A RANDOMISED, DOUBLE-BLIND, MULTIPLE-DOSE TRIAL OF THE EFFICACY OF A GLUTATHIONE WAFER AS A THERAPEUTIC SKIN HEALTH SUPPLEMENT

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Results summary

After 14 days

- ✓ Significant increase in skin luminosity and gloss by up to 60%
- ✓ Significant reduction in eye wrinkles and fine lines by up to 51%

After 28 days

- ✓ Significant increase in skin elasticity by up to 226%

After 56 days

- ✓ Significant increase in skin smoothness with decrease in skin roughness by up to 71%

Based on a clinical trial of 34 female participants with signs of skin ageing on a regime of 200mg sublingual GSH wafers daily for 4 weeks followed by 100mg sublingual GSH wafers for 8 weeks

ABSTRACT

Glutathione (GSH) is a potent master antioxidant and plays a crucial role in various physiological processes within the body, including maintaining skin health and appearance[1,2].

Its powerful antioxidant function neutralizes free radicals and reduces oxidative stress in the skin. Free radicals damage skin cells, collagen and elastin fibres, leading to roughness and premature aging. By neutralizing these free radicals, glutathione may help protect the skin and maintain its smoothness.[3]

Glutathione also stimulates the synthesis of collagen, a protein that provides structure and elasticity to the skin. Increased collagen production improves skin firmness and smoothness, reducing the appearance of fine lines and wrinkles.[4]

Glutathione is involved in the pathway of detoxification processes, in the elimination of toxins and harmful substances. The detoxification helps achieve clearer, healthier skin, contributing to a smoother complexion.[5,6]

GSH is a naturally occurring tripeptide. Literature reports show that GSH can improve skin gloss and luminosity. Several routes of supplementing GSH for these skin treatment purposes are available including intravenous (IV), oral and topical administration.

We investigated a novel patented sublingual wafer containing GSH marketed under the name MeltMed Radiance in a randomised, double-blind, multi-dose trial for efficacy as a therapeutic skin health supplement.

Objectives: The primary objectives of this study were to determine the changes in skin gloss and luminosity, skin elasticity, eye wrinkles and fine lines and skin roughness following the sublingual administration of GSH wafers.

Methods: The 12-week study was conducted on 34 healthy females aged 30 to 65 years old with Fitzpatrick skin type IV or V at one clinical site in Sydney, Australia.

Results: Significant improvements were observed in skin gloss and luminosity (up to 60%), skin elasticity (up to 226%), skin smoothness (up to 71%) and decrease in eye wrinkles and fine lines (up to 51%) within 14 to 56 days of commencing GSH therapy. GSH wafers were considered safe with no serious adverse events reported. All participants rated good to excellent for acceptability of wafers' smell, taste, after-taste as well as the sublingual disintegration rate of the GSH wafers

Conclusions: The authors concluded that the sublingual GSH wafers were safe, tolerable and clinically efficacious in healthy women with the ideal maintenance sublingual dose of 100mg GSH daily after a one month loading dose of 200mg GSH daily.

INTRODUCTION

According to a study by Yang et al,[7] nearly one-third of the 1,434 participants in their study felt unhappy with their skin during the COVID-19 pandemic. Aging skin is a significant and growing concern and the top age-related facial skin concerns include eye puffiness, loose skin, uneven tone, uneven texture, and dry skin. Also, video conferencing, mask wearing, and increased stress during the COVID-19 pandemic have exacerbated their skin concerns for many consumers. Their results revealed the top skin concerns were eye puffiness (86.5%), loose skin (85.1%), uneven tone (84.9%), uneven texture (83.5%), and dry skin (81.4%). Video conferencing (31%), wearing masks (23%), and increased stress (21%) during the COVID-19 pandemic affected how participants felt about their skin.

The study by Luebberding et al[11] revealed a progressive decline in the mechanical properties of skin with aging. These mechanical properties change differently in men and woman over their lifetimes. The obvious morphological sign of skin aging is the development of rhytides, commonly known as wrinkles. The development of facial wrinkles significantly affects men earlier and with greater severity than women. The elasticity of the skin, which influences formation of facial wrinkles and age-related skin laxity, is determined by collagen density and elastin fiber volume. The skin's elastic ability to recover after stretching, smoothness and firmness, are strongly affected by the aging process, the redox status and the GSH levels in the body. GSH has been used to enhance and support overall skin health.

The cellular redox state is altered in a number of pathological conditions, diabetes, cardiovascular diseases (eg atherosclerosis), inflammatory diseases, liver diseases (eg non-alcoholic fatty liver disease or NAFLD) and aging. GSH influences protein synthesis, with GSH deficiency causing changes to the functional and structural properties of cellular proteins, especially collagens. Intracellular redox potential influences the generation of collagen, and also influences gene transcription in mesangial cells, which is important for the functional and structural properties of cellular proteins.[4]

The catalytic activity of enzymes also contribute to collagen synthesis and its secretion are also influenced by the intracellular optimal redox state. GSH is important for maintaining homeostasis and acts as a "redox switch" to maintain this important optimal protein synthesis function. Hence, changes in the cellular redox state can influence collagen anabolism and secretion.[10]

Topical, oral, and IV GSH are available as nutraceutical products for skin health. Oral administration of GSH is not considered optimal due to its very poor bioavailability. The sublingual (SL) form is the most bioavailable compared to other oral forms (tablet, capsule) due to the sublingual absorption bypassing the liver first pass metabolism and the hostile environment of the GI tract. An in vivo study by Daniela Buonocore et al[8] revealed a rapid and efficient uptake of GSH into the blood via the oral mucosa resulting in higher bioavailability. In another in vivo study by Bernard Schmitt et al[9], these researchers

also showed increased plasma GSH levels in the SL group when compared to the oral GSH group. The differences between these two groups were statistically significant ($p < 0.05$).

The SL wafer in our study used a patented wafer matrix technology (WaferiX™) as the GSH carrier. This novel SL GSH wafer was prepared by freeze-drying an aqueous dispersion of GSH containing sodium carboxymethycellulose and amylopectin as the matrix formers. The novel wafer GSH formulation rapidly dissolves sublingually, releasing the GSH into the small saliva volume immediately, adjacent to the sublingual mucosal membranes, resulting in a direct SL absorption with higher bioavailability than other oral formulations.

This study aimed to explore the clinical effect of SL GSH on related skin conditions as a therapeutic skin health supplement.

METHODOLOGY

This study was a randomised, double-blind, multiple-dose study on the efficacy of a sublingual glutathione wafer (MeltMed Radiance™) as a therapeutic skin health supplement. The 12-week study was conducted on 34 healthy females aged 30 to 65 years old with Fitzpatrick skin type IV or V at one clinical site in Sydney, Australia.

The primary efficacy objectives included: (i) skin gloss and luminosity; (ii) skin elasticity; (iii) eye wrinkles/fine lines (crow's feet) and (iv) skin roughness.

Participants were instructed on how to administer the wafers under the tongue for maximum SL absorption. Food and drink were avoided within 10 minutes of administration and the wafers were administered twice daily (morning and evening).

Participants were blinded and randomised in a 1:1 ratio to one of the two cohorts each with seventeen (17) participants. Cohort 1 received 100mg GSH wafer (2 x 50mg GSH wafers plus 1 x placebo wafer) administered twice daily (total daily dose of 200mg) in Week 1 to Week 4. In Week 4 to Week 12, 50mg GSH wafer (1 x 50mg GSH wafer plus 1 x placebo wafer) were administered twice daily (total daily dose of 100mg). This will be referred to as the 200mg/100mg dosing regimen.

Cohort 2 received 150mg GSH wafer (3 x 50mg GSH wafers) administered twice daily (total daily dose of 300mg) in Week 1 to Week 4. In Week 4 to Week 12, 100mg GSH wafer (2 x 50mg GSH wafers) were administered twice daily (total daily dose of 200mg). This will be referred to as the 300mg/200mg dosing regimen.

Assessments were conducted at baseline (Day 0), Week 2 (Day 14), Week 4 (Day 28), Week 8 (Day 56) and Week 12 (Day 84) which was the end of study (+/- 3 days) or at early termination.

Participants were allowed to continue their current skincare regimen without introducing any new or different skincare products or facial treatments. Participants were also asked to avoid sun exposure or to use appropriate sun protection when outdoors (sunscreen, hat, protective clothing). Each participant was provided with product instructions (for use and storage) and a diary to keep track of product use. At each visit participants' compliance and any changes to concomitant medications were recorded.

INSTRUMENTAL MEASUREMENTS

Skin elasticity was measured by Cutometer® Dual MPA 580 (Courage + Khazaka electronic GmbH) and skin luminosity (gloss) was measured by Skin Glossymeter® GL 200 (Courage + Khazaka electronic GmbH).

The skin properties were analysed by digital photographs of the face (front and side facial photographs) using a custom-made digital photography equipment (Canon EOS 60D DSLR camera). Each image was cropped and resized in GIMP to facilitate image analysis. After images were processed, they were imported into Image Pro Premier (IPP) for image analysis (assessment including eye wrinkles, and skin roughness). The IPP provides values in percentage change ("change from baseline" and "percentage change from baseline").

STATISTICAL ANALYSIS METHODS

The instrumental and photos measures were transferred to SAS for analysis. Descriptive statistics were calculated and averaged over participants for each measure and visit. These averages were analysed inferentially using repeated measures analysis of covariance within a mixed model framework. Inferential statistical analysis was performed in order to assess if each parameter (the dependent measure) varied linearly over time. The Baseline value (Visit 1) of the measure, in addition to other demographic variables, were used as covariates within each measure.

Adjusted means, calculated as Least Squared Means in the analyses, are presented in the tables with probabilities less than 0.05 indicating significant differences between selected means. The adjusted means between the first and last visit were compared using a t-test to see if there was an overall change at the end of the study.

Change from Baseline was calculated, and descriptive statistics were also calculated for each measure. This includes number of observations (n), mean, percentage change, standard deviation (SD), minimum, median, and maximum values for each parameter's value and its change from Baseline.

STUDY POPULATION

A total of thirty-four (34) healthy female participants with Fitzpatrick Skin Type IV or V and with some signs of skin ageing (crow's feet, and uneven skin texture) whose written informed consent had been obtained, were screened and enrolled in the study.

RESULTS AND DISCUSSIONS

The average age of the thirty-four (34) female participants enrolled was 44 years (SD = 9.5), ranging from 31 years to 64 years old.

88% of participants had an ethnicity from Asia, 3% from the South Pacific/Papua with 9% from other regions. 24% of participants had normal skin, 18% had dry skin, 11% had oily skin and 47% had combination skin.

56% had 'Phototype IV' skin (olive, moderate brown; burns minimally, always tans well); 44% had 'Phototype V' skin (brown, dark brown; rarely burns, tans profusely).

The following efficacy parameters showed a statistically significant improvement compared to baseline: skin luminosity and gloss (p = 0.04), eye wrinkles (p = 0.04), and skin roughness (p = 0.04).

High positive responder rates across all parameters

We analysed the percentage of participants who had a positive result to GSH therapy within the study period. The study showed an overall high positive responder rate of greater than 70% across all parameters. 100% of participants on the 200mg/100mg regime showed improvements to skin roughness and elasticity within 12 weeks (84 days), while only 100% of participants on the 300mg/200mg regime showed improvements to skin roughness within 12 weeks (84 days).

Due to the high levels of positive response seen in participants on the 200mg/100mg regime across the parameters studied, we postulate that 200mg/100mg regime provides the body with the best optimal homeostatic GSH level. More supplementations (eg 300/200mg dosage regime) may not be necessary, as excessive GSH may be broken down or rechannelled to other cells. GSH has a very complicated pattern of involvement in diverse biological processes/activities.[12]

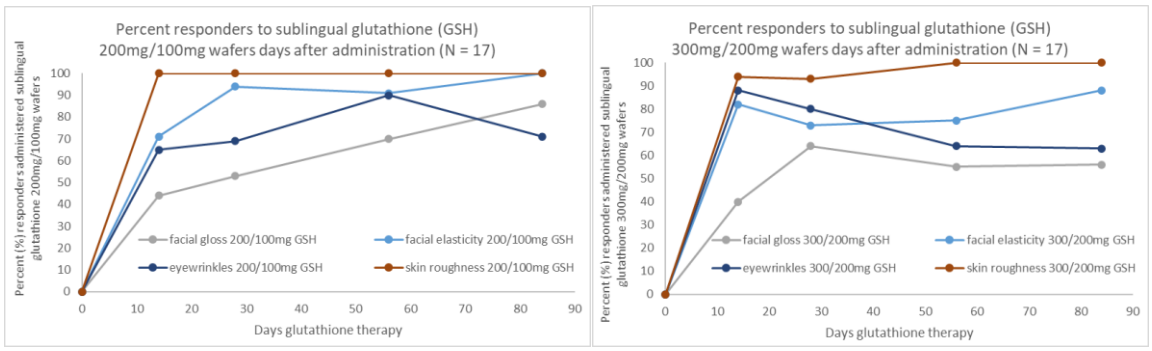

Fig 1. Graph of responder rates (%) from baseline throughout end of study for each efficacy parameter (Left graph = 200mg/100mg sublingual GSH dosage; Right graph = 300/200mg sublingual GSH dosage).

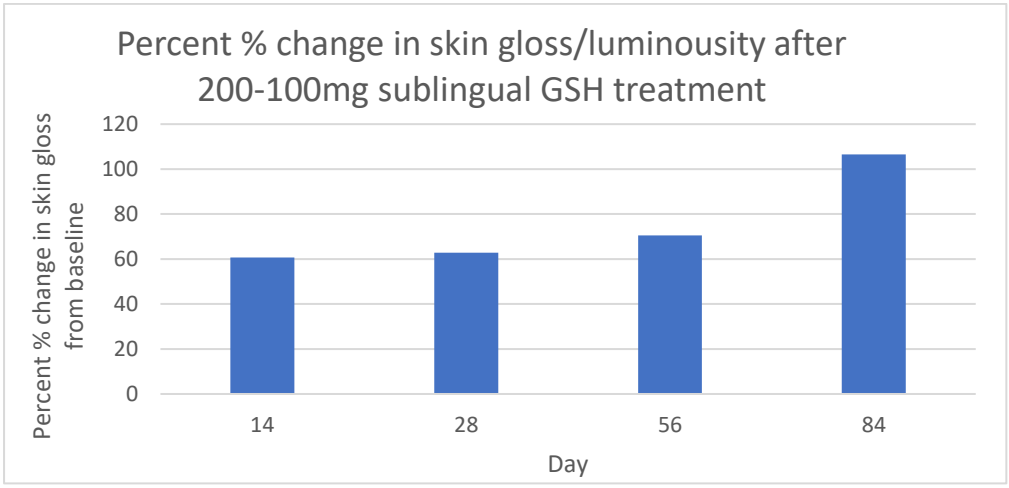

Fig 2. Graph of maximum percent changes from baseline for skin gloss/luminosity for the sublingual 200/100 mg GSH treatment group

Skin gloss and luminosity:

SL GSH therapy resulted in a statistically significant improvement to skin gloss and luminosity in participants compared to baseline (p = 0.04) (see Fig. 2).

Participants on the 200mg/100mg regime achieved rapid and marked increase in skin luminosity and gloss of up to 66% after 4 weeks, increasing up to a remarkable 106% after 12 weeks. By 12 weeks, increased skin luminosity and gloss was observed in 84% of participants.

Based on the mean data showing that maximum improvement was achieved after 12 weeks, continuous SL GSH therapy beyond the study period is expected to result in greater increase in skin luminosity and gloss.

The improvement in skin gloss in this study is in line with other GSH clinical studies[13, 14]. The mode of action of improvement in skin gloss is possibly by GSH inhibiting the tyrosinase enzyme as well as by reducing free radicals damaging the skin cells. Uneven skin gloss can be treated by reducing cells damage caused by UV radiation.

Skin elasticity

SL GSH therapy resulted in a statistically significant improvement in skin elasticity in participants compared to baseline (p = 0.03) (see Fig. 3)

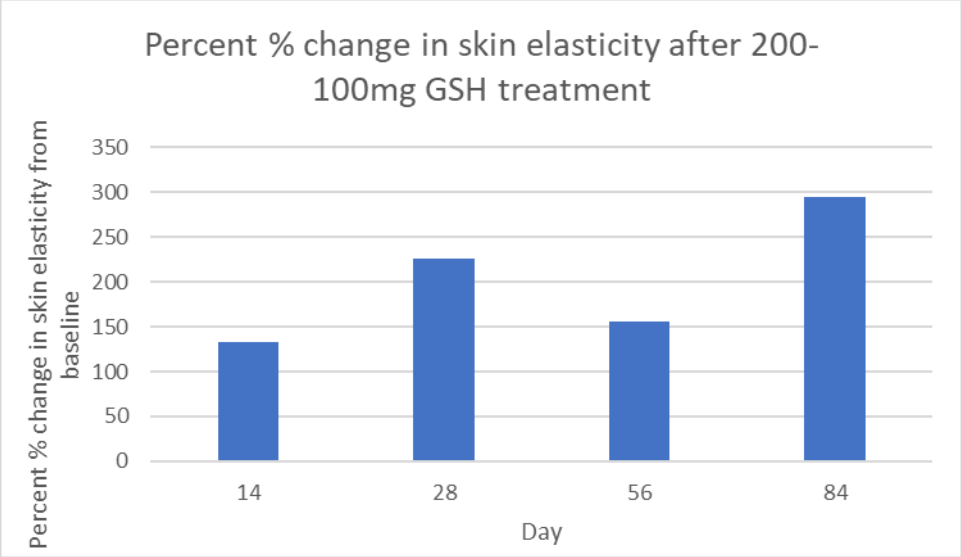

Fig 3. Graph of maximum percent changes from baseline for skin elasticity for the sublingual 200/100 mg GSH treatment group.

Participants on the 200mg/100mg GSH regime achieved significant increase in skin elasticity of up to 226% after 4 weeks and 295% after 12 weeks. By 12 weeks, increased skin elasticity was observed in 100% of participants. Elastin is a fibrous protein found in the dermal skin layer and is responsible for skin elasticity. This study showed that GSH can increase the skin elasticity, most probably due to its antioxidant effect. Weschawalit et al[15] in their study, reported that GSH improves skin elasticity and reduces skin wrinkles in either sun-exposed or sun-protected areas and that GSH is superior to placebo in reducing skin wrinkles.

The primary triggering factor of skin aging and/or skin non-elasticity is oxidative cellular damage caused by increased oxidative stress.[16,17]

Oxidative stress results from an imbalance between reactive oxygen species (ROS) synthesis and defence mechanisms that remove ROS. Enzymes that remove ROS, such as glutathione (GSH), are representative of defence mechanisms against oxidative stress.[18]

Eye wrinkles

SL GSH therapy resulted in a statistically significant reduction in eye wrinkles and fine lines around the eyes compared to baseline ($p = 0.04$) (see Fig. 4).

In the 200mg/100mg regime group, 90% of participants responded positively to treatment at the 8-week mark. The greatest maximum change from baseline in this cohort was 51%, observed at day 14 of the study.

This study demonstrated that glutathione supplementation yields cosmetic benefits such as improvements to skin elasticity and reduction in skin wrinkles, especially around the eyes.

Free radical formation in the cells (cause by UV radiation), if left unchecked or not neutralised (by GSH supplementation) will damage the cells and tissues and may cause inflammation and skin wrinkles, especially around the eye. GSH being a powerful and most important intracellular antioxidant, reduces free radicals and inflammation, resulting in the improvement of the skin wrinkles and skin complexion as shown in this study. This confirms the finding that GSH is effective in reducing facial wrinkles in another study by Weschawalit et al.[15]

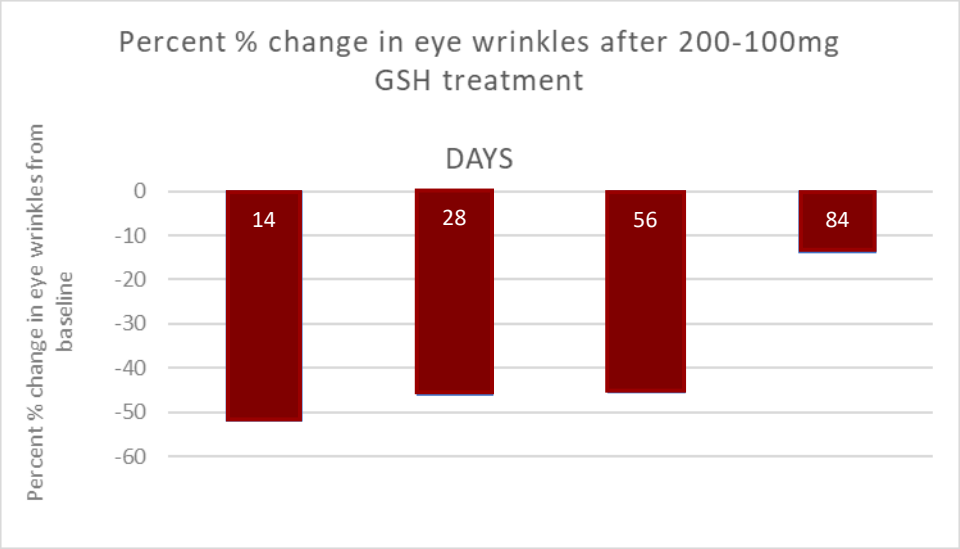

Fig 4. Graph of maximum percent changes from baseline for eye wrinkles for sublingual 200/100 mg treatment group

Skin roughness

SL GSH therapy resulted in a statistically significant reduction in skin roughness compared to baseline (p = 0.04) (see Fig. 5)

Within 14 days, all participants on the 200mg/100mg regime had shown improvements in skin roughness. Within 4 weeks, there was a reduction of up to 64% in skin roughness, which increased to 71% by week 8.

GSH delays skin stiffening and decreases collagen loss from skin injury induced by UV radiation[19]. GSH also assists with skin cell renewal giving the skin a glowing appearance. The aging "dull" skin cells give the skin a rough appearance. GSH assists skin renewal by sloughing away dead cells and giving the new underlying cells a chance to rise to the surface[20]. This study has demonstrated that GSH can improve skin smoothness (or decrease skin roughness) shown by 100% of the participants in the 200/100mg protocol with 14 days of initiation of therapy and maintained till 12 weeks.

The Watanabe et al[21] study showed that GSH significantly increases the moisture content of the stratum corneum plus the suppression of wrinkle formation leading to the improvement in skin smoothness.

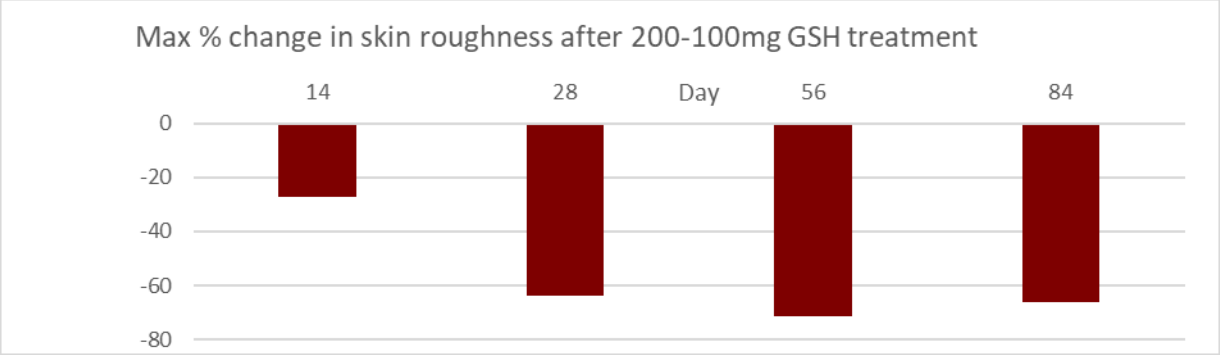

Fig 5. Graph of maximum percent change from baseline for skin roughness for 200/100 sublingual GSH group

Safety and tolerability of the sublingual GSH:

As with previous studies using WaferiX (wafer carrier matrix) there were no abnormal findings of oral cavity tolerability in any subject, with mucosa deemed as normal in all post-dose assessments of oral cavity and sublingual space.[22, 23].

GSH wafers were considered safe with no serious adverse events reported. On the oral symptom questionnaires, there was no sublingual irritation or burning sensation for any subject. This is in sync with several studies where none of the participants demonstrated adverse events during the treatment period of SL GSH.[24, 25]

GSH is a tripeptide consists of cysteine, glycine and glutamate. These three amino acids are bonded together to form a tripeptide called GSH. Cysteine is a sulphur-containing amino acid and some people may taste and/or smell the sulphur which may not be a likable for some people. Our wafer contained taste masking agent incorporated into the formulation to minimise any sulphur taste pertaining to the cysteine residue.

All participants were given questionnaires to rate on acceptability of smell, taste, after-taste as well as the sublingual disintegration rate of the GSH wafers.

70.7% of participants reported that the smell of the GSH wafers was good to excellent. 64.7% of participants reported that the taste of the GSH wafers was good to excellent. Overall tolerability of the GSH wafers was reported as good to excellent in 74.2% of participants.

CONCLUSION

This study showed that GSH sublingual wafers are an effective therapeutic skin health supplement. Statistically significant positive results were observed for skin gloss and luminosity, skin elasticity, eye wrinkles, and skin roughness. All these parameters showed positive therapeutic results within 14 days of therapy with high positive responder rates.

The safety and tolerability of the sublingual GSH after 84 days were good to excellence as rated by the participants.

The ideal initiation dose of GSH is 200mg daily for 28 days (4 weeks), followed by a maintenance dose of 100mg daily thereafter.

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