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HYVIA™: A novel, topical chia seed extract that improves skin hydration

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Abstract

Background: Chia seeds have gained importance as it is the highest known plant source of omega-3 (ω3) polyunsaturated fatty acids. Specifically, chia seeds possess ω3 α-linolenic acid (ALA) and ω6 linoleic acid (LA), together known as Vitamin F, which play an important role in maintaining skin function. Protein phosphatase 2A (PP2A) is a master regulatory protein that plays a critical role in skin barrier function and its activity is modulated by natural lipids.

Aims: Obtain a chia seed extract (HYVIA™) with significant higher levels of Vitamin F, determine in vitro PP2A activity and skin hydration markers compared to other commercial chia seed extracts (CCSEs), and evaluate the potential skin hydration benefits clinically in human subjects.

Methods: A PP2A demethylation assay was utilized to assess PP2A activity. In vitro studies utilizing normal human epidermal keratinocytes (NHEKs) were treated with HYVIA™ and gene expression of hydration markers (AQP3, HAS2) were measured by quantitative PCR (qPCR). A 16-subject clinical trial was performed with 0.1% HYVIA™ formulated in a cream and applied topically to assess its skin moisturizing potential.

Results: We demonstrate here that HYVIA™, ALA, and LA inhibit PP2A demethylation, boosting PP2A activity, while most other CCSEs do not. Unlike other CCSEs, HYVIA™ increases keratinocyte hydration factors aquaporin-3 and hyaluronic acid synthase-2 in vitro. Clinical assessment of 0.1% HYVIA™ cream shows that HYVIA™ improves skin hydration.

Conclusions: HYVIA™ is a novel chia seed extract, enriched for Vitamin F, that modulates PP2A activity and clinically improves skin hydration and barrier function.

KEYWORDS

Chia seed, linoleic acid, PP2A, α-linolenic acid

1 | INTRODUCTION

Chia (*Salvia hispanica* L.) is an ancient crop that has experienced an agricultural resurgence in recent decades due to the high omega-3 (ω3) and omega-6 (ω6) fatty acid content of the seeds and good production potential.[1] While chia has been widely studied as a functional food and reported to have several clinical benefits including cardioprotection, weight loss, and metabolic disorder improvement, very little has been published on its benefits when applied topically, despite the key functions ω3 α-linolenic acid (ALA) and ω6 linoleic acid (LA) (also referred to as Vitamin F) play in skin. To date, only one report has been published where a topical formulation of

4% chia seed oil extract was applied for 8 weeks and the authors concluded that chia was effective for pruritus, xerosis, and beneficial for skin hydration.[2]

Polyunsaturated fatty acids (PUFAs), including ω3 and ω6, play an important role in maintaining normal skin function. The first sign of their critical role was discovered ~90 years ago, where animals on a diet deficient in essential fatty acids (FAs) developed skin abnormalities and decreased hydration.[3] Subsequent studies showed that topical application of LA to animals with chemically comprised skin resulted in a dramatic reduction in transepidermal water loss (TEWL) and reversal of barrier function defects. Moreover, when applied to human skin, a gamma-LA rich cream performed better than placebo in reducing pruritus.[4] Thus, supplying these FAs are critical for maintaining optimal skin function. It was later determined that both ω3 and ω6 are essential FAs that must be obtained from exogenous sources because the skin lacks the enzymes to place double bonds at the n-3 and n-6 positions. LA is the parent compound of the ω6 PUFAs, and ALA is the parent compound of the ω3 PUFAs. Together, these two essential FAs are the precursors in the skin for synthesis of longer chain compounds that also play key roles in skin structure and function. For example, LA is the most abundant PUFA in the epidermal layer and serves as a precursor for the synthesis of ceramides that are uniquely found in skin.[5] ALA and its derivatives are mainly located in cell membrane phospholipids and play a significant immunological role in the epidermis in several skin disorders including atopic dermatitis, psoriasis, acne, and photoaging.[6] These findings have led to the concept that PUFAs in topical formulations are beneficial as precursors to structural skin lipids participating in the repair of a suboptimal barrier resulting from PUFA deficiency or compromised skin metabolism.

Protein phosphatase 2A (PP2A) is a heterotrimeric protein comprised of A (structural), C (catalytic), and B (regulatory) subunits. The C subunit is subject to methylation at its carboxyl terminus, and this modification drives the association of all three subunits which is necessary for optimal enzyme activity. PP2A is a master regulator whose critical role and function has been mostly studied in neurodegeneration (reviewed in[7]), cancer (reviewed in[8]), and metabolic disorders (reviewed in[9]). However, more recent studies demonstrate this protein also plays a critical role in skin barrier function, oxidative stress signaling, and inflammation. PP2A activation is required for proper epidermal barrier formation during late embryonic development.[10] Conversely, a decrease in PP2A activity has been shown to lead to failure in filaggrin processing, which is essential for epidermal barrier homeostasis,[11] and has been proposed to contribute to the development of harlequin ichthyosis. Moreover, LA is an essential precursor for the synthesis of skin ceramides, which play a critical role in skin barrier function and have been reported to activate PP2A.[12] PP2A also regulates skin inflammation. For example, reactive oxygen species have been shown to inactivate PP2A,[13] resulting in activation of NF-κB mediated pro-inflammatory signaling. In human dermal fibroblasts, oxidative stress has been shown to induce PP2A demethylation, driving the disassociation of the fully active PP2A holoenzyme trimer to the less active dimeric form.[14] Altogether, these data suggest that preventing PP2A inactivation by ensuring it remains in a highly methylated more active state is critical for combating oxidative stress and promoting skin health.

Here, we demonstrate for the first time the identification and characterization of a novel chia seed extract called HYVIA™. We report HYVIA™ has significantly higher levels of ω6-LA and ω3-ALA PUFAs than other commercial chia seed extracts. Moreover, HYVIA™ modulates PP2A methylation, promoting its active form and increasing important hydration factors such as aquaporin-3 (AQP3) and hyaluronic acid synthase 2 (HAS2) better than commercial chia seed extracts. Lastly, clinical results in human subjects demonstrate that HYVIA™ formulated at 0.1% and applied topically is well tolerated and significantly increases skin barrier function and hydration compared to vehicle.

2 | MATERIALS AND METHODS

2.1 | Reagents

All chemicals were obtained from Sigma-Aldrich Co. Chia seeds were obtained from BI Nutraceuticals; Pure Lead (PowerNutri Shop, CA); Food to Live; Chiaseedsdirect.com (Scottsdale, AZ); Gigawatt Spices; CPX PERU (Surquillo, Peru); Tierra Overseas (Maharashtra, India); Heartland Chia (Franklin, KY); Naturkost de Mexico (Jalisco, Mexico); Nutiva (Richmond, CA); InkaSeed (Lima, Peru); Namaskar SAC (Lima, Peru); and Salba Smart Natural Products (Littleton, CO). Commercial chia seed extract and oil were obtained from Botanical Beauty (Miami, FL) and US Organics (Englewood Cliffs, NJ), respectively. Ethanolic extracts from each chia seed source (aka commercial chia seed extracts or CCSEs) were prepared using 80% ethanol 1:10 (w/v).

2.2 | HYVIA™ production

HYVIA™ was isolated from *Salvia hispanica* L. (Salba) seed by suspending the material in 80% ethanol 1:10 (w/v) and heating at 50°C for 18 hours. The suspension was filtered and concentrated to dryness on a rotary evaporator. The mass of the dried oil was calculated and then re-solubilized in ethanol (1:10 w/v). Dry fractionation was performed by heating filtrate to 75°C for 1 hour and slowly cooling to 4°C for 15-18 hours and then chilling sample further to −20°C for 2 hours. Cold filtration apparatus under vacuum was utilized to remove crystals formed during dry fractionation. The filtrate was evaporated to dryness using rotary evaporation.

2.3 | Protein purification

PP2A-AC dimer was prepared as previously described. Additional purification was performed with a HiPrep™ 16/60 Sephacryl® S100 HR in a buffer composed of 50 mmol/L MOPS pH 7.2,

1.0 mmol/L EDTA, 1.0 mmol/L dithiothreitol (DTT), and 0.50 µg/mL each of aprotinin, leupeptin, and pepstatin. Leucine carboxyl methytransferase-1 (LCMT-1) and protein phosphatase methylesterase-1 (PME-1) were prepared using the methods as previously described.

2.4 | Demethylation of PP2A by PME-1

[^{3}H]-labeled methylated PP2A AC dimer was prepared by incubating PP2A, LCMT1, and [^{3}H]-SAM (PerkinElmer) in 50 mmol/L MOPS-Na (pH 7.2), 5 mmol/L $MgCl_2$, and 1 mmol/L DTT at room temperature for 1 hour. Demethylation of PP2A by PME-1 was measured using the radioactive filter binding assay format. 20 nmol/L PME-1 was incubated for 15 minutes with extract or compound, and then, 20 nmol/L of [^{3}H]-labeled methylated PP2A AC dimer was added. Reactions were run at room temperature for 30 minutes and then applied to a 96-well filter plate (Millipore Co.) containing 30% TCA where proteins were precipitated and separated from the excess of [^{3}H]-SAM by washing with 70% ethanol. [^{3}H]-incorporation was measured using TopCount NXT scintillation counter (PerkinElmer; Waltham, MA). IC_{50} values were generated from dose-response curves using a four-parameter logistic curve fit in SigmaPlot (Systat Software, Inc).

2.5 | HPLC analysis

HPLC analysis was performed as previously described by isocratic gradient utilizing Acetonitrile:Methanol:Hexane (90:8:2) + 0.02% acetic acid as mobile phase over 35 minutes. Separation was performed on a Luna 5 µm C18(2) 100 Å 250 × 4.6 column (Phneomenex) at a flow rate of 1 mL/min, monitored by absorbance at 208 nm using Agilent 1290 Infinity. Standard curves of linoleic acid (LA) and α-linolenic acid (ALA) were utilized for quantification.

2.6 | Monolayer NHEK cell culture

Normal human epidermal keratinocytes (NHEKs) from neonatal donors were obtained from Thermo Fisher and cultured in EpiLife® media supplemented with keratinocyte-growth supplement and 60 µM calcium (Thermo Fisher). Cells were cultured until the second passage and seeded on 6-well plates for 24 hours before treatments.

2.7 | Gene expression

NHEKs were treated for 24 hours in EpiLife® media without growth factors. Cells harvested after treatments were homogenized in RNA lysis buffer. Total RNA was extracted using the RNAqueous kit (Thermo Fisher), and cDNA was obtained using the High Capacity RNA-to-cDNA kit (Thermo Fisher). Quantitative PCR (qPCR) was performed using the TaqMan® Fast Advanced Master Mix (Thermo Fisher) and specific TaqMan®-probes human gene primers for AQP3, HAS2, and GAPDH to calculate the relative gene fold expression change per treatment. Gene expression analysis was performed using the comparative Ct method (2–[delta][delta] Ct) approach by comparing the Ct values of the treated samples with vehicle-only treated samples and normalized to GAPDH gene expression as endogenous housekeeping gene.

2.8 | Clinical study

A clinical trial in healthy volunteers was conducted at Princeton Consumer Research Group (PC) in accordance with the intent and purpose of Good Clinical Practice regulations described in Title 21 of the US Code of Federal Regulations (CFR), the Declaration of Helsinki, and/or PCR Standard Operating Procedures. This was a single-blinded vehicle-controlled study, with 16 subjects completing the study (5 males/11 females, age range from 18 to 60 years and all enrolled subjects finished the study). Subjects reported to the testing facility for baseline screening at which time informed consent and demographics were obtained. Subjects attended PCR Corp on Day −3 of the study to enroll into the study and complete their informed consent. They were given a bland soap product (eg, Simple® Soap) to use on their lower legs for the three days prior to the active phase. They were instructed not to use any other treatment products on their lower legs for the next 3 days. These products included moisturizing foam baths, shower gels or soaps, lotions and creams, and depilatory products. Prior to application of the test articles in the lower legs, the test sites were wiped with a soft tissue. A 15-minute warm-up period was allowed before measurements. Each test article (50 µL) was randomly applied to two 25 cm^2 areas and was spread evenly across the area using a gloved finger. Moisturization measurements to study the humectant properties and transepidermal water loss (TEWL) measurements of the test articles were performed using the Corneometer® CM825 and Tewameter® TM300 (Courage and Khazaka, Germany), respectively. Four measurements were made using each of the instruments at each of the test sites: prior to application of the test article, and at 2, 8, and 24 hours following application of the test article.

2.9 | Statistical analysis

For enzymatic and gene expression analysis, samples were assayed in triplicate. Statistical significance was determined by ANOVA followed by a Dunnett multiple comparisons test using $P < .05$ as significant difference. For the clinical study, the paired one-way ANOVA was used to assess within-subject improvements over baseline (0 hours), and the unpaired one-way ANOVA to compare responses between HYVIA™ and vehicle treatments. $P < .05$ was considered significant.

3 | RESULTS

3.1 | α-Linolenic and linoleic acids regulate PP2A

Filaggrin degradation into natural moisturization factors 2-pyrrolidone-5-carboxylic acid (PCA) and urocanic acid (UCA) is catalyzed by skin proteases that are induced by the active methylated form of PP2A. PP2A is inactivated by demethylation in response to oxidative stress.[15] Previous reports have identified regulators of PP2A[12,16] that are structurally related to ALA and LA. While most of these compounds are saturated, we asked whether PUFAs might have a potential new role in skin hydration via the modulation of PP2A activity. In cells, the methylation state of PP2A is determined by the relative activities of leucine carboxyl methytransferase-1 (LCMT-1) which adds methyl groups and PP2A methylesterase-1 (PME-1) which removes them. Therefore, to determine whether ALA and LA can regulate PP2A methylation to maintain its active form, ^{3}H-methyl group-labeled PP2A was generated with LCMT-1 and was then incubated with PME-1 and PUFAs. We demonstrate for the first time that ALA and LA dose dependently inhibit PP2A demethylation by PME-1 with an IC_{50} of 4.9 and 1.4 μg/mL, respectively (Figure 1), thus maintaining PP2A in its active form.

3.2 | HYVIA™, a chia seed extract high in PUFA's prevents demethylation of PP2A

Chia seeds provide a potential natural source of high levels of ALA and LA. Therefore, we sought to determine whether PUFAs could be extracted from chia at levels that would increase PP2A activity. Ethanolic extracts from a variety of chia seed sources (aka commercial chia seed extracts or CCSEs) were quantitatively analyzed for ALA and LA content using previously published HPLC methods. These levels were compared for their potency to inhibit the demethylation of PP2A measured as described above. We observed that most extracts had insufficient concentrations of ALA and LA to be active in our PP2A demethylation assay. However, for extracts containing higher levels of ALA and LA, a positive correlation between the PUFA levels and the potency of the extract for inhibiting PP2A demethylation emerged (Table 1). Of the 13 initial CCSEs investigated, 10 had no effect on PP2A methylation status, exhibiting IC_{50}s greater than the highest tested extract concentration of 50 μg/mL (Table 1). Of the 3 active CCSEs that had both higher ALA/LA levels and potent inhibition of PP2A demethylation, Salba Chia was selected for further fractionation and development over the other active sources because it was the only non-GMO chia seed of the group that could provide the quality and volume required for future commercial development. We tested two different Salba Chia seed batches, and both provided high enough ALA/LA levels to significantly modulate PP2A methylation levels. Altogether, these data suggest that ALA ≥~6 μg/mL and LA ≥~2.25 μg/mL are required in chia seed extracts to effectively inhibit PP2A demethylation (Table 1).

While chia seeds have potential as a source for ALA and LA with the capability to regulate PP2A, these results demonstrate that standard extraction methods do not produce commercially viable yields. Therefore, given our established consistent results with Salba Chia seeds, we sought to develop an extraction process utilizing

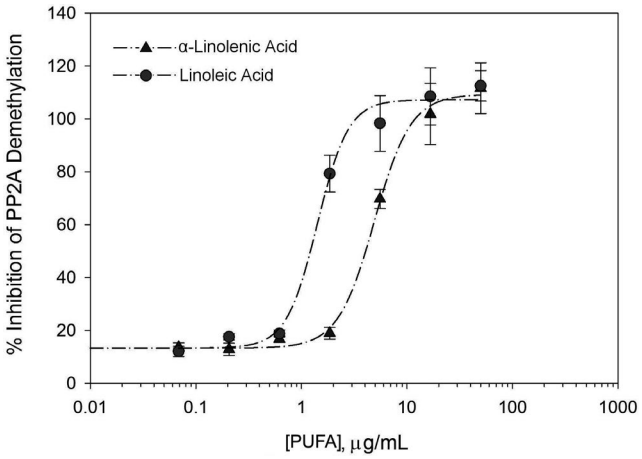

FIGURE 1 Polyunsaturated fatty acids Linoleic (ω6) and α-linolenic (ω3) acids inhibit demethylation of PP2A. The PP2A demethylating enzyme, PME1 and ^{3}H methyl tagged PP2A, was incubated with various concentrations of PUFA. Dose-dependent inhibition was monitored by ^{3}H retained on methylated PP2A. The data represent the mean ± SD

TABLE 1 HPLC analysis for ALA and LA (Vitamin F) levels and PP2A demethylation activity of HYVIA™ and other commercial chia seed extracts

Chia Sample	[ALA] μg/mL	[LA] μg/mL	PP2A demethylation (IC_{50})
BI Nutraceuticals	1.73	LOQ	>50
Pure Lead (Mexico)	2.14	LOQ	>50
Food to Live (Argentina)	1.94	LOQ	>50
Chiaseedsdirect.com	1.94	LOQ	>50
Gigawatt	0.98	LOQ	>50
CPX Chia Seed oil	0.94	LOQ	>50
Tierra Overseas	0.97	LOQ	>50
Heartland Chia	2.54	LOQ	>50
Naturkost de Mexico (aka CCSE #1)	2.12	LOQ	>50
Nutiva	4.56	LOQ	>50
InkaSeed Peru	9.83	4.74	11.8
Namaskar SAC/ Agrillatin Peru	10.12	4.55	5.3
Salba Parent (Batch #1)	6.96	2.37	18.3
Salba Parent (Batch #2)	5.17	2.71	27.3
HYVIA™	31.22	8.86	3.2

Abbreviation: LOQ = Limit of Quantitation < 1μg/mL.

these seeds to obtain an extract with even greater concentrations of ALA and LA. We were able to develop a two-step extraction process via solid-liquid fractionation followed by dry fractionation which resulted in a new chia extract called HYVIA™. HYVIA™ is enriched for ALA and LA, with levels 5 to 6 times higher than the first-step intermediate product of the extraction, named "Salba Parent Extract" (Table 1). Correlating with increased PUFA levels, HYVIA™ also exhibits a PP2A demethylation IC_{50} of 3.2 μg/mL (Figure 2). This is ~6 to 8 times more potent than the Salba Parent Extract, which had an IC_{50} of 18.3 and 27.3 μg/mL for two different batches (Figure 2). Moreover, HYVIA™ is at least ~15-fold more potent in PP2A demethylation than CCSE #1 (selected as a representative CCSE from those tested in Table 1) (Figure 2). Thus, our new extraction method yielding HYVIA™ produces an extract with significantly higher concentrations of PUFA and PP2A-activating activity than other CCSEs.

3.3 | HYVIA™ increases skin hydration marker expression in keratinocytes

Given the previous literature suggesting that chia seed extract may provide skin moisturizing benefits, we sought to determine whether HYVIA™ could increase traditional molecular markers of skin hydration such as aquaporin-3 (AQP3) and hyaluronic acid synthase 2 (HAS2) in vitro. We investigated gene expression activity in primary normal human epidermal keratinocytes (NHEKs) that were treated with HYVIA™ at several different concentrations for 24 hours. After incubation, cells were collected, and gene expression was assessed by quantitative PCR (qPCR). After 24 hours, HYVIA™ at ≥1 μg/mL significantly increased AQP3 gene expression and at ≥10 μg/mL significantly increased HAS2 expression (Figure 3). Conversely, neither CCSE #1 nor the Salba Parent extract had any significant activity on either of these key skin hydration markers.

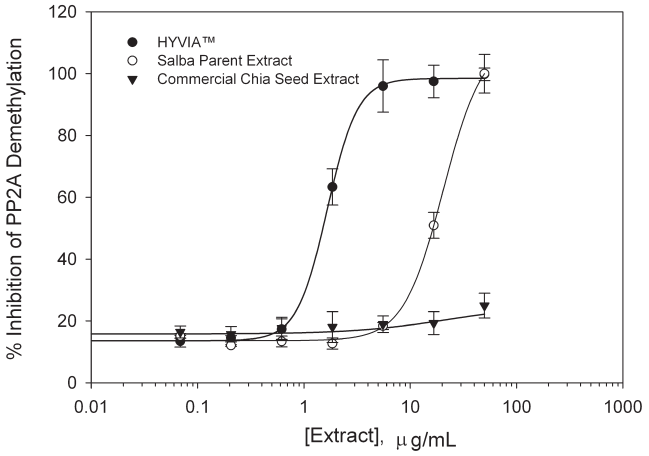

FIGURE 2 HYVIA™, a PUFA-enriched chia extract, inhibits PP2A demethylation greater than commercial chia seed extracts. HYVIA™ inhibits PP2A demethylation significantly better than commercial chia seed extract and the Salba Parent extract. The data represent the mean ± SD

3.4 | HYVIA™ improves skin hydration in human subjects

Since HYVIA™ demonstrated potential skin moisturizing activity in keratinocytes in vitro, we sought to determine whether it could provide similar benefits when applied topically to human skin. We performed a proof of concept moisturization clinical study via a third-party dermatology CRO (Princeton Consumer Research). This single-blind clinical trial was conducted in 16 healthy volunteers to evaluate the potential hydrating properties of HYVIA™. Skin hydration was measured using a Corneometer® and skin barrier function via transepidermal water loss (TEWL) using a Tewameter®. HYVIA™ was formulated at 0.1% in a cream, and single applications of HYVIA™ and vehicle cream were applied to each subject at two distinct sites on the leg, and skin assessments were performed at 0 (baseline), 2, 8, and 24 hours postapplication. Results show HYVIA™-treated sites produced statistically significant higher mean Corneometer® values when compared to vehicle-treated sites (Figure 4A). Specifically, HYVIA™ 2 hours postapplication improved skin hydration by 16% over vehicle-treated sites, and at 24 hours postapplication, HYVIA™ treated skin was 58% more hydrated than vehicle. We did not run a vehicle group for TEWL assessment. However, when compared to untreated skin, HYVIA™ produced statistically significant lower TEWL values, demonstrating an increase in skin barrier function (Figure 4B). Altogether, these results indicate HYVIA™ when topically applied improves skin hydration and barrier function.

4 | DISCUSSION

We demonstrate in this study that HYVIA™, an extract of chia seeds which is highly enriched for Vitamin F, increases hydration in healthy skin when applied topically. Based on the data presented here, this can best be explained by a novel mechanism of PUFA stimulation of PP2A methylation and activation that modulates signaling pathways which regulate the expression of genes involved in hydration. Despite the recent increased use of chia seed extracts and oils in topical products and the anecdotal benefits of chia seeds for skin, to date, there has only been one peer-reviewed publication demonstrating the potential benefits of this native Central American plant when applied topically. Previously, chia seed oil formulated at 4% was shown to be an effective moisturizing agent for pruritic skin; however, the study had several limitations.[2] The clinical study was performed using only 10 subjects (two groups of five healthy volunteers and five disease subjects with end-stage renal disease (ESRD)), and the functional measurements for moisturization: TEWL, skin capacitance, and skin surface pH, showed no statistically significant change from baseline for the healthy volunteers.[2] Moreover, no placebo group was utilized to account for any potential vehicle effects, making it difficult to interpret the benefits of chia in this study. Here, we demonstrate for the first time that a novel chia seed extract enriched for both ALA and LA, called HYVIA™, effectively hydrates the skin when applied topically to healthy subjects at 0.1% significantly

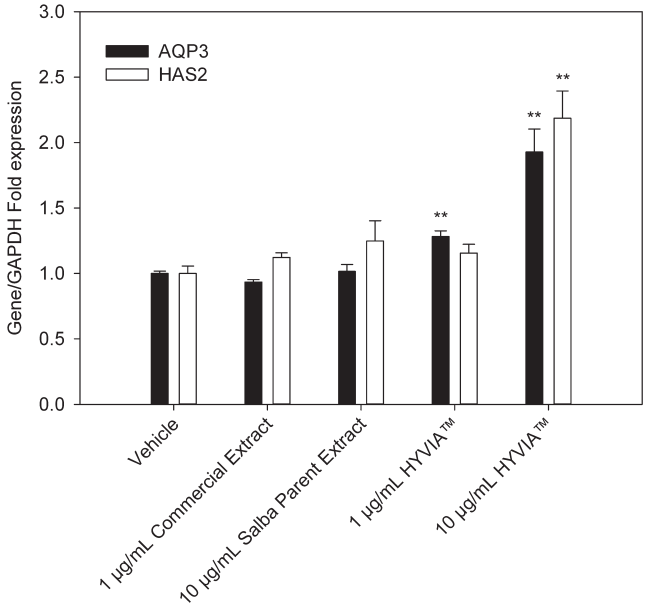

FIGURE 3 HYVIA™ increases skin hydration gene expression markers in a dose-dependent manner. NHEKs were treated with the indicated concentrations for 24 hours and harvested for gene expression analysis. The level of AQP3 and HAS2 gene expression was quantitated by qPCR normalizing to level of GAPDH the control housekeeping gene. The data represent the mean ± SEM of cumulative from three independent experiments. $^{**}P \leq .01$ indicates a statistically significant difference compared to vehicle-treated cells

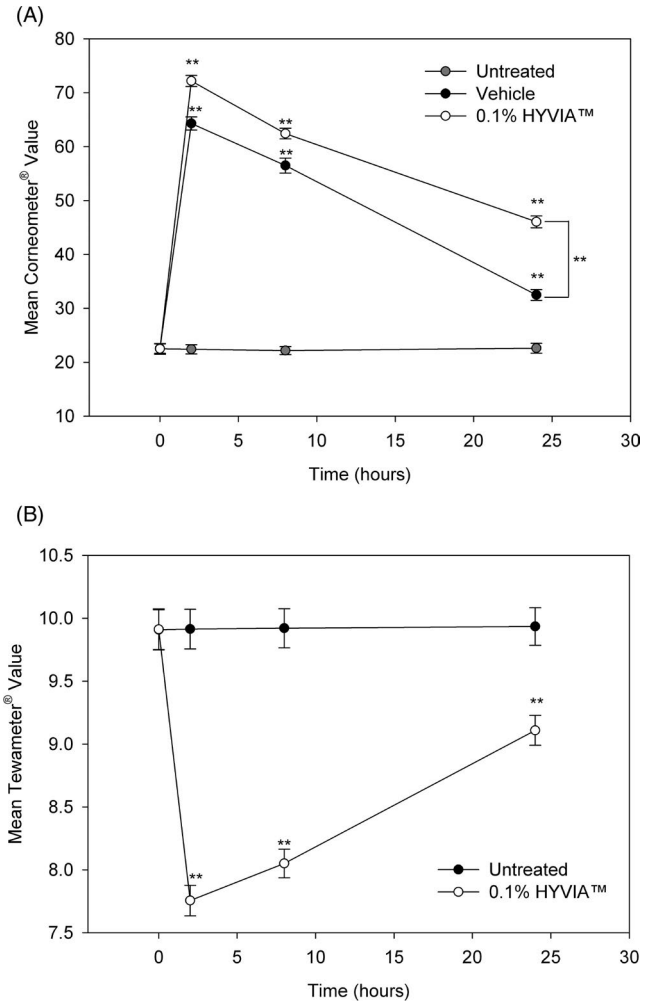

FIGURE 4 Clinical study utilizing HYVIA™ shows improvement in (A) transepidermal water loss over vehicle and (B) hydration level over nontreated skin (note: vehicle was only used for Corneometer readings but not Tewameter measurements). The data represent mean ± SEM. $^{**}P \leq .01$ indicates a statistically significant difference compared to baseline (time = 0 h) reading

better than vehicle-treated skin (Figure 4A). Given the important role ω3 and ω6 PUFAs like LA and ALA play in barrier function and skin health, the data presented confirm topical application of oil/extract as an effective means of delivering PUFAs to the skin. Similar to chia seed oil, sunflower seed oil is also rich in LA. When applied to patients with essential fatty acid deficiency (EFAD), sunflower seed oil increased LA content of the epidermis, lowered the rate of TEWL, and reduced skin scaliness after two weeks of daily application. However, when applied to healthy volunteers, sunflower seed oil showed no change.[17] Topically applied olive oil, rich in monounsaturated fatty acid (MUFA) oleic acid, also did not have any effect on EFAD patients.[17] Conversely, HYVIA™ applied to healthy skin effectively and immediately decreased TEWL (Figure 4B). This suggests that oils and/or extracts richer in PUFAs LA and ALA, like this chia seed derived extract, may be more effective in promoting skin hydration and barrier function over similar PUFA-rich and MUFA-rich extracts.

ALA and LA when topically applied in a cream to hairless mice lower erythema compared to vehicle-treated mice.[18] Moreover, docosahexaenoic acid (DHA), a longer chain derivative of ω3 ALA, when applied to mice skin before irradiation induces the expression of heme oxygenase-1 (HO-1) and protects against UVB-induced inflammation.[19] In another study, DHA when topically administered inhibits COX-2 and NOX-4,[20] both important factors in inflammatory and oxidative stress signaling. Thus, ALA and other ω3 PUFAs function to modulate the inflammatory response in skin, while LA and the other ω6 PUFAs help maintain barrier function. Therefore, combining PUFAs may be advantageous for promoting skin health. Here, we establish for the first time a new and potentially important target for ω3 and ω6 PUFAs in the skin that suggests that they are doing more than acting as precursors for more complex skin lipids. Both ALA and LA effectively inhibit PP2A demethylation, maintaining it in its more active state (Figure 1). Given the important role PP2A methylation plays in oxidative stress signaling[21] and skin barrier integrity,[10] ALA and LA's effects on skin may be due in part to their modulation of the methylation state of this key master regulator protein. Moreover, ALA and LA have no effect on PP2A active site turnover, while other FAs such as stearic, palmitic, myristic, and behenic acid inhibit PP2A phosphatase activity.[22] This suggests these FAs have some specificity for influencing PP2A phosphatase activity directly or indirectly by regulating its methylation status. Additionally, in dermal fibroblasts, it has been shown that oxidative stress and inflammation generates reactive oxygen species that induce demethylation of PP2A,

disassembly of active PP2A heterotrimer, and therefore increases stress response signaling.[14] Furthermore, LA is a key building block of skin-specific ceramides, which are a group of lipids that help form the skin's barrier and retain moisture. It has been previously shown that ceramides bind and activate PP2A.[23] Thus, LA may promote PP2A activity in two distinct manners: (a) directly by inhibiting PP2A demethylation and (b) indirectly by yielding ceramides that bind and activate PP2A. Based on the research presented here, we propose ALA, LA, and extracts enriched for these compounds, like HYVIA™, that inhibit the PP2A demethylating enzyme and boost PP2A methylation will be effective in keeping PP2A in its optimal, more active trimeric state that acts to turn off stress responses. Given the emerging evidence of the important role PP2A plays in skin function, the effect of ceramides on PP2A activity certainly merits additional attention.

As we show in Figures 2 and 3, the mere presence of chia seed extract is not enough to modulate PP2A activity or confer hydrating properties, as most commercial chia seed extracts that were screened did not have activity. Moreover, two of the three CCSEs (Inkaseed Peru and Namaskar SAC/Agrillatin Peru) that did have initial potency were determined to be less viable sources than Salba Chia for development into a commercial product. Factors contributing to this determination included that Salba Chia is not GMO and the fact that this source does not entail supplier pooling of seeds from different strains and growth regions, leading to their inconsistent ALA and LA levels and other types of lot-to-lot variability. PUFA levels, such as ALA and LA, can be altered in chia seeds based on variables in their cultivation and conditions such as temperature, elevation, and maturity at harvest. For instance, different microwave settings and/or roasting vs nonroasted of chia significantly influences PUFA levels.[24,25] Controlling for all these conditions is difficult when trying to develop a commercial product that requires batch to batch reproducibility, but we identified a consistent, scalable source with Salba Chia seeds. Utilizing Salba Chia as starting material, we were able to develop a reproducible two-step extraction product (HYVIA™) that is significantly better than other CCSEs and the Salba Parent (one-step) extract in modulating PP2A activity (Figure 2, Table 1) and upregulating key hydration markers (Figure 3). While the primary goal of our extraction process was to enrich for ALA and LA levels, it is likely that there are other PP2A-activating FAs in HYVIA™, as well as other compounds that could affect PP2A methylation. Our next goal is to characterize the composition of HYVIA™ and compare it to commercial chia seed extracts as this may provide further insights for its enhanced hydration properties.

5 | CONCLUSION

We demonstrate HYVIA™, a Vitamin F enriched chia seed extract that possesses higher PP2A demethylation inhibition activity compared to commercial chia seed extracts. This activity is positively correlated with PUFA levels. In addition, HYVIA™ increases AQP3 and HAS2 gene expression in primary keratinocytes to enhance skin hydration and elevate skin barrier function. Lastly, in a human clinical study, topical application of 0.1% HYVIA™ provides several benefits to skin including increased moisturization, barrier function, and elasticity.

CONFLICT OF INTEREST

All authors for this manuscript are paid employees for Signum Biosciences which is where the research was performed. All authors contributed to drafting the manuscript or revising the manuscript and all gave final approval before submission. Lastly, all authors agree that they are accountable for all aspects of the work presented in this paper.

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