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PHARMACOGNOSY

Clinical trial of a natural and bioactive melon SuperOxide Dismutase (SOD B Dimpless®) on cellulite.

Etude clinique d'une SuperOxyde Dismutase de melon naturelle et bioactive (SOD B Dimpless®) sur la cellulite

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Abstract A randomized, double blind, placebo controlled clinical study was conducted on 41 women with cellulite aged between 31 and 50 years old. They were orally supplemented with SOD B Dimpless®, a natural dry melon juice highly concentrated in SuperOxide Dismutase (SOD) at 40 mg per day (480 IU SOD), during 56 consecutive days. Cellulite was measured visually by scoring fat nodes on stomach and thighs, according to a linear scale. The oral supplementation with SOD B Dimpless® significantly reduced cellulite on thighs compared to the placebo after 28 days. This reduction is amplified after 56 days of supplementation. The mechanism of action may involve the induction of endogenous antioxidant enzymes expression, leading to the inhibition of fibrosis and the stimulation of lipolysis. Further investigations are necessary to document the way of action of SOD B Dimpless® against cellulite.

Key words Oxidative stress – Antioxidant enzymes – Fibrosis – Lipolysis

Résumé Une étude clinique, randomisée en double aveugle contre placebo, a été conduite sur 41 femmes ayant de la cellulite, âgées de 31 à 50 ans. 21 d'entre elles ont été supplémentées oralement avec SOD B Dimpless®, un jus de melon séché hautement concentré en SuperOxyde Dismutase (SOD), à 40 mg par jour (480 IU SOD) pendant 56 jours consécutifs. La cellulite a été mesurée par l'évaluation visuelle des capitons graisseux sur le ventre et les cuisses, à l'aide d'une échelle linéaire. La supplémentation orale avec SOD B Dimpless® a significativement réduit la cellulite sur les cuisses en comparaison avec le groupe placebo après 28 jours. Cette réduction est amplifiée après 56 jours de supplémentation. Le mécanisme d'action pourrait impliquer l'induction de l'expression des enzymes antioxydantes endogènes, conduisant à l'inhibition de la fibrose et à la stimulation de la lipolyse. D'autres investigations sont nécessaires afin de confirmer le mode d'action de SOD B Dimpless® contre la cellulite.

Mots Clés : Stress oxydant - Enzymes antioxydantes - Fibrose - Lipolyse

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Introduction

In a society where appearance plays an increasingly important role, cellulite is a major beauty problem responsible for the development of emotional and psychological imbalance in many women. Cellulite is a phenomenon that affects more than 80% of post-pubescent women, highlighting the strong demand for an effective solution against the development of cellulite [1-3].

Even if its exact mechanism is not fully elucidated, cellulite results from the expansion of fat lobules, adipose tissue protrusions into the dermis, and changes in connective tissue fibers leading to fibrosis. All these phenomena lead to the well-known orange peel-like appearance of the skin, especially on the buttocks, thighs, flanks, and stomach [4-6].

One of the fundamental causes for the appearance of cellulite is related to adipocyte hypertrophy, that is to say, the increase in the size of fat cells [2]. This is partly due to a disturbance in lipid metabolism with inhibition of lipolysis, *i.e.* fat degradation [7]. Even if cellulite should not be confused with obesity, some authors have observed that cellulite was more pronounced in obese women [8]. This worsening of cellulite with overweight, and the correlation between Body Mass Index (BMI) and severity of cellulite [8], reflects the expansion of adipose tissue in the dermis when the volume of fat is increased [1].

These changes in adipocyte metabolism lead to an increased production of Reactive Oxygen Species (ROS) and a decrease in antioxidant defenses, leading to oxidative stress [9, 10]. Siems *et al.* showed an increase in oxidative damage to lipids and proteins in women with cellulite [11].

Further studies have shown that antioxidant compounds capable of removing ROS, can improve lipid metabolism [12, 13]. Pina-Zentella *et al.* have shown that taurine stimulates the process of lipolysis in adipocytes [14]. As the first line of antioxidant defenses, SOD could also be efficient in inhibiting adipocyte hypertrophy. Carillon *et al.* have reported stimulation of lipolysis in obese hamsters orally supplemented with SODB, a dried melon juice highly concentrated in SOD [15].

The second main cause of the cellulite appearance is linked to the development of fibrosis of the connective tissue, as a result of the evagination of fat lobules in the dermis [5, 6]. Indeed, when subjected to pressure changes, fat cells must adapt their shape without changing their volume. The gradual accumulation of fats leads to the destruction of collagen fibers and the development of thickened and stiffened fibrous strands limiting the evagination of fat lobules [16]. This process leads to an accumulation of connective tissue, which is characteristic of a localized fibrosis in areas affected by cellulite (stomach, thighs, flanks) [7, 16].

There is a close relationship between the change in oxidative status and fibrosis [11, 17]. One of the main mechanisms responsible for fibrosis is the release of ROS, such as superoxide anion ($O_2^{\bullet -}$) and the hydroxyl radical ($OH^{\bullet}$) by inflammatory cells [18, 19].

Due to their high instability, ROS will bind to adjacent structures and cause damage to the connective tissue and vascular network [20].

The generation of oxidative damages has been reported in many cases of fibrosis observed in animal models but also in humans [21]. Therefore the use of antioxidants is a solution to reduce fibrosis. Siems *et al.* have demonstrated the correlation between the reduction of oxidative stress and improvement of the skin mechanical properties, leading to a smoother skin appearance [11]. Thus, antioxidant enzymes such as SOD may be effective in preventing the accumulation of ROS and their effects on fibrosis [19]. The anti-fibrotic properties of SOD have been extensively studied and are now well established [19, 22-25]. In the 1990s, SOD was even used as a drug in injectable form (Orgotein®, Pegorgotein®, and Ormentein®) to prevent and treat radiotherapy-induced fibrosis. Vozenin-Breton *et al.* have especially shown that the mechanism of action of SOD was correlated with the inhibition of the expression of the pro-fibrotic cytokine Transforming Growth Factor-β1 (TGF-β1) [24]. This mechanism leads to the degradation of collagen and the dissolution of fibrotic network [26].

Based on this scientific background, the objective of this study is to clinically test the efficacy of an oral supplementation with SOD B Dimpless®, an encapsulated dried melon juice highly concentrated in SOD, on the reduction of cellulite.

Material and Methods

Study design

The study was carried out in a French private clinical experimentation laboratory on 41 healthy women with cellulite aged between 31 and 50 years old.

Main inclusion criteria were as follows: healthy non-smoker women between 30 and 50 years old, with visible fat nodes on stomach and/or thighs.

Main non-inclusion criteria were: women with skin conditions in the study areas, suffering from cardiovascular disease, taking drugs acting on subcutaneous fat or who have stopped treatment there is less than a month, taking any types of topical or systemic treatments in the previous weeks that could interfere with the evaluation of tolerance and efficacy of the product, taking drugs continuously 7 days before the inclusion, taking medication for more than two weeks in the month preceding the inclusion, taking food supplement in the two months prior the inclusion, and with a known allergy to one of the components.

Women were divided into two distinct groups:

- CL group (n= 21), supplemented with 40 mg per day of SOD B Dimpless® (480 IU SOD), during 56 days;
- PL group (n= 20), orally supplemented with a placebo during 56 days.

The subjects could not modify their diet or sport habits during all the study.

Tested product

SOD B Dimpless® is an encapsulated dry melon juice highly concentrated in SuperOxide Dismutase (12,000 IU SOD/g), obtained from a specific variety of Cantaloup melon (*Cucumis melo* L.), non-GMO, and exclusively cultivated by BIONOV (Avignon, France).

Global tolerance

At the beginning of the study, at D0, the overall health status of the subjects and their medical history were assessed by a clinical examination performed by the physician in charge of the study, to check the compatibility of their health status with the studied product.

After 56 days of supplementation, at D56, the possible felt and/or observed effects were postponed to allow assessment of the global tolerance. This evaluation takes into account the relevant factors reported by the subject (functional and physical signs) and those noted during the examination (clinical signs). The comparison of these signs was conducted to conclude the final tolerance of the studied product. The overall tolerance of the studied product was defined as the least favorable outcome.

Scoring of fat-nodes

Fat nodes were evaluated on the thighs and stomach at D0, D28 and D56.

In the literature, cellulite severity is generally evaluated with various visual and photographic methods, although accepted standards have not yet emerged.

In the present study, the practitioner evaluated visually cellulite according to a non-contact method, that is to say without pinching the skin. Three-dimensional (3D) images are obtained according to a tridimensional laser scanning technology, localized on a linear platform. The outer aspects of both thighs and stomach were scanned while subjects sat on a level surface with knees bent at a 90° angle to avoid compression of the thigh. Images were taken from the back of the thighs as well as from the side of the thighs, where cellulite is best visible. The scanner operates on the principal of triangulation. As a constant laser light source passes through two cylindrical lenses, the highlighted profile is reflected from the image mirrors to a video sensor and digitized.

The scanner moves along a linear trajectory performing 500 individual surface contour scans (402 X 170 mm) in equal increments. Shadows, ambient light, and skin color do not affect the laser scanned data.

The obtained images have been observed and cellulite has been assessed according to an unstructured linear scale defined by the practitioner, classified from 0 to 10 depending on the cellulite severity, as follows:

- 0 means "no cellulite",
- 1 to 3 means "slight cellulite"
- 4 to 6 means means "moderate cellulite"
- 7 to 10 means "severe cellulite".

The evaluation of the skin aspect and cellulite incidence gives a good indication of the visual effectiveness of the product.

Statistical analysis

The action of SOD B Dimpless® on one side, and of the placebo on the other, were assessed over time by successive steps according to the ANOVA method, followed by pairwise comparisons of three times (D0, D28, D56) using Fisher's test. The comparison between the CL and PL groups was performed using a factorial ANOVA on each change (D28-D0 & D56-D0). A value of $P< 0.05$ is considered as statistically significant.

Results

Studied Population

41 women, aged 31-50 years (mean 44) with a BMI of 23 to 30, participated in this study. There was no statistical difference between the two groups at baseline in scores for fat nodes confirming the homogeneity of groups at D0.

Tolerance

Only one subject in the CL group and two subjects in the PL group have reported transient discomfort that may be related to the product or placebo, respectively ingested. No significant change in blood pressure and pulse rate were observed whatever the group.
Under the conditions of the study, conducted under clinical control, the product SOD B Dimpless® and placebo were globally well tolerated.

Fat nodes score

Groups were substantially similar at D0.

Stomach

Visual assessment of the laser-scanned images of cellulite by the physician revealed that SOD B Dimpless® did not induce a significant visual decrease in fat nodes on stomach.

No significant evolution of cellulite on stomach was observed in the CL group compared to the PL group at D28 or D56. The data are reported in Table 1.

Table 1 Visual cellulite assessment on stomach at D0, D28, and D56, in women orally supplemented with SOD B Dimpless® (CL), compared to the placebo (PL). Values are mean ± SEM.

	D0	D28	D56
CL (n=21)	5.3 ± 0.2	5.1 ± 0.2	5.0 ± 0.2
PL (n=20)	5.9 ± 0.2	5.4 ± 0.3	5.5 ± 0.2
P Value	NS	NS	NS

Thighs

When compared with the basal value (D0), SOD B Dimpless® induces a visual reduction in cellulite on thighs by -3.4% on average on D28 ($P= 0.007$) and by -6.8% on average on D56 ($P= 0.039$). Results are shown in the Table 2.

When compared to the PL group, SOD B Dimpless® significantly reduces cellulite on thighs by 9.5% and 11.3%, respectively at D28 and D56. The improvement was observed in 71% of subjects at day 28 and 67% on day 56. The comparative statistical analysis showed a significant reduction of cellulite score in the CL group compared to the PL group on day 28 ($P= 0.0152$) and day 56 ($P= 0. 0217$). These results, shown in the Figure 1 and Table 2, illustrate the effectiveness of SOD B Dimpless® in the reduction of cellulite on thighs after 28 and 56 days of use.

Figure 1: Visual assessment of fat nodes on thighs in orally supplemented women with SOD B Dimpless® (CL, n=21) compared to the placebo (PL, n=20) Values are mean of scores SEM. NS: non-significant; *, $P< 0.05$.

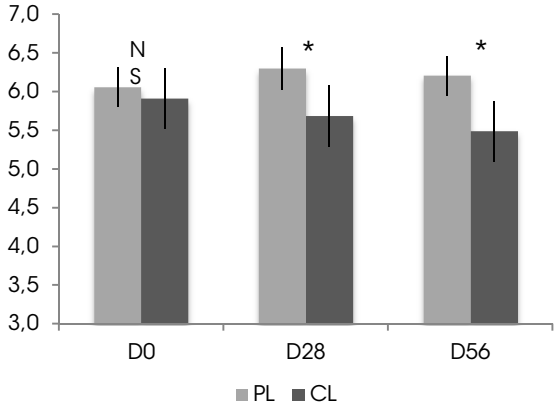

Table 2 Visual cellulite assessments on thighs at D0, D28, and D56, in women orally supplemented with SOD B Dimpless® (CL), compared to the placebo (PL). Values are mean ± SEM.

	D0	D28	D56
CL (n=21)	5.9 ± 0.4	5.7 ± 0.4	5.5 ± 0.4
PL (n=20)	6.1 ± 0.3	6.3 ± 0.3	6.2 ± 0.3
P Value	NS	0.0152	0.0217

Discussion

The main objective of this study was to evaluate the anti-dimples effect of SOD B Dimpless® compared to a placebo after 28 and 56 days of use. The results show a steady and continuing decline in visible cellulite on thighs in women supplemented with SOD B Dimpless® compared to placebo at D28 and D56.

The lack of effect of SOD B Dimpless® on cellulite on the stomach compared to the placebo could be due to the fact that fat nodes in this area are much less pronounced than on thighs. In this present study, the average rating of fat nodes is 5.6 on stomach against 6.0 on the thighs at D0. A significant visual difference is more difficult to obtain on the stomach and can explain the non-significativity of the results.

These results demonstrate the effectiveness of a natural ingredient administered orally in reducing cellulite in comparison with a placebo. These results also indicate that SOD B Dimpless® has a quick and lasting action: a visible reduction of cellulite on thighs is obtained only after 28 days, and amplified after 56 days of supplementation.

Several *in vivo* studies performed by Carillon *et al.* have shown that an oral supplementation with SODB, a melon juice highly concentrated in SOD, induced the expression of the three endogenous antioxidant enzymes, which are SOD, catalase (CAT) and glutathione peroxidase (GPx), in different target tissues (liver [27], adipose tissue [15], heart tissue [28]).

Carillon *et al.* highlighted the stimulation of the expression of SOD, CAT and GPx in the adipose tissue of an obese hamster's model, orally supplemented with SODB (10 IU SOD/day, corresponding to 400 IU SOD/day for a human of 60 kg) during 28 days [15]. Carillon *et al.* added that such an induction is linked to the inhibition of oxidative stress, as illustrated by the decrease in $O_2^{\bullet-}$ production by 67% [15]. This inhibition of ROS generation is associated with a stimulation of lipolysis by 43%, a reduction in adipocytes size by 54%, and an inhibition of fibrosis by 52% in SODB supplemented obese hamsters compared to untreated obese animals [15]. The decrease in fibrosis and adipocyte hypertrophy could be explained by the antioxidant action of SOD, CAT and GPx, and the inhibition of ROS production.

These results have established a link between the induction of antioxidant enzymes endogenous expression, and the reduction of oxidative stress and inflammation [28]. This way of action could explain the reduction of cellulite observed in this study. These results strongly suggest that a restoration of its pool of endogenous antioxidant defenses, following an oral supplementation with SOD B Dimpless®, could be an active strategy against cellulite by fighting its two main identified causes: adipocytes hypertrophy and fibrosis.

To go further in this theory, complementary studies on the mechanism of action of SOD B Dimpless® could help to confirm its clinical efficacy on cellulite. Moreover, studies that highlight the link between anti-fibrotic properties of SOD and cellulite reduction could help to support the mechanism of action of this antioxidant enzyme against this aesthetic issue.

Similarly, additional data on the possible action of SOD against fat cells hypertrophy would provide additional evidence of its anti-dimples efficiency.

Conclusion

To conclude, this clinical study reports the positive effect against cellulite of an oral supplementation with SOD B Dimpless®, an encapsulated dry melon juice highly concentrated in SOD. This study demonstrates that women orally supplemented with SOD B Dimpless® (40 mg daily for 56 days) have less visible cellulite on thighs than women who received the placebo.

The possible mechanism of action of SOD B Dimpless® includes the induction of endogenous antioxidant enzymes expression, the inhibition of inflammation and oxidative

stress, and the reduction of fibrosis and adipocyte hypertrophy. This hypothesis should be confirmed by further studies.

Disclosure Statement

The authors Sébastien Le Quéré, Benoit Lemaire, and Dominique Lacan work for Bionov.

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