® to inhibit PDE-4 (phosphodiesterase-4) and PDE-5 (phosphodiesterase-5) in physiological concentrations (Weidner & Weidner, 2015).

cAMP is a classical second messenger, involved in processes such as airway regulation, inflammation and pathway signaling among others (Billington & Hall, 2012). Increased cAMP leads to an increased anti-inflammation/ immunomodulatory response (Homeostasis, 2013). PDE-4 is a major cAMP degrader and PDE-4 degradation of cAMP can cause immune cell activation and the release of proinflammatory mediators such as TNF- α , IL-17, IL-22 and IFN- γ (Homeostasis, 2013; Inflammation, Mechanisms, & Inflammation, 2013). PDE-4 is found to be active especially in keratinocytes and PDE-4 inhibition showed great effect against diseases such as atopic dermatitis (Rundfeldt, Steckel, Sørensen, & Wlaź, 2012). PDE-5 is involved in the hydrolysis of cGMP to 5-GMP. Thus, inhibition of PDE-5 will lead to a higher level of cGMP, which promotes vascular repair in multiple models of tissue damage and wound repair (Zhu et al., 2008). Thus, inactivation of PDE-5 will lead to an increase in vascular development, thus increasing the blood flow (Sahara et al., 2010).

Blackcurrant is already known to have an effect on skin parameters, with increased cutaneous blood flow (ASHIGAI et al., 2018; Denev et al., 2014) whereas redcurrant is less known for such effects. Others have shown that polyphenols and flavonoids have a great biochemical effect on oxidative stress as well as inflammation and diseases such as cancer (Denev et al., 2014; Park et al., 2012).

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In the current study we suggest a new extract from black- and redcurrants having an effect on the skin. The data is a combination of cellular assays showing effects of the active compound as well as a study showing change in the skin parameters after merely 84 days of intake. The effect of MitoBeauty™, is evaluated *in vitro* based on enzymatic parameters as well as the effect on the skin. The skin parameters include cutaneous blood flow, the skin miniaturization, the redensifying effect of the skin, anti-wrinkling based on clinical analysis and subject's statement of tolerance for the product and the efficacy of the product. We suggested a possible connection between a PDE-4 and PDE-5 inhibitor and physiological effects on the skin, but further studies needs to be performed to verify the effect.

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Method and Materials:

PDE4A1A Assay Kit and PDE5A Assay Kit:

The assay was performed according to the BSP Bioscience protocol [BSP Bioscience, Data Sheet PDE4A1A Assay Kit, Catalog #60340). The assay is based on fluorescence AMP generated by PDE4A1A. Thus a compound inhibiting the PDE4A1A will lead to lower fluorescence. A fluorescence plate reader (Appliskan, Thermo Scientific) was used to detect the fluorescence at excitation of 485 nm and emission of 530 nm, with a measuring time of 500 ms.

Skin Study:

The evaluation of the cutaneous microcirculation, cutaneous hydration and anti-wrinkle effect was performed by Pharmascan (Pharmascan, Lyon, France) in accordance with ISO9001-2008.

The study was performed with 50 subjects, being given a daily dose of 30 ml MitoBeauty™ in a glass of water during the evening meal. The subjects were instructed to come to the laboratory without any product on face or forearms.

Cutaneous microcirculation:

Cutaneous microcirculation is measured by face tissue viability imaging TiVi600®. The imaging is based on the fact that linear polarized light, in the visible wavelength region, is reflected directly by the skin surface and partly diffused by the deeper located dermal tissue matrix. The technology of TiVi600® depends on the wavelength of absorption of the red blood cell.

Study of cutaneous hydration:

The cutaneous hydration is evaluated using the corneometer CM 825 (Courage & Khazaka, Germany). This instrument determines the humidity level of the most external cutaneous layers of the stratum corneum. In this study the forearm was analyzed.

Anti-wrinkle effect

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The anti-wrinkle effect is measured using the PRIMOS (phaseshift Rapid *in Vivo Measurement of Skin*) lite, on the skin of the foot of the test subjects. The technique calculates a phase image from images with interference projection. This image then allows to determine the height of each point.

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Results

In vitro:

Ribetril A® inhibition of PDE-4 and PDE-5:

	IC50 value
PDE-4	2.95 µM
PDE-5	2.1 µM

The values are measured using a fluorescence assay in 5 dilutions to determine the IC50 value for the two enzymes. The data shows an inhibitory effect of PDE-4 and PDE-5 upon exposure to Ribetril A®.

In vivo

A significant increase in red blood cells in the skin of 6 % is observed in 78 % of the subjects after 84 days. A significant increase in cutaneous hydration rate of 9 % is observed in 69 % of the subjects after 84 days.

Table 1 Anti-wrinkle and smoothing effect of MitoBeauty after 42 and 84 days of exposure. As seen from the data is a 4 % decrease found for both conditions in between 63 and 69 % of the subjects

Parameters Observed	Kinetics	Effects Observed	Product efficacy	% of subjects having the excepted effect
Average Roughness Parameters	Day 42	Smoothing effect	-4%	69
	Day 84		-4%	63
Average relief Parameters	Day 42	Anti-wrinkle effect	-5%	69
	Day 84		-4%	67

Subjective analysis:

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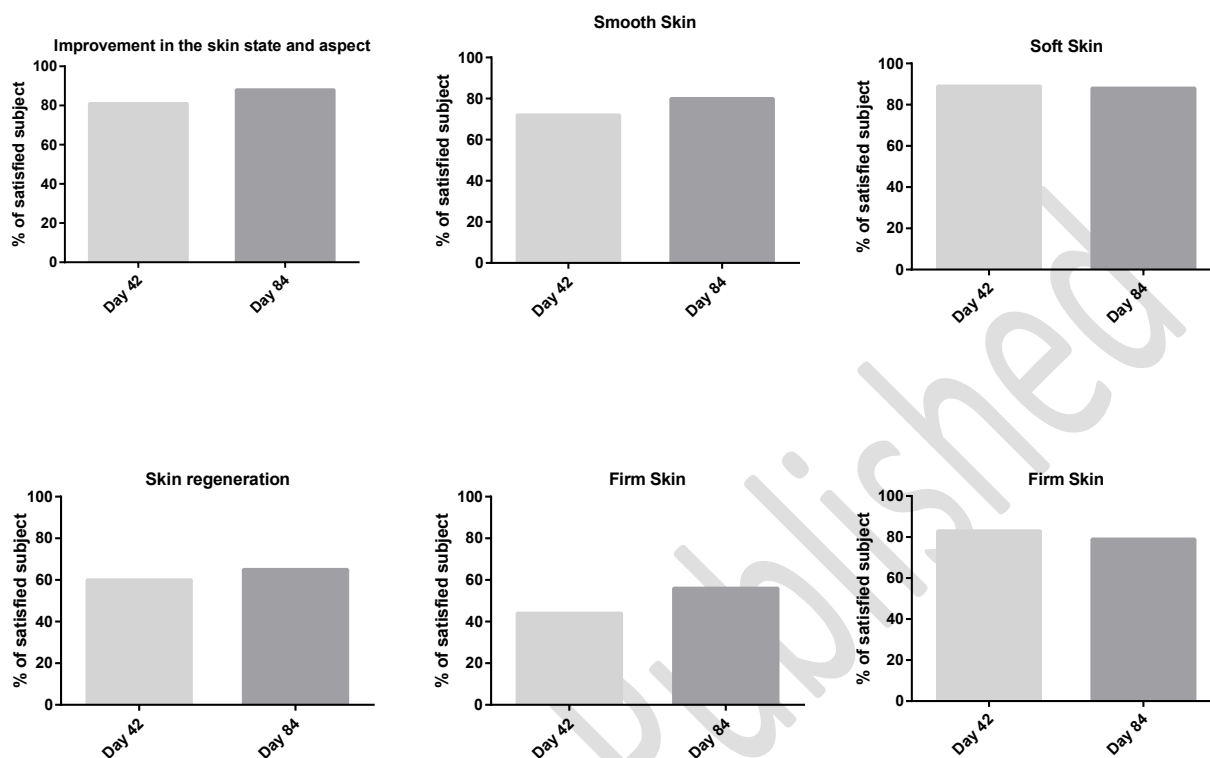


Figure 1 displays the subjective data obtained from the people in the study. For all except the firm skin is a general increase found in the subjects with the highest effect after 84 days.

Discussion:

MitoBeauty™ is a natural concentrate produced from specific species of black- and redcurrants. The active compound Ribetril A® was shown to inhibit PDE-4 and PDE-5 *in vitro*. In chronic skin patients with psoriasis or psoriatic arthritis elevated level of PDE-4 in the skin have been shown (Kumar, Goldminz, Kim, & Gottlieb, 2013). It is well known that inhibition of PDE-4 will lead to an increased cAMP level which decreases the inflammation in the body (Heng, Zuo, & Tang, 2018; Kumar et al., 2013). Furthermore, it has been shown that flavonoids inhibit PDE-4 (Kumar et al., 2013). In this study we have shown that Ribetril A® inhibits PDE-4 and PDE-5 *in vitro* and we have correlated this with increased cutaneous blood flow, increased physiological parameters such as skin regeneration as well as improvement in skin state and aspect. It has been shown the medical inhibitors such as apremilast have great effect on chronic skin disease, though problems with headache, abdominal pain and nausea have been observed (Heng et al., 2018; Kumar et al., 2013; Rundfeldt

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et al., 2012). Interestingly, the active compounds from the medical industry have activity around the 26 pM level to 2.2 nM level (Heng et al., 2018), where Ribetril A® have an effect on 2.95 and 2.1 µM for PDE-4 and PDE-5, respectively, with no adverse effect observed. Furthermore, MitoBeauty™ is a full berry extract and the effect of the formulation could also be a combination of Ribetril A™ and the rest of the polyphenol, anthocyanin and flavonoids in the extract. It is previous known from the literature that fruits will have effect on the general health of the body. With polyphenols believed to have antioxidant property (Denev et al., 2014), anthocyanins having effect on oxidative stress as well as lipoprotein oxidation and platelet aggregation (Kong, Chia, Goh, Chia, & Brouillard, 2003) and flavonoids having effect on the risk of cardiovascular disease (Nakamura, Matsumoto, & Todoki, 2002). The full spectrum of active compounds might explain the full effect of MitoBeauty™ on the skin. The results also showed a significant cutaneous blood flow increase of 6 % as well as a significant hydration rate of 9 %. This could be explained by the inhibition of PDE-5, PDE-5 inhibition leads to an intra-cellular increase of cGMP which leads to increase of blood vessels relaxation and smoothing (Krawutschke, Koesling, & Russwurm, 2015). Furthermore, the effect of PDE-4 inhibition will lead to increased cAMP level, which is associated with increased anti-inflammatory effects in the body as well as the skin (Denda et al., 2004; Grandoch, Roscioni, & Schmidt, 2010; Rundfeldt et al., 2012).

Increased cAMP also leads to increased MB (mitochondrial biosynthesis) (Whitaker, Wills, Stallons, & Schnellmann, 2013). This correlates with Ribetril A that was shown to increase mitochondrial biosynthesis, and this could be explained by decreased activity of PDE-4.

Although results in this study cannot be directly correlated to the inhibition of PDE-4 and PDE-5 in the skin, it is seen from the data that the subjective skin parameters are dramatically increased upon intake of MitoBeauty™. Figure 1 shows that the skin smoothness and the general skin aspects are increased. Furthermore, an increase in skin regeneration is observed. This is in agreement with inhibition of PDE-4 leading to increased skin parameters due to increased cAMP levels (Denda et al.) and inhibition of PDE-5 resulting in increased cutaneous blood flow due to an increased level of cGMP (Denda et al., 2004; Whitaker et al., 2013). Studies have shown that increased cGMP leads to signal and blood vessel relaxation which could be correlated with the subject data obtained in the current study (Krawutschke et al., 2015). Other polyphenols have also been analyzed for this effect, with Resveratrol probably being the most commonly known, which has been shown to have effect on breast cancer cells *in vitro* due to change in the cAMP levels. Furthermore, *in vivo* studies have suggested increase level of resveratrol both muscle adipose tissue (Park et

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al., 2012), indicating that resveratrol indeed can be absorbed into the body. Where increased cAMP level is found upon resveratrol, *in vitro* data from the current study suggest not only an effect on cAMP but also on cGMP level, as seen from table 1.

The study shows a change in skin parameters from intake of MitoBeauty™ with increased cutaneous blood flow and increased hydration. The observed effect of MitoBeauty™ in this study could be explained by a PDE inhibition, this however, would need more testing, with PDE-4 and PDE-5 measured in the subject as well as the cAMP level in the subjects of the intake of the extract. Such results correlated with the results from this study would show that the effect on skin parameters is caused by of Ribetril A® and the MitoBeauty™ extract.

Conclusion:

From this study we have shown that Ribetril A® one of the active compounds in MitoBeauty™ have an inhibitory effect on two intracellular enzymes PDE-4 and PDE-5, with IC50 values of 2.95 and 2.1 µM on PDE-4 and PDE-5, respectively. Furthermore, skin parameters of subjects changed after 84 days of exposure with increased hydration level of 9 % as well as increase cutaneous blood flow of 6 %. From the study it is observed that a natural product can change the skin parameters from both a scientific as well as a subjective point of view. The study also shows a decrease in wrinkles based on a purely natural product from berries.

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