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Anti-Inflammatory Activities of Colloidal Oatmeal (*Avena sativa*) Contribute to the Effectiveness of Oats in Treatment of Itch Associated With Dry, Irritated Skin

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ABSTRACT

Background: Oat (*Avena sativa*) in colloidal form is a centuries-old topical treatment for a variety of skin conditions, including skin rashes, erythema, burns, itch, and eczema; however, few studies have investigated the exact mechanism of action for the anti-inflammatory activity of colloidal oatmeal.
Methods: Four extracts of colloidal oatmeal were made with various solvents and tested in anti-inflammatory and antioxidant assays. In addition, an investigator blind study was performed with twenty-nine healthy female subjects who exhibited bilateral mild to moderate itch with moderate to severe dry skin on their lower legs. Subjects were treated with a colloidal oatmeal skin protectant lotion.
Results: Extracts of colloidal oatmeal diminished pro-inflammatory cytokines in vitro and the colloidal oat skin protectant lotion showed significant clinical improvements in skin dryness, scaling, roughness, and itch intensity.
Conclusions: These results demonstrate that colloidal oat extracts exhibit direct anti-oxidant and anti-inflammatory activities, which may provide the mechanisms for observed dermatological benefits while using the colloidal oatmeal skin protectant lotion.

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INTRODUCTION

Oats (*Avena sativa*) have been cultivated since the Bronze Age, and have been used in traditional medicine for centuries. As a topical treatment, colloidal oatmeal has emollient and anti-inflammatory properties, and is commonly used for skin rashes, erythema, burns, itch, and eczema.[1-3] Historically, investigations into the phytochemical constituents of oat have focused primarily on their value as a food. For example, β-glucan is the "soluble fiber" that makes oats a heart-healthy food. β-glucans have also been used as scaffolds for the growth of bioartificial skin, and are known to assist in wound healing, response to injury and infection, and have a great water retention capacity.[4]

Colloidal oatmeal is the finely ground whole oat kernel or groat, and is an active natural ingredient covered by the FDA OTC Skin Protectant monograph in the US. The oat grain is ground and processed until no more than 3% of the total particles exceed 150 µm and no more than 20% exceeds 75 µm.[5] The composition of colloidal oatmeal largely consists of starch (65-85%), protein (15-20%), lipids (3-11%), fiber (5%) and β-glucans (5%).[3,6]

Oat lipids are primarily composed of triglycerides, along with polar lipids and unsaturated free fatty acids. Oat triglycerides are rich in omega-3 linoleic and omega-6 linolenic acids and essential fatty acids[7] which are necessary for normal mammalian health and important for skin barrier function.[8-10] In addition, oat lipids contain important mammalian cell membrane components, such as phospholipids, glycolipids, and sterols. Lipid oxidation protection is supplied by mixed tocopherols (vitamin E) and tocotrienols.

Colloidal oatmeal is also a rich source of phenolic antioxidants and saponins. Avenanthramides, nitrogen-containing phenolic compounds specific to oats, are potent antioxidants and anti-inflammatory agents that have been previously shown to inhibit NF-κB and IL-8 release in a dose dependent manner.[3,11-13] Saponins are glycosylated metabolites which help to protect oat plants from disease,[14] and which can also help create stable emulsions when colloidal oatmeal is used in a formulation.

Despite a rich history of traditional use, there remain some gaps in the understanding of the exact mechanisms that give colloidal oatmeal its clinical benefits; we conducted a series of *in vitro* experiments and a clinical study to help identify the mechanism of action for the clinical benefit of colloidal oatmeal. We made four extracts of colloidal oatmeal with organic and aqueous solvents to concentrate constituents based on compound polarity, and subjected them to antioxidant and anti-inflammatory assays. In addition, an investigator-blind clinical study was conducted to evaluate the efficacy of a colloidal oatmeal skin protectant lotion in alleviating extra dry, itchy skin.

Healthy female subjects with bilateral itch and moderate to severe dry skin on their lower legs were enrolled in a 2-week study. Subjects applied a set amount of the lotion to the lower leg

area twice a day. Benefits of the lotion were assessed by clinical evaluations, instrumental measures and self-assessments. Itch intensity was monitored daily through patient diaries.

This clinical study showed that the colloidal oatmeal skin protectant lotion was effective at relieving itch and alleviating moderate to severe dry skin while improving stratum corneum function in patients with itchy, extra dry skin. In addition, extracts of colloidal oat demonstrate anti-inflammatory and antioxidant activity, which may account for some of the observed clinical benefits.

MATERIALS AND METHODS

Primary human keratinocytes were obtained from Lonza Walkersville, Inc. (Walkersville, MD) and Promocell (Heidelberg, Germany). Human recombinant TNF-α was procured from Peprotech Inc. (Rocky Hill, NJ).

Preparation of Extracts of Colloidal Oatmeal

Four extracts of colloidal oatmeal were prepared using HPLC-grade hexanes, aqueous acetone, aqueous methanol, and water to generate extracts enriched in phytochemicals based on polarity. Approximately 10 g of colloidal oat was suspended in 100 mL of solvent and placed on a shaker to maintain the slurry in constant motion. After 24 hours, the suspensions were centrifuged, filtered, and concentrated on a rotary evaporator under reduced pressure at a temperature not exceeding 40 °C. The extracts were then transferred to a tarred container, dried under nitrogen, and lyophilized to remove all traces of solvent. For cell culture experiments, stock solutions of each extract were dissolved in DMSO (50 mg/mL) and diluted into media so that DMSO concentrations less than 0.01%.

TNF-α induced IL-8 Release

Primary normal human keratinocytes were treated with test samples in DMSO for 2 hours before exposure to solar ultraviolet light (1000W-Oriel solar simulator equipped with a 1-mm Schott WG 320 filter; UV dose applied: 70 kJ/m^2 as measured at 360 nm). After 24 hours, supernatants were analyzed for IL-8 cytokine release using commercially available kits (Millipore Corp., Billerica, MA). Statistical significance ($P< 0.05$) was determined by 2-tailed t-test.

NF-κB Luciferase Promoter Assay in Keratinocytes

Primary normal human keratinocytes were transiently transfected with 0.25 ug/well total DNA containing pNF-κB-Luc reporter plasmid (Stratagene, La Jolla, CA) and the internal control Renilla luciferase reporter (pRL-TK; Promega Corporation, Madison, WI) using Lipofectamine 2000 transfection reagent (Invitrogen Corporation, Carlsbad, CA). At 24 hours post transfection, cells were pre-treated with extracts for 2 hours, followed by treatment with 100 ng/ml TNF-α (Peprotech Inc., Rocky Hill, NJ) for 24 hours. Statistical significance ($P< 0.05$) was determined by 2-tailed t-test.

UV-Induced Reactive Oxygen Species Production in Keratinocytes

Primary normal human keratinocytes were incubated for 30 min with 5 μM of the hydrogen peroxide-sensitive fluorescent probe 5-(and-6)-chloromethyl-2′,7′-dichlorodihydro-fluorescein diacetate, acetyl ester (CM-H2DCFDA; Invitrogen Carlsbad, CA). After incubation, cells were washed 2X with PBS to remove excess probe and treated with extracts at indicated concentrations; the plate was then exposed to UV (1000W-Oriel solar simulator equipped with a 1 mm Schott WG 320 filter; UV dose applied 4.2 kJ/m^2 as measured at 360 nm). The plate was read immediately post-UV exposure on a fluorescent plate reader set at wavelengths 485 nm excitation/530 nm emission to detect basal peroxide formation. Statistical significance ($P< 0.05$) was determined by 2-tailed t-test.

Gene Expression

Primary human keratinocytes were treated for 24 hours in the presences of 1.2 mM $CaCl_2$. RNA was extracted using Qiagen RNeasy kit with DNase I digestion (Valencia, CA). Reverse transcription was performed using High Capacity cDNA kit (Life Technologies). 40 to 60 ng of cDNA samples were used in a QPCR reaction to measure IL-8. Taqman gene expression assay was purchased from Life Technologies (Grand Island, NY). QPCR reaction were performed using ABI 7500 fast amplifier. All gene expression data were normalized by reference genes, polymerase (RNA) II polypeptide A (POLR2A). Statistical significance ($P< 0.05$) was determined by one-way ANOVA.

"Unlike other lipid-rich plants, whole oat extracts contain components that not only protect oat lipids from spoilage, they also form a synergistic mixture that enhances oat oil's capacity to protect and nurture healthy skin."

Clinical Study Design

The study was an investigator-blind design conducted in Colorado, USA. Subjects applied a colloidal oatmeal skin protectant lotion (Skin Relief 24Hr Moisturizing Lotion, Aveeno®, Skillman, NJ) to the lower leg area twice a day for the two-week study period. Benefits of the colloidal oatmeal skin protectant lotion were established by clinical evaluations, diaries, self-assessments, TEWL, corneometer, and imaging at day 0, followed by determinations at days 1, 7, and 14. Written informed consent was obtained from each subject prior to enrollment in the study. The protocol and informed consent agreement for this study were reviewed and approved by an Institutional Review Board (IRB).

TABLE 1.

Summary of Colloidal Oat Extracts

Extraction solvent	Abbreviation	Expected Phytochemistry	IL-8 Production	NF-κB Promoter	ROS Production	IL-8 Transcription
Hexanes	HCO	Oils and lipophilic compounds	Decrease	n/a	n/a	Decrease
80% Acetone	ACO	Mid-polar phenolics	n/a	Decrease	Decrease	Decrease
80% MeOH	MCO	Phenolics	Decrease	n/a	n/a	Decrease
Water	WCO	Proteins and carbohydrates	Decrease	n/a	n/a	n/a

Population

Twenty-nine healthy female subjects between 18 and 60 years of age completed the study. Mean age was 44.5. All subjects exhibited bilateral mild to moderate itch with moderate to severe dry skin on their lower legs. Exclusion criteria included those with acute inflammatory lesions on the lower leg area, individuals on medications that could interfere with the outcome of the study and those with known allergies/sensitivities to the test product.

Clinical Evaluations

Clinic evaluations were conducted at baseline, day 1, day 7, and day 14. Participants also conducted self-evaluation questionnaires.

Clinical Grading

Subjects were clinically graded on the right or left lower legs for the following objective irritation parameters: cracking, scaling, dryness, erythema, and roughness. Subjects assessed the following subjective irritation parameters on the lower leg area: burning/stinging, tightness, and itching.

Corneometer Measurements

Triplicate Corneometer CM 825 (Courage + Khazaka Electric GmbH) measurements were obtained on the lower leg (midway between the major joints) to quantify moisture content in the stratum corneum.

TEWL Measurements

Trans-epidermal water loss (TEWL) measurements were taken on the lower leg area using the Dermalab (Cortex Technologies) in conjunction with a computer.

Hi-Scope Images

A high-definition microscope [Hi-Scope KH-2400R videomicroscope (100x)] was used to capture images of the skin at designated areas on the right and left lower lateral leg.

Biostatistics

Mean values for objective/subjective irritation parameters, instrumental measurements (Corneometer and TEWL), and self-assessment questionnaires at day 1, day 7, and day 14 were statistically compared to mean baseline values using a paired t-test at the $P \leq 0.05$ significance level. Average percent change from baseline and incidence of positive responders were calculated for each time point.

RESULTS

Preparations of Extracts

The hexane extract of colloidal oatmeal (HCO) generated an oily residue (3.8% yield); the aqueous acetone extract of colloidal oatmeal (ACO) generated a sticky amorphous powder (2.6% yield); the aqueous methanol extract of colloidal oatmeal (MCO) generated a dry amorphous powder (2.5% yield); the water extract of colloidal oatmeal (WCO) generated a white powder (0.7% yield). An approximate qualitative composition of each extract based on the nature of extraction processes is presented in Table 1 along with a summary of bioactivities.

Gene Expression

The effect of the colloidal oat extracts on the basal (unstimulated) gene expression of IL-8 by human keratinocytes were investigated. MCO, ACO, and HCO oat extracts (50 µg/mL) significantly decrease the mRNA transcript levels of IL-8 ($P < 0.001$, $P < 0.001$, and $P < 0.01$, respectively) in unstimulated keratinocytes (Figure 1A).

TNF-α induced IL-8 release

Treatment with TNF-α induced a significant increase in interleukin-8 (IL-8) production by human keratinocytes. Pre-treatment with MCO or WCO oat extracts significantly inhibited the expression of IL-8 ($P = 0.004$ and $P = 0.0002$, respectively) (Figure 1B).

NF-κB Luciferase Promoter Assay in Keratinocytes

Treatment with TNF-α induced a significant increase NF-κB promoter activity in primary human keratinocytes. Pre-treatment with ACO oat extract resulted in a significant inhibition of NF-κB promoter levels over the TNF-α treated controls at 25 and 50 µg/mL doses ($P < 0.05$) (Figure 2A).

UV-induced ROS Assay

Exposing human keratinocytes to UV light resulted in a significant increase in reactive oxygen species (ROS) generation by the cells. There was a dose-dependent decrease in UV-induced

FIGURE 1. (A) At 50 µg/mL, HCO, MCO, and ACO decreased the mRNA transcript levels of interleukin-8 (IL-8) in primary human keratinocytes. **(B)** In primary human keratinocytes induced with TNF-α, MCO and WCO led to inhibition of the production of the inflammatory IL-8. (** P<0.01; *** P<0.001)

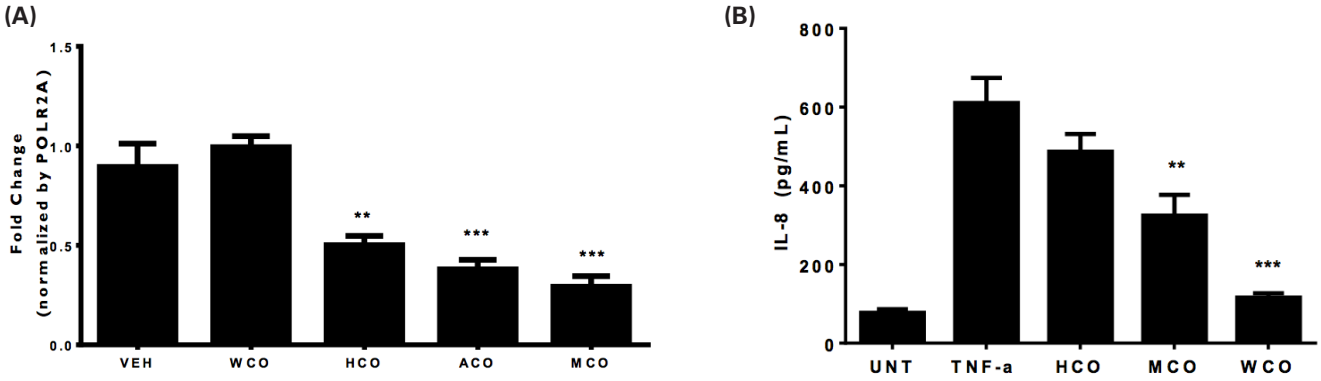
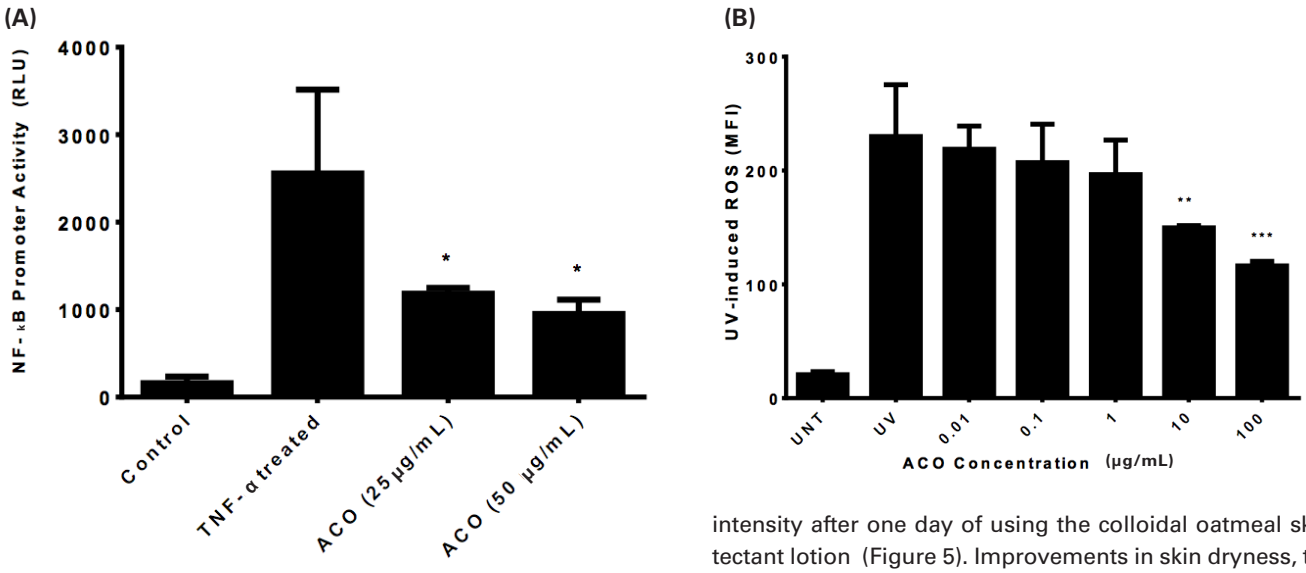


FIGURE 2. (A) At 25 and 50 µg/mL, ACO led to inhibition of TNF-α induced NF-κB promoter activity in primary human keratinocytes. Data expressed as Relative Luminecence Units (RLU). **(B)** ACO led to a dose-dependent inhibition of UV-induced reactive oxygen species (ROS) in primary human keratinocytes. Data expressed as Mean Fluorescence Intensity (MFI). (* P< 0.05; ** P< 0.01; *** P< 0.001).



ROS generation in cells treated with ACO oat extract (Figure 2B). At 10 and 100 µg/mL concentrations, there was a significant decrease (P = 0.0071 and P = 0.0004, respectively).

Clinical Results

Clinical evaluations of the subjects' lower legs showed significant improvements (P< 0.05) in skin dryness, scaling, roughness, itching and cracking after one day of use of the oat-containing skin lotion when compared to baseline mean values (Figure 3). This improvement increased over time (7 and 14 days). After 14 days, all subjects showed improvements in cracking, scaling, and skin dryness. In addition, TEWL and corneometer measurements improved significantly (P< 0.05) at 7 and 14 days (Figure 4).

Study participants also completed surveys and assessments. Subjects perceived a significant (P< 0.05) mean reduction in itch intensity after one day of using the colloidal oatmeal skin protectant lotion (Figure 5). Improvements in skin dryness, textural parameters, and itch were maintained throughout the remainder of the study. Subjects also noticed significant improvements (P< 0.05) in roughness, dryness, itch and scaling of their skin as early as the day 1 time point. And finally, high-resolution digital imaging showed dramatic visible improvements in skin textural properties including dryness and flaking after two weeks of using the colloidal oatmeal skin protectant lotion (Figure 6).

DISCUSSION

Oats have been extensively used for dermatological treatment of inflammatory skin conditions, yet few studies have delineated the anti-inflammatory mechanism of action of colloidal oatmeal. In the current study we report that extracts of colloidal oatmeal show anti-inflammatory and antioxidant activity, as demonstrated by a decrease in NF-κB promoter activity, decreased ROS generation, and reduced IL-8 production.

FIGURE 3. At all evaluation timepoints, subjects using the colloidal oatmeal skin protectant lotion showed significant improvements in cracking, scaling, dryness, and roughness. *P< 0.05

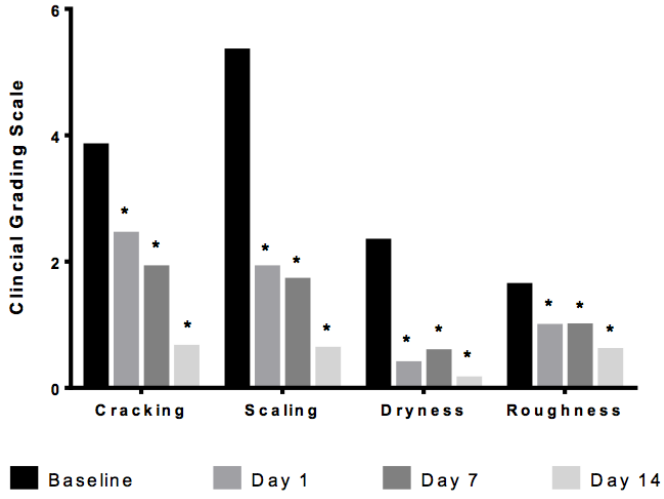
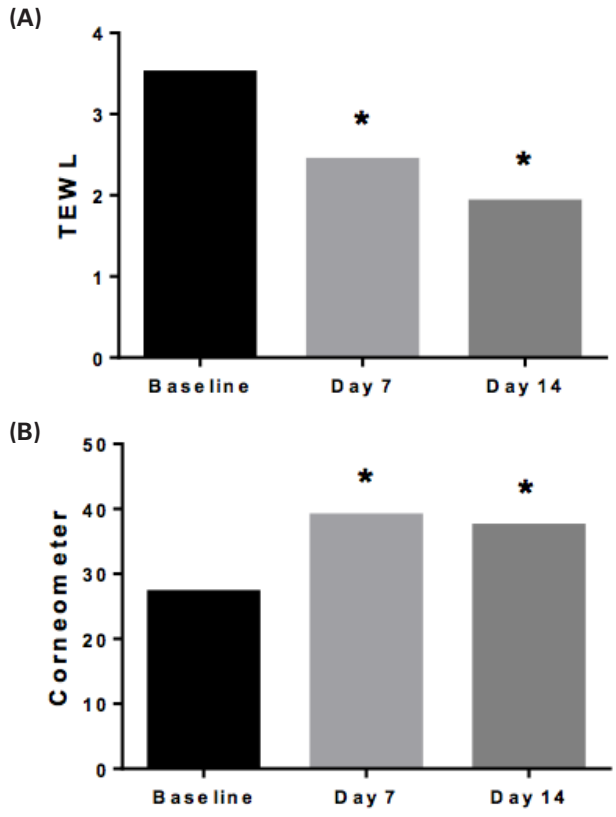


FIGURE 4. (A) Transepidermal water loss (TEWL) and **(B)** corneometer measurements improved significantly (P< 0.05) over the course of the 14 days study using the colloidal oat containing lotion.



NF-κB is a key nuclear receptor driving the expression of many pro-inflammatory and oxidative pathways during inflammation. Overexpression of the inflammatory cytokine IL-8 has been linked to itch and pruritic skin disease.[15] Itch

FIGURE 5. Significant improvements with itchy skin were observed over the course of the 14-day treatment (P< 0.05).

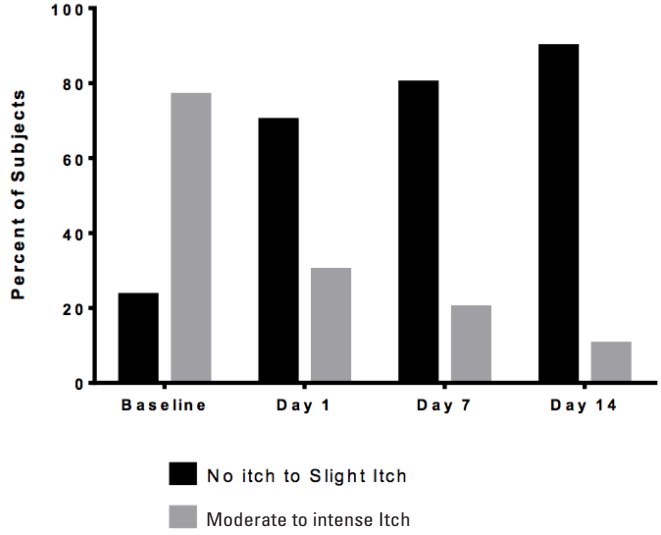
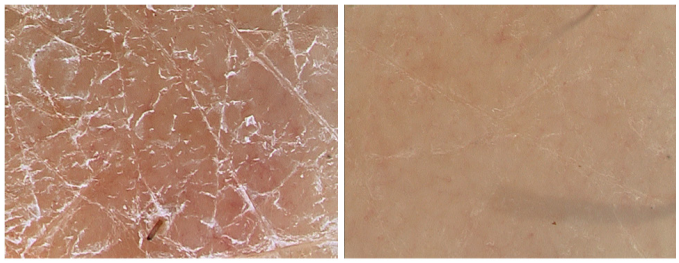


FIGURE 6. High resolution digital images before and after use of the colloidal oat skin protectant lotion.



(pruritus) leads to scratching, which in turn causes damage to skin barrier and integrity; this damage creates a feedback loop and an increased release of pro-inflammatory mediators that will, in turn, increase the feeling of itch. Compositions with colloidal oatmeal reduce skin inflammation and therefore may help stop the itch-scratch cycle.

Our studies indicate that fractions rich in different oat phytochemicals can also mediate biochemical pathways important in assuaging clinical challenges. Oat has the highest oil content (upwards of 10%) of any grain, and is rich in antioxidants and other phytochemicals.[7] Unlike other lipid-rich plants, whole oat extracts contain components that not only protect oat lipids from spoilage, they also form a synergistic mixture that enhances oat oil's capacity to protect and nurture healthy skin.

HCO represents the oil fraction of colloidal oatmeal, and demonstrated both a decrease in IL-8 transcription and cytokine production. Phenolics (flavonoids, avenanthramides) and alcohol-soluble albumin proteins will be found in MCO and ACO, which demonstrated decreases in ROS production, IL-8

transcription and production, and NF-κB promoter activity. In addition, WCO, rich in water-soluble oat proteins (globulins and prolamines) and carbohydrates, demonstrated the strongest decrease in IL-8 production of any extract ($P = 0.0002$). To our knowledge this is the first study to report that oat proteins may directly contribute to the anti-inflammatory skin benefits of oatmeal. Although these fractions were studied independently in the current study, all of these extracts are contained in whole colloidal oatmeal. It is expected that when formulated into colloidal oatmeal-containing lotions, the proteins, phenolic antioxidants, and lipids would contribute to the overall clinical efficacy of colloidal oatmeal.

The reduction of inflammatory mediators in skin may assist in the relief of a variety of skin conditions that result in dryness, eczema, irritation, and inflammation. Our clinical study demonstrated that a colloidal oatmeal skin protectant lotion was able to significantly alleviate the itch ($P < 0.05$) and improve the condition of moderate to severely dry skin, and was well tolerated in subjects with compromised itchy, dry skin. Clinical evaluations showed that the colloidal oatmeal skin protectant lotion significantly improved dryness, scaling and roughness as early as 1 day after use, and these improvements were maintained over the duration of the study with continued use of the lotion. Subjects perceived a significant mean reduction in itch intensity after only 1 day of use of the colloidal oatmeal skin protectant lotion, and also reported that their itch significantly improved shortly after each application of the lotion as demonstrated by comparison between pre-application and post-application scores on days 0 through 3. Finally, transepidermal water loss rates at days 7 and 14 showed a significant improvement ($P < 0.05$) in skin barrier function when compared to baseline values. Taken together, these *in vitro* results demonstrate that colloidal oatmeal can reduce inflammatory mediators associated with skin inflammation; the inhibition of inflammatory mediator production may in turn help enhance the skin benefits of colloidal oatmeal for dry, irritated, and eczematous skin.

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DISCLOSURES

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