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A Multifunctional Solution for Acne Prone Skin with a Single Natural Active Ingredient

S. Hettwer, E. Besic Gyenge, B. Suter, S. Breitenbach, B. Obermayer

Introduction

Prevention of acne or reduction of oily skin are challenging tasks for the cosmetic industry. Salicylic acid, benzoyl peroxide and retinoids are all specialized ingredients to either control corneocyte shedding, the microflora or to reprogram the cell's biochemistry. All of them are efficient specialists but no all-rounders. However, there are at least four trigger factors for the development of acne: activity of sebocytes, dihydrotestosterone level, inflammation and skin microbiota. It is difficult to develop active ingredients, which are suitable to cover all necessary needs to improve oily skin and as such, they are rare on the market. Additionally, very effective agents tend to irritate the skin and, like retinoic acid, are in most countries only available as prescriptive drugs from the dermatologist in effective concentrations.

Acne vulgaris affects about 80 % of adolescents and young adults aged 11-30 years [1]. Also in aged people, persistent acne is common in the Western civilizations. According to "The International Dermal Institute" 40 to 55 percent of the adult population in the age of 20-40 have signs of persistent acne and oily skin. It is a major problem leading to discomfort, lowered self-confidence and the tendency to distract oneself from social life. Inflammation, hormonal state, activity of sebocytes and the skin microflora have influence on the development of acne. To prevent acne formation, normalization of oily skin is key. Oily skin is mainly caused by a chronic subliminal inflammatory state of the skin leading to a subtle change of the expressome of sebocytes. This leads to excess sebum – the ideal nutrition for unwanted microbes like *Propionibacterium acnes*, which in turn aggravates inflammation and further increases sebum production [2]. As such, also a disturbed microflora can trigger the appearance of acne. Additionally, the hormonal state of the skin plays an important role: excess of dihydrotestosterone, derived from testosterone or 17-hydroxyprogesterone fuels the sebum production [3] and inflammation [4]. With SEBOCLEAR™-MP (INCI: Propanediol, Bioflavonoids), all four trigger factors of acne development are controlled to reach superior efficacy on acne prone skin.

The Skin Types: Role of the Steroid 5α-reductase

Whether we have dry, normal or oily skin is determined by the sebum deposition on the skin's surface. By becoming adolescent, the amount of sebum increases. One main trigger is the production of the male sex hormone testosterone, which is increasing during puberty in both male and female (**Fig. 1**).

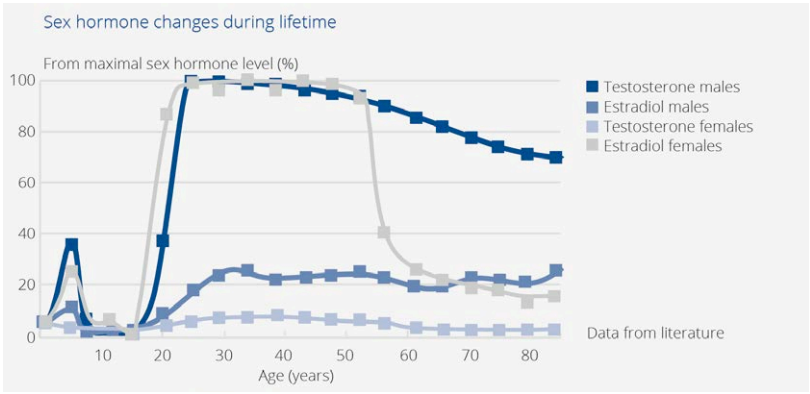

Fig. 1 The hormonal state of our body changes during lifetime. Starting with puberty, the sex hormones estradiol (♀) and testosterone (♂) rise dramatically in females or males, respectively. While in males, testosterone declines only subtle within the second half of life, women experience a rapid drop of estradiol during menopause. Data compiled from [5-8].

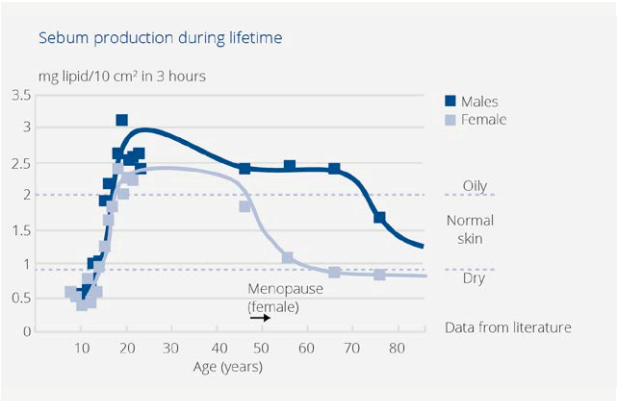

Fig. 2 Sebum production during lifetime. The sebum amount corresponds roughly to the sex hormone levels of androgens in males and estrogens in females. Data compiled from [5-8].

Males in general suffer from more oily skin starting at puberty, which lasts until their 70's (**Fig. 2**). Then, the sebum production declines to normal level, in parallel with a decrease in testosterone. In contrast, women can have a dual sebum problem through their lifetime: Starting with puberty, the skin may become oily until menopause. Then, in very short time, the sebum production can decline and reach a low level, giving rise to dry skin, in parallel to the concentration of estrogens. This in contrast to the fact that estrogens are believed to counteract the effects of testosterone [5-8]. Despite of this, it is clear that one of the most important enzymes in sebum regulation is the steroid 5α-reductase. The enzyme converts testosterone into dihydrotestosterone (DHT). This highly active steroid binds to the androgen receptor, which activates the corresponding genes to increase the sebum production. As such, typically males suffer from oily skin. Many women experience this during their menses cycle due to changing hormonal levels as well [9].

An Elevated Inflammatory State is an Underlying Condition of Acne Prone Skin

Unlike formerly believed, it is not the colonization of sebum with *P. acnes* that drives acne formation [10]. This only aggravates the situation. It is suggested, that an increased sebum production caused by a subliminal inflammatory state is causative for the development of acne. In acne prone skin, the inducible enzyme cyclooxygenase 2 (COX-2) and 5-lipoxygenase (5-LOX) are increased in sebaceous glands [11]. These enzymes (including COX-1) convert arachidonic acid into the inflammatory mediators prostaglandins and leukotrienes, of which the first group predominantly creates pain and systemic inflammation and the latter predominantly attract immune cells. Prostaglandin E2 is known to induce sebaceous gland hyperplasia and overshooting of sebum production. Non-steroidal anti-inflammatory drugs (NSAIDs) like aspirin, ibuprofen or diclophenac only block the COX-pathway. As a con-

sequence, arachidonic acid is converted to leukotrienes, which predominantly attract immune cells to the site of synthesis. This still aggravates the situation on acne proine skin. To completely suppress the inflammatory condition, the inhibition of both pathways – COX and 5-LPO – is necessary (**Fig. 3**).

SEBOCLEAR™-MP is an extract of *Maclura cochinchinensis*, which is a well-known medical plant from Asia with anti-microbial properties. *Maclura* bark and roots contain multiple bioflavonoids with the corresponding activity. We used the leaves for extraction, which ensures a sustainable handling of the resource. We found three prenylated isoflavones as active ingredients (Bioflavonoids). Besides having anti-microbial activity, these compounds also inhibit the production of inflammatory mediators from arachidonic acid. Indeed, we found that the Bioflavonoids can block both cyclooxygenases COX-1 and COX-2 and, additionally, the 5-LPO. As such, the inflammatory state of the skin is reduced. Additionally, the prenylated isoflavones inhibit the steroid 5α-reductase making them powerful agents to control the sebum production of the sebaceaous gland.

Materials and Methods

Inhibition of the Steroid 5α-reductase

LC-MS analysis of 5α-androstanedione converted by recombinant human 5α-reductase from 4-androstene-3,17-dione according to [12]. In brief, HEK 293 cell homogenates stably expressing the isoform I of human steroid 5α-reductase were incubated in assay buffer containing 4-androstene-3,17-dione with different concentrations of test compounds. The reaction was stopped by addition of an equal volume of acetonitrile and the concentration of 5α-androstanedione was determined with LC-MS analysis.

Inhibition of Arachidonic Acid Processing Enzymes

Fluorimetric enzyme inhibition assay on human recombinantly produced COX-1, COX-2 and 5-lipoxygenase (5-LOX or ALOX5; 5-LPO) enzymes. For COX-1 and COX-2, 10-Acetyl-3,7-dihydroxyphenoxazine (ADHP) was used as detector molecule. Arachidonic acid is converted by COX-1 and COX-2 into Prostaglandin G2 (PGG2). The peroxidase reaction between PGG2 and ADHP is also catalyzed by the COX enzymes and produces the highly fluorescent compound resorufin from supplemented dihydroresofurin, which is analysed with an excitation wavelength of 530–540 nm and an emission wavelength of 585-595 nm [13].

For the determination of 5-LOX activity, the fluorescent probe rhodamine-123 was used in a similar way as described for the COX enzymes. 5-LOX converts arachidonic acid into leukotriene A_4 (LTA_4) with an intermediate peroxidation step, where the fluorescent rhodamine-123 is produced from dihydrorhodamine-123 [14].

In vivo Study

The *in vivo* study was conducted in accordance with the principles of good laboratory practice (GLP), good clinical practice (GCP), and in compliance with the quality assurance system requirements. The study was in accordance with the World Medical Association's Declaration of Helsinki. All study participants signed a written informed consent at the beginning of the study.

Bioflavonoids Reduce Signs of Acne (*in vivo* Study)

In a double-blind, placebo-controlled, randomized hemi-face study, 21 female subjects with healthy, oily Caucasian skin, 16–24 years (average 19.4) applied an emulsion with 3 % *Maclura cochinchinensis* extract or 0 % (placebo) twice daily on the hemi-face. Inflammatory spots were assessed with the red channel of a VISIA photographic system while porphyrines were detected in the UV channel.

Lesions in the face after 28 days and 84 days of application were assessed by a dermatologist. The number of open comedones (black heads), closed comedones (whiteheads) and papules and pustules were determined.

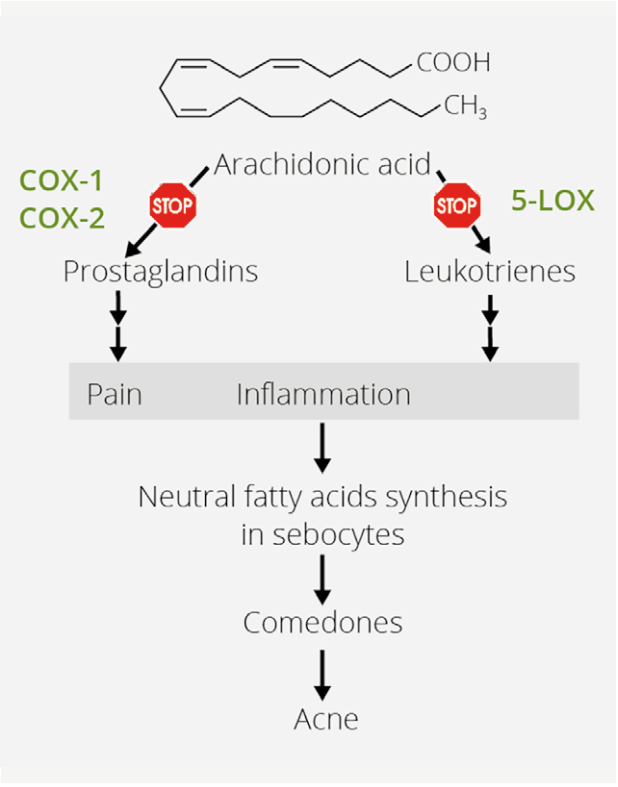

Fig. 3 Inhibition of arachidonic acid processing counteracts the development of acne. The inflammatory mediators prostaglandins and leukotrienes are produced by the COX-1/COX-2 and 5-LPO enzymes from arachidonic acid. Inhibition of both pathways is required to shut down inflammation and to prevent an acne prone skin condition.

Results

Bioflavonoids Directly Inhibit Steroid 5α-reductase

Sebocytes strongly express the type I isoform of steroid 5α-reductase [15]. This enzyme converts testosterone to dihydrotestosterone (DHT), which activates the androgen receptor (AR). An increased concentration of DHT in scalp leads to alopecia. In sebocytes, activated AR promotes sebum over-production. The *Maclura cochinchinensis* extract reasonably inhibits the steroid 5α-reductase (**Fig. 4**). With increasing concentration, the activity of the enzyme is completely blocked. At 0.1 %, the inhibition is 32 %, at 0.5 % *Maclura cochinchinensis* extract inhibition is already 90 %. The IC_{50} is at 0.12 %.

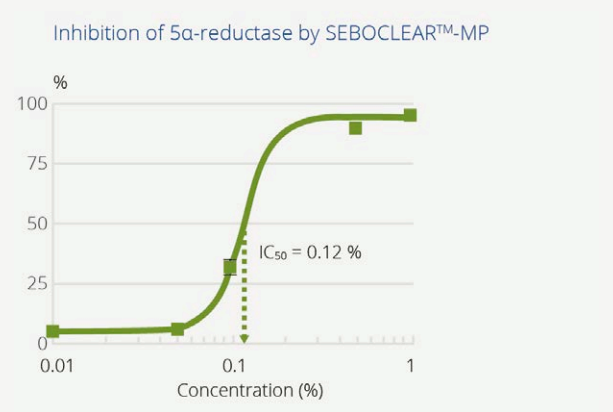

Fig. 4 *Maclura cochinchinensis* extract blocks the activity of steroid 5α-reductase. It prevents the generation of the highly active dihydrotestosterone from testosterone.

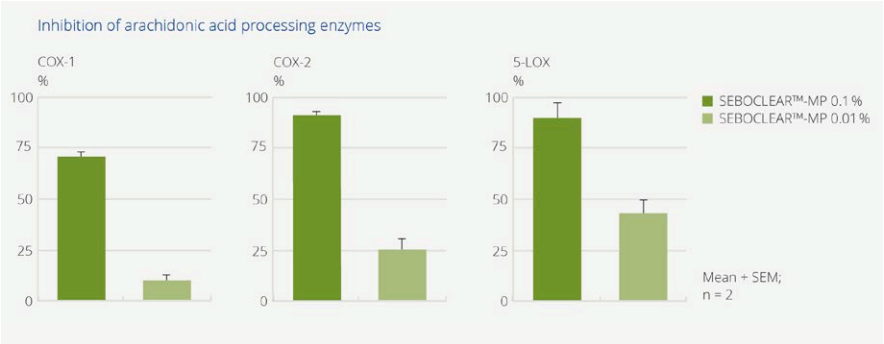

Fig. 5 *Maclura* bioflavonoids inhibit both pathways of inflammatory mediator generation from arachidonic acid. At a concentration of 0.1 %, *Maclura cochinchinensis* extract significantly inhibits the constitutively expressed COX-1, as well as the inducible COX-2, elevated in acne prone and ageing skin. Also the activity of 5-lipoxygenase (5-LOX) is almost completely blocked.

Bioflavonoids Shut Down Inflammation by Blocking Arachidonic Acid Processing Enzymes

To investigate the anti-inflammatory effect of the bioflavonoids from *Maclura cochinchinensis* on key enzymes for the arachidonic acid processing inflammatory pathways, the enzymes COX-1, COX-2 and 5-LOX were investigated. COX-2 is an inducible enzyme, which is upregulated especially in acne conditions and ageing skin.

The bioflavonoids inhibit both pathways of inflammatory mediator generation from arachidonic acid by direct inhibition of these key enzymes (see also **Fig. 3**). The presence of 0.1 % *Maclura cochinchinensis* extract leads to significant inhibition of COX-1, COX-2 and 5-LOX between 71 % and 91 % (**Fig. 5**).

Maclura cochinchinensis Extract Reduces Signs of Acne (*in vivo* Study)

Additionally to the reduction of DHT and inflammatory reactions, *Maclura cochinchinensis* extract is capable of selectively reducing *P. acnes* and coryneform bacteria of the skin's mi-

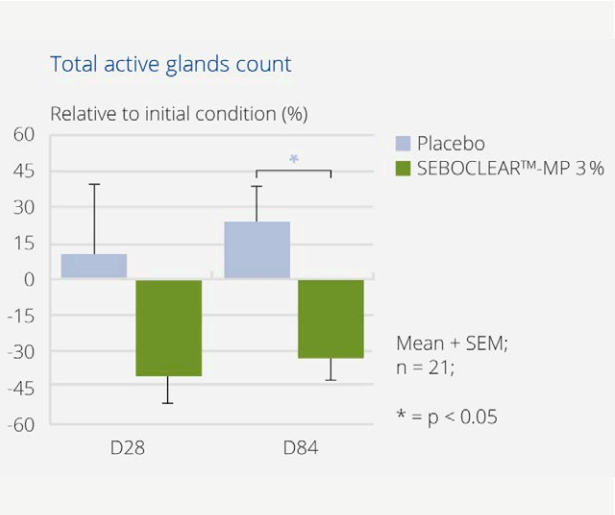

Fig. 6 *Maclura* bioflavonoids decrease the number of active sebaceous glands. Compared to placebo, the number of active glands was significantly reduced after 84 days. Wilcoxon signed rank test.

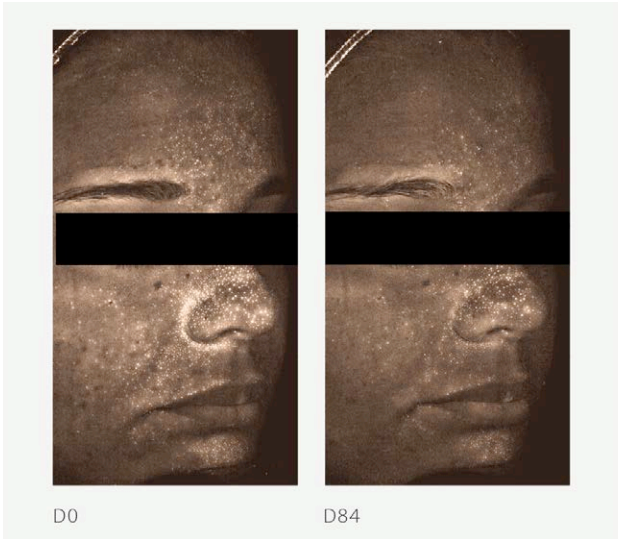

Fig. 8 *Maclura* bioflavonoids reduce porphyrines. After 84 days, the porphyrins were significantly reduced proving a reduction of sebum and settlement of *P. acnes*.

crobiota [16], of which the first is responsible for aggravating acne due to settlement in the sebum of comedones and potentiating the inflammatory condition. Black head comedones, which are frequently driven by 5α-reductase activity (sebum over-production) and white head comedones are already a condition of acne. In a double-blind, placebo-controlled, randomized hemi-face study, 21 female subjects applied placebo or 3 % *Maclura cochinchinensis* extract for 84 days and the comedones were evaluated by a dermatologist. Additionally, inflammatory spots were investigated. Sebum production was measured as number of active glands with Sebufix.

In the entire population, the number of active glands decreased by more than 40 % after 28 days of application, whereas the number of active glands increased when using placebo in a hemiface approach. After 84 days, the number of active glands reduction leveled in by 33.5 % significant over placebo (**Fig. 6**). As a result, the number of comedones were significantly reduced by 38 % over baseline and placebo as judged by a dermatologist (not shown). After 84 days, 41 % of the open (blackheads) and 36 % of the closed (whiteheads) comedones disappeared significantly (**Fig. 7**). The effect was significant or limit significant (p = 0.08) over placebo. This was also reflected by a reduction of the porphyrin count (**Fig. 8**), a measure for the metabolic activity of *P. acnes* in sebum. After 84 days, the total combined non-inflammatory and inflammatory lesion reduction after 84 days was 35.4 %, which was significant over baseline and placebo (**Fig. 9**) and goes in line with the observations made with a VISIA device. After 28 days of application, the bioflavonoids already decreased

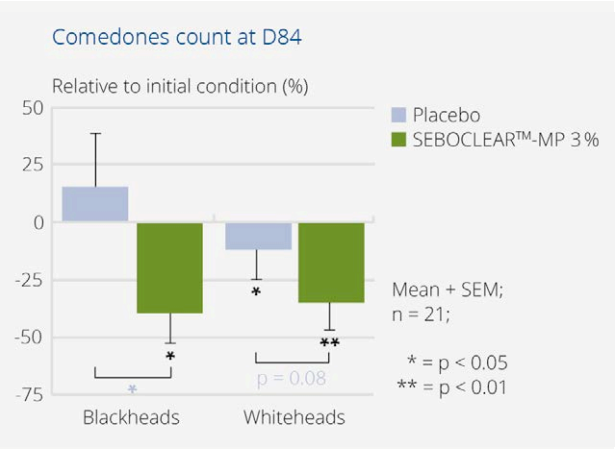

Fig. 7 *Maclura* bioflavonoids are effective against open and closed comedones. After 84 days, open and closed comedones were significantly reduced. Wilcoxon signed rank test. Statistical values in black refer to comparison with baseline while values in blue refer to comparison with placebo.

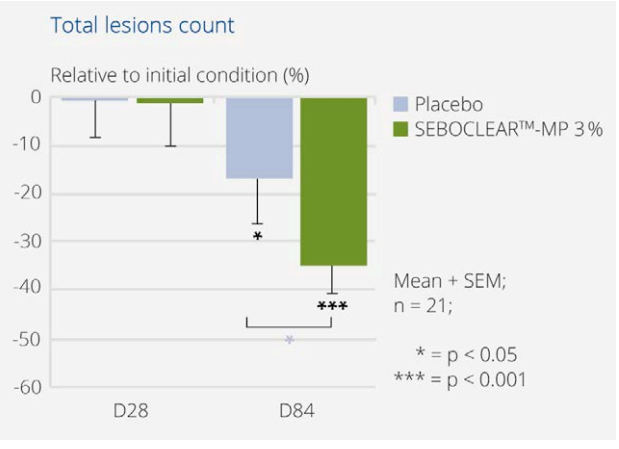

Fig. 9 The total lesion count is significantly reduced after application of 3 % *Maclura cochinchinensis* extract. After 84 days, the total lesions including inflammatory spots were reduced by 35 %. Wilcoxon signed rank test. Statistical values in black refer to comparison with baseline while values in blue refer to comparison with placebo.

the red spots count by 8 %. After 84 days, these spots were reduced significantly over baseline and placebo by 22 % while the condition with placebo did not significantly change (**Fig. 10**).
The dermatological investigation of different skin parameters associated with oily and acne prone skin revealed a superior result for *Maclura cochinchinensis* extract over placebo. Pore visibility was reduced by 15.6 %, sebum quantity and shine by 5.4 % or 4.3 %, respectively after 84 days.

Discussion

Oily skin is prone to develop spots and pimples. Puberty, dysbalanced hormonal state or Western diet can cause an increased sebum production, hyperkeratinisation and trapping of sebum in the pores. Application of comedogenic cosmetics clog the pores and create an anaerobic environment suitable to be colonized by *P. acnes*. Stress, UV radiation and intrinsic ageing lead to an increased inflammatory state originating from COX-2 overexpression. By that, prostaglandins and leukotrienes aggravate the inflammatory state and increase sebum production and hyperkeratinisation. Once the pore is clogged, *P. acnes* induces an increased attraction of immune cells leading to spots. The result is flawed and oily skin. Prenylated isoflavones from SEBOCLEAR™-MP not only inhibit COX-1 and COX-2 but also block the possible alternative routing via 5-LOX. As such, the generation of the pro-inflammatory mediators prostaglandins and leukotrienes is blocked. Additionally, the steroid 5α-reductase is inhibited, as well. The result is a normalization of sebaceous gland activity. The retinoid-like activity of *Maclura* bioflavonoids promotes a sustainable reprogramming of sebocytes and keratinocytes into a normal proliferative state (not shown, [17]). Additionally, by having regulatory activity on the skin's microbiome, *Propionibacterium acnes* is hindered on colonizing clogged pores, preventing the formation of acne pustules (not shown, [16]).
In summary, SEBOCLEAR™-MP reduces spots and comedones on acne prone skin. The number of porphyrins, a measure of *P. acnes* colonization is reduced as well as the number of active sebaceous glands. This leads to a lower burden oily skin to develop the uncomely signs of acne.

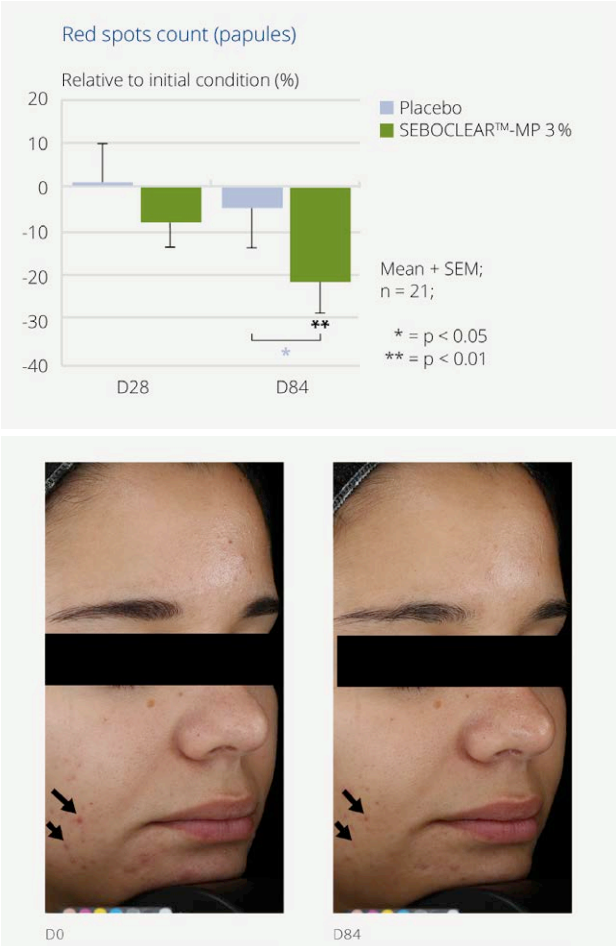

Fig. 10 *Maclura* bioflavonoids reduce inflammatory spots. After 84 days, red spots were significantly reduced by 22 % over baseline and placebo. Wilcoxon signed rank test. Statistical values in black refer to baseline while values in blue refer to comparison with comparison with placebo.

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