Peer-reviewed version available at *Cosmetics* **2018**, *5*, 60; doi:10.3390/cosmetics5040060

Anti-aging efficacy of melatonin-based cream: Clinical and instrumental skin evaluation.

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Words count:	2500
Pages:	10
Figures:	5
References:	31

Peer-reviewed version available at Cosmetics 2018, 5, 60; doi:10.3390/cosmetics5040060

Abstract

Background: Melatonin is a potent mitochondrial, cytoprotective and antioxidant molecule with potentially strong anti-aging properties. Topical melatonin has shown to improve the clinical signs of skin aging. Melatosphere™ is a new lipid-based delivery system able to improve stability and skin penetration of melatonin when used in topical formulations. No clinical studies, using objective instrumental data, are available so far regarding the positive effect of Melatosphere™ in improving wrinkles in women with mild-to-moderate skin aging. **Study Aim**: We evaluate, in an open prospective, evaluator-blinded trial, the effects on skin texture of 2 months treatment with a Melatosphere™ based cream. **Subjects and Methods**: 15 women aged >45 years with mild to moderate facial skin aging (Glogau score ≥2) participated in the trial, after their informed consent. An ANTERA 3D computer-assisted skin analysis evaluation for the assessment of coarse and fine wrinkles of the periorbital area and melanin content was performed at baseline and after two months of treatment. An evaluator-blinded Investigator Global assessment of skin elastosis, roughness, level of dyscromia, skin dryness and presence of actinic damage was also performed at the same time points using a 4-grade score from 0 (no sign) to 3 (severe sign). **Results**: At baseline the mean (SD) IGA score was 8.2(1.0). After 2 months the IGA score significantly decrease to 4.2(1.4) (49% reduction) (P=0.0007). ANTERA 3D evaluations showed a significant reduction in skin coarse and fine wrinkles volume in the target area of -31% and -18%, respectively. Melanin content was reduced significantly by -17%. **Conclusion**: Topical melatonin carried in Melatosphere improves in the short-term signs of skin aging evaluated clinically and by ANTERA 3D device in women with mild to moderate skin aging.

Key words: melatonin, skin aging, ANTERA 3D.

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Introduction

Several studies have supported the concept that skin is a complete and independent melatoninergic system[1]. Mel receptors (MT1, MT2 and MT3) are expressed in both animal and human skin[2]. At the skin level, melatonin (Mel) acts as a relevant antioxidant and cytoprotective substance[3]. In particular, Mel is an active mitochondrial, cytoprotective and antioxidant molecule[4]. Mel is both a direct and indirect anti-oxidant molecule[5,6]; Mel can increase the production of anti-oxidative enzymes like superoxide dismutase[7]. Furthermore, Mel potentiates the activity of Sirtuin 1, an anti-degenerative molecule[8]. Several studies[9],[10] support the concept that Mel can prevent skin sun damage when administered before UV irradiation. Mel could also counteract hyperpigmentation processes through direct and indirect actions[11]. The pleiotropic biochemical actions of Mel at skin level could represent an effective anti-aging strategy[12],[13]. Topical application of Mel is considered meaningful, since it can easily penetrate the stratum corneum[14]. A recent clinical trial has shown that topical Mel improves clinical signs of skin aging[15]. Melatonsphere™ is a new delivery system able to improve stability and skin penetration of melatonin when used in topical formulations. No clinical studies, using objective instrumental data, are available so far regarding the positive effect of Melatosphere™ cream in improving coarse wrinkles in women with mild-to-moderate skin aging.

Study Aim

We evaluate, in a pilot, open prospective, evaluator-blinded trial, the effects on skin texture of 2 months treatment with a Melatosphere™ based cream (a cream containing glycerin and 0.1% melatonin in oleospheres of opuntia ficus indica seed oil and persea gratissima oil) in 15 women aged >45 years with mild to moderate facial skin aging (Glogau score ≥2). The study aimed to assess the antiaging effect of Mel-based cream evaluated by clinical (IGA score) and instrumental (ANTERA 3D CS; Miravex, Dublin, Ireland) evaluations. The ANTERA 3D CS images system could measure in an objective and operator-independent manner the volume of skin depressions (fine and coarse wrinkles) and the melanin content of a prespecified area[16].

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Subjects and Methods

Fifteen women with mild to moderate facial skin aging were enrolled after their informed consent. The subjects also provided consent to the publication of any pictures. The inclusion criterion was women aged ≥45 years with moderate–severe facial skin aging (Glogau score of 2-4). The exclusion criteria were any acute or chronic skin conditions, which could interfere with the parameters of evaluation and a positive history of allergic contact dermatitis to any of the component of the tested cream. This study was conducted in compliance with the ethical principles originating in or derived from the Declaration of Helsinki and in compliance with the guidelines of Good Clinical Practice. All subjects provided signed informed consent. The local institutional review board (IRB; Independent Ethical Committee, c/o Derma Medica, Modena, Italy) approved the trial protocol on February 2018 (Approval number 01/DMP/18). The compliance to the treatment was evaluated counting the returned used cream tubes at the final visit. Melatosphere™ based cream was applied on the entire face twice daily (2 Finger Tip Units per application) for 2 consecutive months. An ANTERA 3D computer-assisted skin analysis evaluation for the assessment of coarse and fine wrinkles of the periorbital area and melanin content was performed at baseline and after 2 months of treatment. ANTERA 3D is a device which contains a camera for image acquisition and corresponding software for analysis of the skin. It measures an area of 3136 mm2 (56×56 mm) and can shoot any part of the body. The skin topography and the chromophores concentration are derived from the spatial and spectral analysis of the acquired image data, obtained by illuminating the skin with LEDs of different wavelengths from different directions. The ANTERA 3D uses computer to analyze the differences between these images and reconstruct the surface in two and three dimensions. ANTERA 3D images can be used to evaluate skin color, wrinkle, texture, melanin, hemoglobin, pore, depression, and elevation. A target area, for the ANTERA 3D evaluations, was identified at baseline visit. An evaluator-blinded Investigator Global assessment of skin elastosis, roughness, level of dyschromia, skin dryness and presence of actinic damage was also performed at the same time points using a 4-grade score from 0 (no sign) to 3 (severe sign). Statistical analysis was performed using GraphPad Statistical Software (GraphPad Software, Inc., La Jolla, CA, USA). Continuous

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variables were expressed as mean ± standard deviation (SD). According to the fact that this was a pilot study, not a formal sample size calculation was performed. We decided to enroll at least 15 subjects.

Results

The trial was conducted between March 2018 and September 2018 in an outpatient derma-esthetic clinic. All the 15 subjects completed the study. At baseline the mean (SD) IGA score was 8.2(1.0). After 2 months the IGA score significantly decrease to 4.2(1.4) (a 49% reduction) (P=0.0007; Wilcoxon test) (*Figure 1*). In comparison with baseline, ANTERA 3D evaluations at month 2 showed a significant reduction in coarse (*Figure 2, a and b*), fine (*Figure 3, a and b*) and periorbital (*Figure 4, a and b*) wrinkles volume in the target area of -31%, -18% and -17% respectively. Target area coarse wrinkles volume decreased from 25.7 mm3 to 17.8 mm3. Target area fine wrinkles decreased from 9.0 mm3 to 6.0 mm3. Melanin content was also reduced significantly by 17% (*Figure 5, a and b*), from 0.6(0.03) to 0.5(0.03).

Discussion

Melatonin is a potent anti-inflammatory and anti-oxidant molecule[17]. In particular, melatonin acts as a direct, receptor-independent potent antioxidant[18]. The skin can produce and metabolize melatonin[19]. Skin cells present, also, specific receptors for melatonin (MT1, MT2 and MT3)[20]. For these reasons skin is defined as a complete and independent melatoninergic system[21]. At skin level endogenous melatonin could perform anti-aging and regenerative actions[22]. Melatonin is also a potent protecting molecule for mitochondria[23],[24]. Mitochondrial functional activity declines with age, mainly due to chronic and cumulative oxidative damage[25]. Therefore, the mitochondrial protective action of melatonin could slow down the aging process[26],[27]. Melatonin increase the expression of sirtuin 1, counteracting degenerative and pro-apoptotic mechanisms[28]. Melatonin suppresses ultraviolet (UV)-induced damage in human skin and human derived cell lines (e.g. keratinocytes, fibroblasts)[29]. Skin melatonin declines with age and therefore topical supplementation of melatonin could an interesting anti skin-aging strategy. A randomized, controlled split-face trial has shown that topical melatonin creams (a day and a night products) improves clinical sign of skin aging of the face. However, in this trial the

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primary outcomes were evaluated mainly clinically. So far, no trials have been performed to assess the effect on skin aging parameters of topical melatonin be means of objective, operator-independent, instrumental evaluations. In our trial we evaluated the anti-aging effects of melatonin cream by means of a computerized, operator-independent skin analysis system. The results of the present trial show that topical melatonin can reduce significantly coarse and fine wrinkles. Interesting we also documented a reduction in melanin content in the target area. The discovery of Mel by Lerner et al[30] in 1958 was characterized by the fact that this "pineal factor" was able to lighten melanocytes. Some limitations should be taken in account in evaluating our results. We have performed an open non-controlled trial. To increase the internal validity of the trial results, we have used an objective procedure to evaluate the efficacy of the tested product (skin wrinkles profilometry and melanin content). Photodamage skin is characterized by the presence of coarse and fine wrinkles, hyper and hypo pigmentation, roughness and sallowness[31]. ANTERA 3D CS device could measure all these parameters in a reproducibly and sensitively way.

Conclusion

Topical melatonin vehiculated in Melatosphere improves, in the short-term, signs of skin aging evaluated clinically and instrumentally in women with mild to moderate skin aging.

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Figure 1. Evolution of IGA score from baseline to month 2

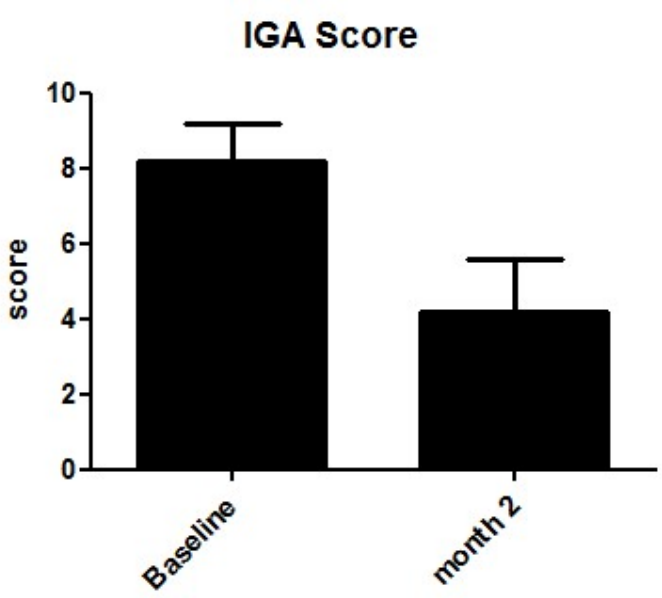

Figure 2. Coarse wrinkles at baseline (A) and after 2 months of application of melatonin cream (B)

A B

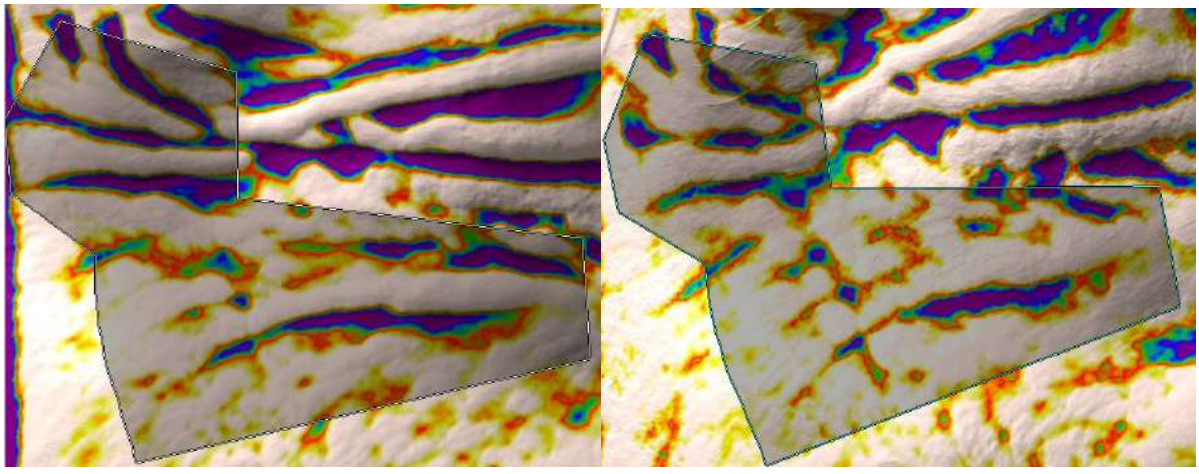

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Figure 3. Fine wrinkles at baseline (A) and after 2 months of application of melatonin cream (B)

A B

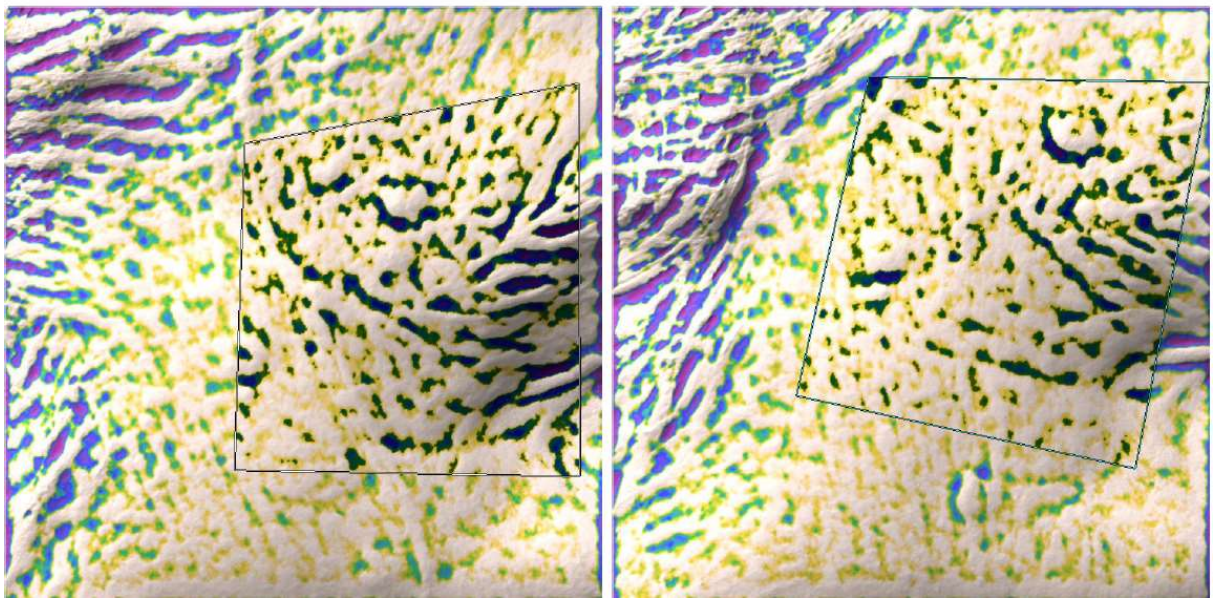


Figure 4. Periorbital wrinkles at baseline (A) and after 2 months of application of melatonin cream (B)

A B

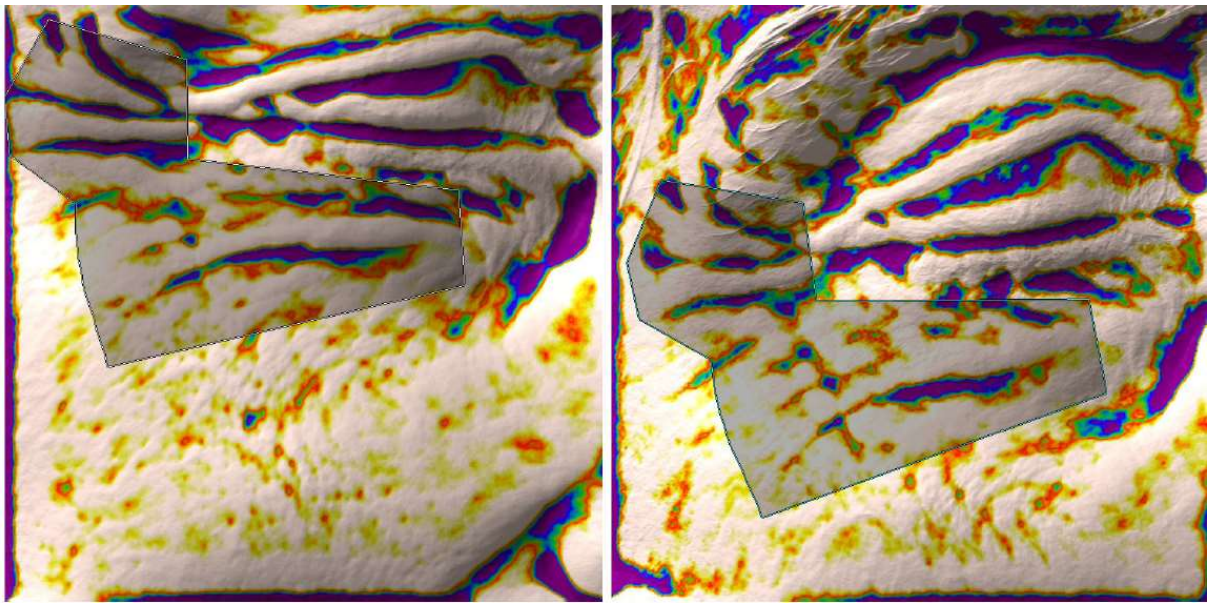
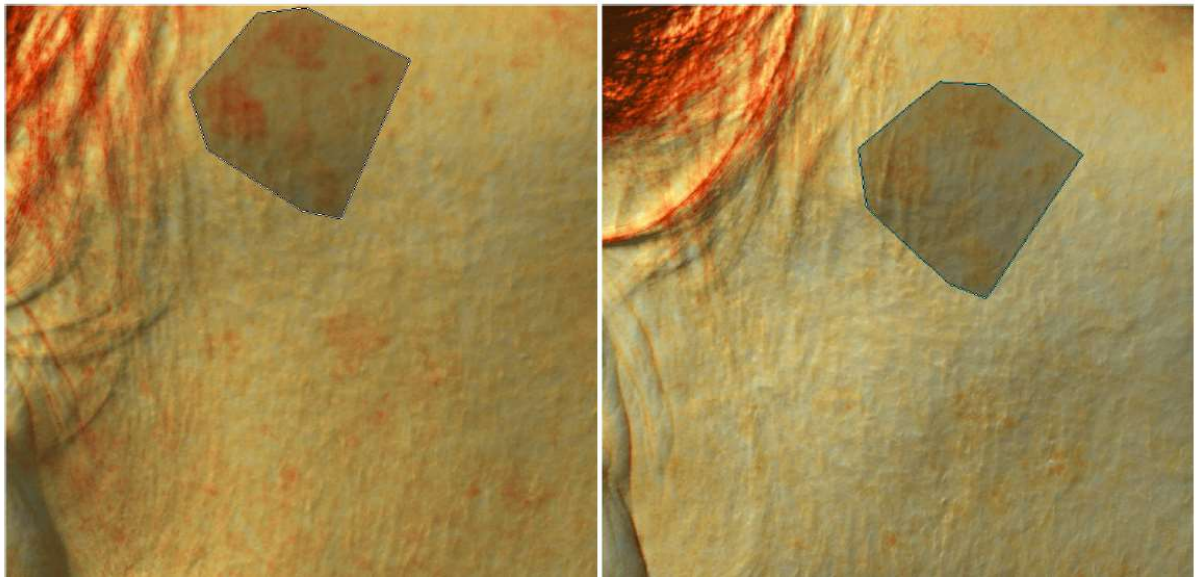


Figure 5. Melanin content at baseline (A) and after 2 months treatment with melatonin cream (B)

A B



References

[1] Slominski, A., Pisarchik, A., Semak, I., Sweatman, T., Wortsman, J., Szczesniewski, A., Jing, C. (2002). Serotoninergic and melatoninergic systems are fully expressed in human skin. The FASEB Journal, 16(8), 896-898.

[2] Slominski, A., Pisarchik, A., & Wortsman, J. (2004). Expression of genes coding melatonin and serotonin receptors in rodent skin. Biochimica et Biophysica Acta (BBA)-Gene Structure and Expression, 1680(2), 67-70.

[3] Pandi-Perumal, S. R., Srinivasan, V., Maestroni, G. J. M., Cardinali, D. P., Poeggeler, B., & Hardeland, R. (2006). Melatonin: Nature's most versatile biological signal? The FEBS journal, 273(13), 2813-2838.

[4] Acuna Castroviejo, D., C Lopez, L., Escames, G., López, A., A Garcia, J., & J Reiter, R. (2011). Melatonin-mitochondria interplay in health and disease. Current topics in medicinal chemistry, 11(2), 221-240.

[5] Reiter, R. J., Tan, D. X., Osuna, C., & Gitto, E. (2000). Actions of melatonin in the reduction of oxidative stress. Journal of biomedical science, 7(6), 444-458.

[6] Galano, A., Tan, D. X., & Reiter, R. J. (2011). Melatonin as a natural ally against oxidative stress: a physicochemical examination. Journal of pineal research, 51(1), 1-16.

[7] Tan, D. X., Manchester, L. C., Reiter, R. J., Qi, W. B., Karbownik, M., & Calvo, J. R. (2000). Significance of melatonin in antioxidative defense system: reactions and products. Neurosignals, 9(3-4), 137-159.

[8] Tajes, M., Gutierrez-Cuesta, J., Ortuno-Sahagun, D., Camins, A., & Pallàs, M. (2009). Anti-aging properties of melatonin in an in vitro murine senescence model: involvement of the sirtuin 1 pathway. Journal of pineal research, 47(3), 228-237.

[9] Fischer TW, Sweatman TW, Semak I, Sayre RM, Wortsman J, Slominski A. Constitutive and UV-induced metabolism of melatonin in keratinocytes and cell-free systems. FASEB J 2006; 20:1564-6;

[10] Fischer TW, Slominski A, Zmijewski MA, Reiter RJ, Paus R. Melatonin as a major skin protectant: from free radical scavenging to DNA damage repair. Exp Dermatol 2008; 17:713-30;

[11] Rendon, M. I., & Gaviria, J. I. (2005). Review of skin-lightening agents. Dermatologic surgery, 31, 886-890.

[12] Hardeland, R., Cardinali, D. P., Srinivasan, V., Spence, D. W., Brown, G. M., & Pandi-Perumal, S. R. (2011). Melatonin—A pleiotropic, orchestrating regulator molecule. Progress in neurobiology, 93(3), 350-384.

[13] Kleszczynski, K., & Fischer, T. W. (2012). Melatonin and human skin aging. Dermato-endocrinology, 4(3), 245-252.

[14] Dubey, V., Mishra, D., Asthana, A., & Jain, N. K. (2006). Transdermal delivery of a pineal hormone: melatonin via elastic liposomes. Biomaterials, 27(18), 3491-3496.

[15] Milani, M., & Sparavigna, A. (2018). Antiaging efficacy of melatonin-based day and night creams: a randomized, split-face, assessor-blinded proof-of-concept trial. Clinical, cosmetic and investigational dermatology, 11, 51.

[16] Linming, F., Wei, H., Anqi, L., Yuanyu, C., Heng, X., Sushmita, P., <li, L. (2018). Comparison of two skin imaging analysis instruments: The VISIA® from Canfield vs the ANTERA 3D® CS from Miravex. Skin Research and Technology, 24(1), 3-8.

[17] Tan DX, Chen LD, Poeggeler B, Manchester LC, Reiter RJ. Melatonin: a potent, endogenous hydroxyl radical scavenger. Endocr J 1993; 1:57 - 60

[18] Reiter RJ, Tan DX, Maldonado MD. Melatonin as an antioxidant: physiology versus pharmacology. J Pineal Res 2005; 39:215 - 6;
[19] Slominski A, Wortsman J, Tobin DJ. The cutaneous serotoninergic/melatoninergic system: securing a place under the sun. FASEB J 2005; 19:176 - 94
[20] Dubocovich, M. L., & Markowska, M. (2005). Functional MT 1 and MT 2 melatonin receptors in mammals. Endocrine, 27(2), 101-110.
[21] Slominski, A., Fischer, T. W., Zmijewski, M. A., Wortsman, J., Semak, I., Zbytek, B., Tobin, D. J. (2005). On the role of melatonin in skin physiology and pathology. Endocrine, 27(2), 137-147.
[22] Majidinia, M., Sadeghpour, A., Mehrzadi, S., Reiter, R. J., Khatami, N., & Yousefi, B. (2017). Melatonin: A pleiotropic molecule that modulates DNA damage response and repair pathways. Journal of pineal research, 63(1), e12416.
[23] Leon, J., Acuña-Castroviejo, D., Escames, G., Tan, D. X., & Reiter, R. J. (2005). Melatonin mitigates mitochondrial malfunction. Journal of pineal research, 38(1), 1-9.
[24] López, A., García, J. A., Escames, G., Venegas, C., Ortiz, F., López, L. C., & Acuña-Castroviejo, D. (2009). Melatonin protects the mitochondria from oxidative damage reducing oxygen consumption, membrane potential, and superoxide anion production. Journal of pineal research, 46(2), 188-198.
[25] Balaban, R. S., Nemoto, S., & Finkel, T. (2005). Mitochondria, oxidants, and aging. Cell, 120(4), 483-495.
[26] Karasek M, Reiter RJ. Melatonin and aging. Neuro Endocrinol Lett 2002; 23:Suppl 1 14 - 6
[27] Armstrong, S. M., & Redman, J. R. (1991). Melatonin: a chronobiotic with anti-aging properties?. Medical hypotheses, 34(4), 300-309
[28] Chang, H. M., Wu, U. I., & Lan, C. T. (2009). Melatonin preserves longevity protein (sirtuin 1) expression in the hippocampus of total sleep-deprived rats. Journal of pineal research, 47(3), 211-220..
[29] Bangha, E., Elsner, P., & Kistler, G. S. (1996). Suppression of UV-induced erythema by topical treatment with melatonin (N-acetyl-5-methoxytryptamine). A dose response study. Archives of dermatological research, 288(9), 522-526.
[30] Lerner AB, Case JD, Takahashi Y, Lee TH, Mori W. Isolation of melatonin, a pineal factor that lightens melanocytes. J Am Chem Soc 1958; 80:2587
[31] Griffiths, C. E. M. (1992). The clinical identification and quantification of photodamage. British Journal of Dermatology, 127(S41), 37-42.