Mitochondrial Revitalization for Skin Rejuvenation by a Proprietary, Cold-Pressed *Nigella sativa* Seed (Black Cumin) Oil Standardized to 3% Thymoquinone

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ABSTRACT

Medicinal herbs have been used as natural healing and cosmetic remedies since ancient times. The annual flowering plant, *Nigella sativa*, is native to southeastern Europe, western Asia, the Middle East, and northern Africa. It is also cultivated in various parts of the world for nutritional and cosmetic purposes, traditional medicine use, and as a source of its unique constituents. Oil from the small black seeds of *N. sativa* has been used for medicinal and beauty treatments since the days of the ancient Egyptian pharaohs and Cleopatra. More recently it has been studied for its many health and cosmetic, anti-inflammatory, antioxidant, and anti-aging benefits. To harness the incredible power of the *N. sativa* seed oil, its active constituent, thymoquinone, and their demonstrated benefits, a patent-pending cold-pressed extract high in thymoquinone has been developed to deliver full-spectrum black seed oil, standardized to 3% thymoquinone and very low free fatty acids.

Branded as B'utyQuin™ for cosmetic use, this cold-pressed black seed oil has been studied in vitro to determine mechanisms related to mitochondrial biogenesis and revitalization. This has been followed by clinical research to establish the safety, compatibility, and efficacy of B'utyQuin as a topical anti-aging cosmetic aid for human skin. B'utyQuin's ability to positively impact several characteristics of aging human skin has been revealed. This placebo-controlled clinical trial demonstrates topical application to a variety of healthy skin types over 28 days yields statistically significant improvements in skin hydration, luminosity, firmness, and elasticity, when compared to a placebo cream, resulting in a more flawless appearance.

KEYWORDS

Nigella sativa, black seed oil, thymoquinone, anti-aging, mitochondrial biogenesis, mitochondrial health, skin rejuvenation, B'utyQuin

INTRODUCTION

Medicinal herbs have been used as natural healing and cosmetic remedies since ancient times. The annual flowering plant, *Nigella sativa* (*N. sativa*, family Ranunculaceae), is native to southeastern Europe, western Asia, the Middle East, and northern Africa. It is also cultivated in various parts of the world for nutritional and cosmetic purposes, traditional medicine, as well as a source of its unique constituents.[1,2] Oil from the small black seeds of *N. sativa*, also known as black seed oil, has been used for medicinal and beauty treatments since the days of the Egyptian pharaohs and Cleopatra and is featured in the Bible and Koran as the "curative black cumin." More recently, black seed oil has been studied for its many health and cosmetic, anti-inflammatory, antioxidant, and anti-aging benefits.[3-7] Determination of the major chemical components of *N. sativa* seed,[8] has resulted in a growing interest in *N. sativa* as a therapeutic agent for a variety of conditions.

Nigella sativa seeds contain two distinguishable oil fractions: fixed oil and essential oil, with the essential oil containing one of the most active constituents, a monoterpene aromatic, known as thymoquinone (TQ).[9,10] Many of the important pharmacological activities of *Nigella sativa* are attributable to thymoquinone, including its powerful antioxidant and anti-inflammatory properties.[11-16]

To harness the incredible power of the active constituent thymoquinone and its demonstrated benefits, TriNutra™, Ltd has cultivated a high in TQ *N. sativa* strain to deliver full-spectrum, cold-pressed black seed oil standardized to 3% thymoquinone and very low free fatty acids, the highest quality and most potent thymoquinone concentration in cold-pressed oil available on the market. TriNutra's farm-to-finished black seed oil features full sustainability and traceability by ensuring that farm history, cultivation of varietals, growing and harvesting conditions, seed storage, extraction, and processing techniques are completely controlled in one location to guarantee a superior quality material. TriNutra's cold-pressed black seed oil is branded as B'utyQuin™ for cosmetic use and ThymoQuin® for nutraceuticals.

B'utyQuin's black seed oil (BSO) is formulated to contain a proprietary, patent-pending composition of constituents with superior potency that act synergistically to maximize the anti-inflammatory, antioxidant, and antimicrobial effects of the oil.

BSO PROPRIETARY COMPOSITION KEY CONSTITUENTS

Fatty Acids Profile

- Palmitic Acid – 11-13%
- Oleic Acid – 19 - 25%
- Linoleic Acid – 53 - 63%
- Very Low Free Fatty Acids – <2%

Aromatics Profile

- Standardized Thymoquinone (min.) – 3%
- P-Cymene – >1%
- Carvacrol – <0.1%

To understand and elucidate the mechanisms of action of B'utyQuin on and in the skin, both in vitro studies and clinical trials have been conducted to assess this BSO's role in mitochondrial revitalization and rejuvenation, boosting ATP production, reducing oxidative stress and inflammation, all having protective benefits for aging skin. The clinical trial assessed the efficacy as well as safety and compatibility of a topical cosmetic product containing 3% B'utyQuin in an Anti-Aging Facial Cream (Active Cream), after application under normal conditions of use.

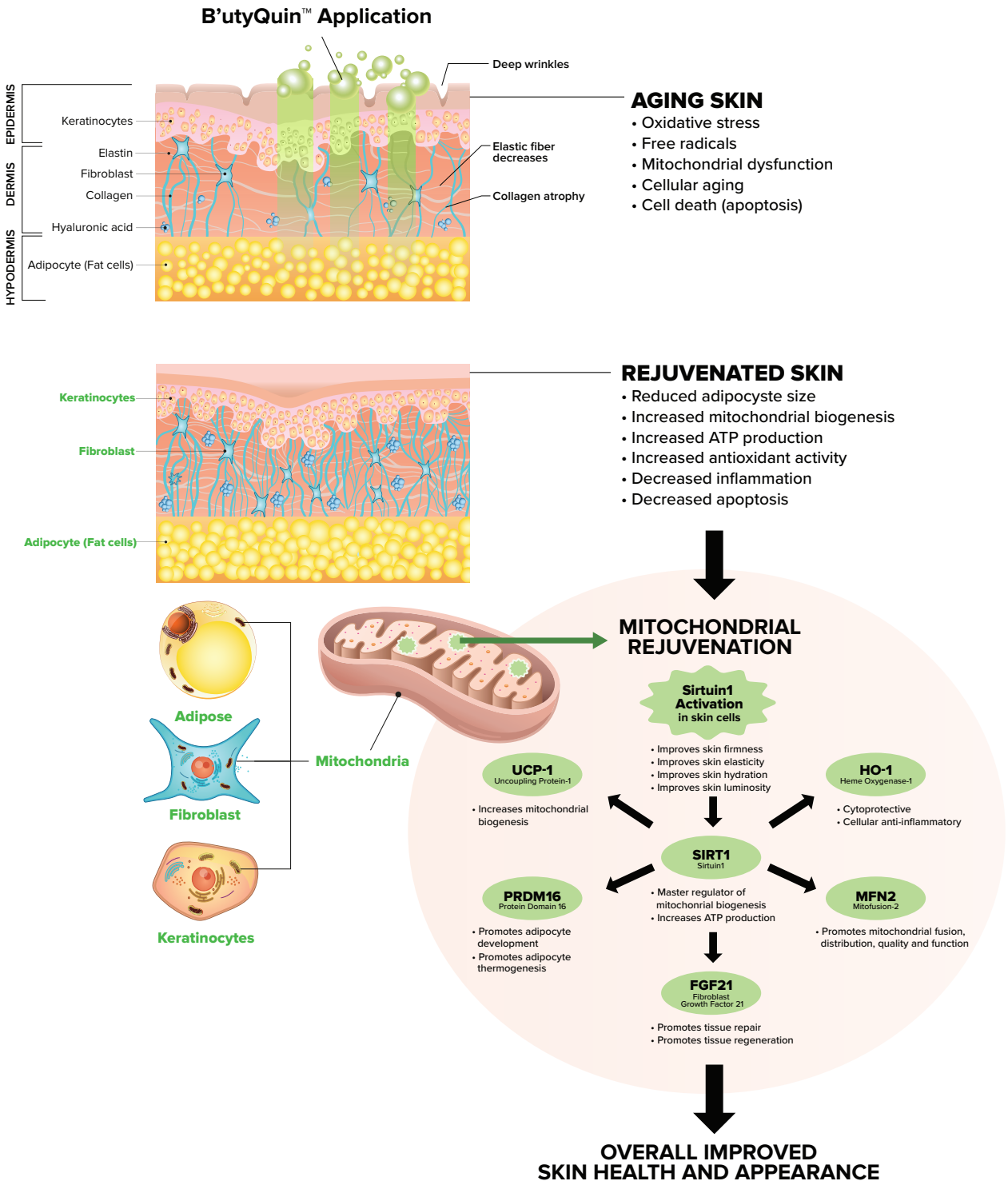
STUDY DESIGNS

In Vitro Research

In vitro studies were conducted to determine the mechanisms of action behind BSO's remarkable benefits for aging skin (Figure 1).

FIGURE 1

Mechanism of Action Elucidation Behind B'utyQuin - Black Seed Oil with 3% Thymoquinone.



A variety of human cell lines were used to evaluate B'utyQuin's in vitro effects on markers of mitochondrial activity including ATP production (Figure 2), antioxidant activity, inflammation, and mitochondrial biogenesis (Figure 3). In vitro research utilizing a human adipocyte cell line (3T3-L1) also evaluated BSO's effect on size and composition changes (Figure 4).

Results demonstrated that BSO boosted mitochondrial function and ATP production, significantly inhibited nitric oxide production, and inhibited inflammation. It also activates Sirtuin1, a "master regulator" of mitochondrial biogenesis involved in the cellular response to inflammatory, metabolic, and oxidative stressors. Research elucidated BSO's beneficial and powerful effect on

FIGURE 2

The effect of BSO with 3% thymoquinone (BQ) on production of ATP in Keratinocytes (HaCaT cells) compare to baseline (Naïve cells) and positive control (N-Acetylcysteine (NAC) 1mM).

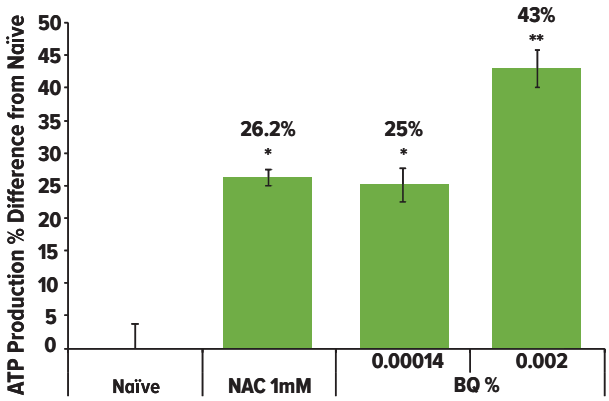


FIGURE 3

Effects of BSO with 3% thymoquinone (TQ) treatment on the protein expression of beige adipocytes and key regulators of mitochondrial biogenesis in 3T3-L1 adipocytes. Representative and graph of uncoupling protein 1 (UCP-1), PR domain containing 16 (PRDM16), and fibroblast growth factor 21 (FGF21) (A–D); Sirtuin 1 (Sirt1) Mitofusion 2 (Mfn2), and heme oxygenase-1 (HO-1) protein expression (E–H). 3T3-L1 adipocytes were treated with TQ 2 µM, for six days (n=4).

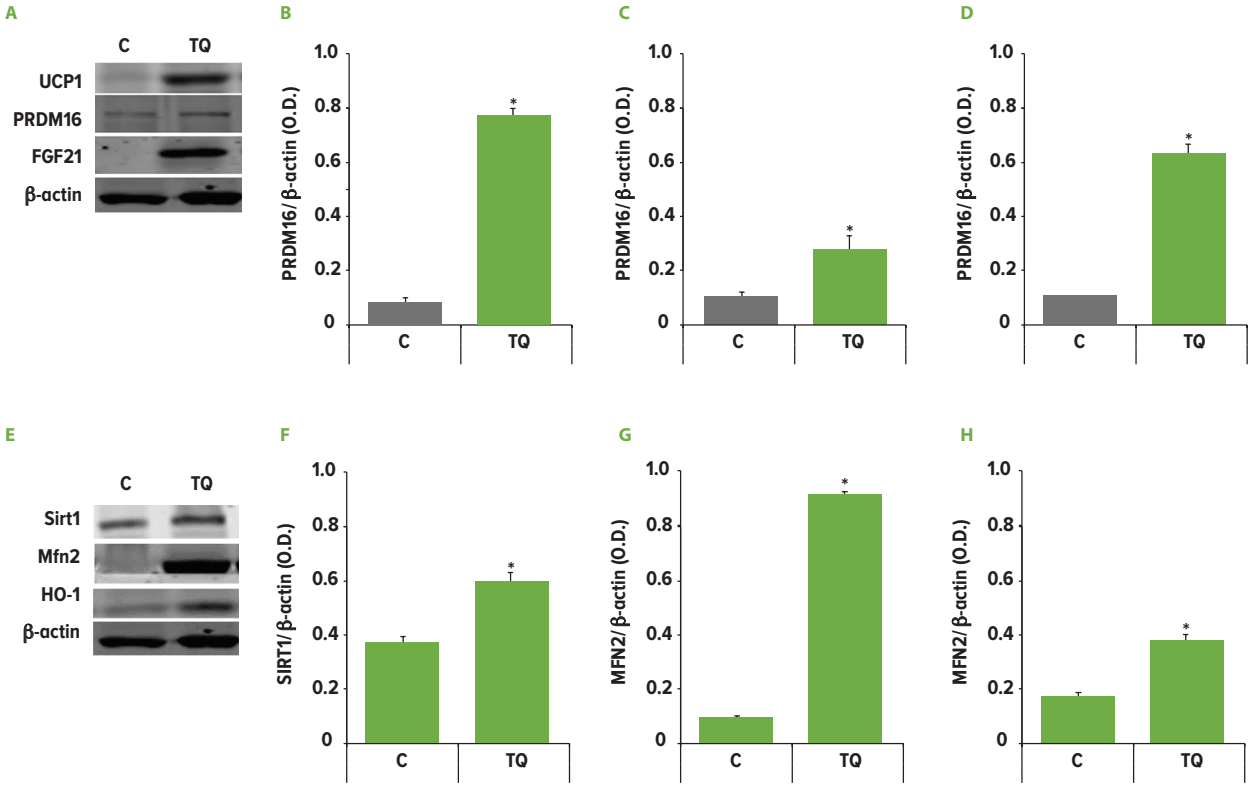
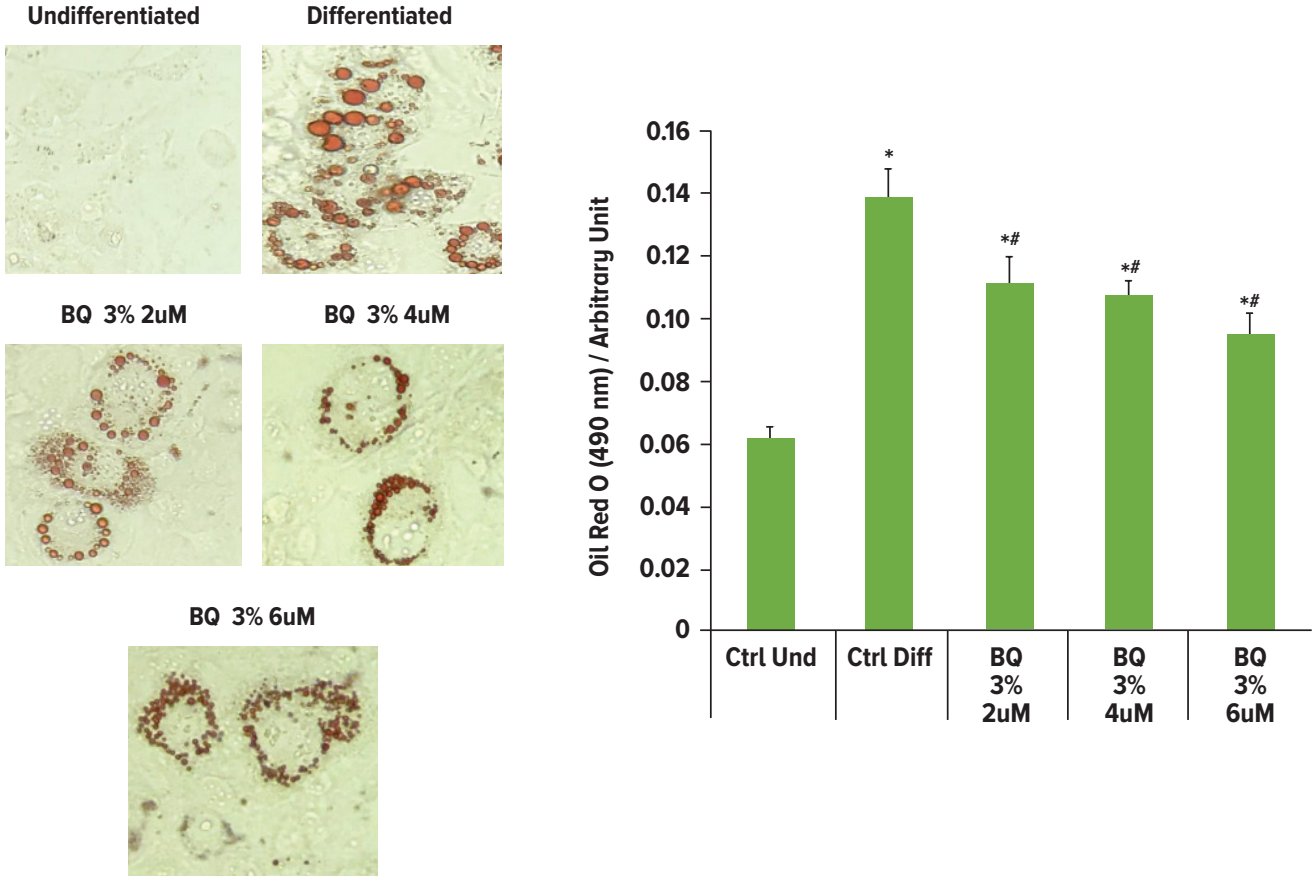


FIGURE 4

*Effect of BSO with 3% thymoquinone oil on oil droplets formation in 3T3 adipocytes showed a significant reduction of lipid droplets formation in 3T3 adipocytes at day 6. (n=4), *#p<0.05 vs. control.*



uncoupling protein 1 (UCP1), and PRDM16, key regulators of mitochondrial biogenesis and thermogenesis. It also positively impacted Fibroblast Growth Factor 21 (FGF21), a hormone involved in mitochondrial energy production and important metabolic pathways, and heme-oxygenase-1 (HO-1), a protective antioxidant enzyme that prevents cellular apoptosis. BSO's beneficial effect on mitochondrial revitalization and cellular hydration in established in vitro models of human skin health was followed by clinical research to establish the safety, compatibility, and efficacy of BSO as a topical anti-aging cosmetic support for human skin[11,12]

CLINICAL RESEARCH

A single-center, randomized, blinded, placebo-controlled trial was conducted with 22 healthy male and female subjects, aged 35-65 years with all types of skin. All subjects were compliant with the non-inclusion criteria. The trial duration was 28 days and subjects received samples of both standardized topical containing 3% Active Cream (*Nigella sativa* seed oil) and a placebo cream. All subjects applied both B'utyQuin product and the placebo, one on each half of the face. Products were attributed for half-face applications (left/right) to the subjects in a randomized manner. Subjects were assessed at baseline and 28 days via individual daily observation sheets, questionnaires, physical skin examination, photography, and measurements by dermatologists. The active cream was found to be completely safe and compatible with all skin types of study participants.

The efficacy of the Active Cream (Anti-Aging Facial Cream with 3% B'utyQuin) and the placebo was assessed using a variety of standard dermatological examination techniques and equipment. These evaluations determined the degree of change in skin firmness and elasticity (Cutometer), hydration (Corneometer), skin color and luminosity (Chromameter CR-400), and general skin appearance (standardized images VISIA-CA) over the course of the 28-day study.

MEASURABLE SKIN CHANGE EFFICACY:

The application of the Active Cream presented an 11.2% improvement in skin firmness after 28 days of application, compared to only 5.5% firmness improvement with the placebo cream (Figure 5A). The comparison between the two products shows a statistically significant difference in favor of the Active Cream.

The application of the Active Cream over the course of the study presented a 6.8% increase in skin elasticity, compared to only a 1% improvement in elasticity for the placebo cream, representing a statistically significant improvement with the application of the Active Cream (Figure 5B).

After 28 days of application, the Active Cream presented a 22.2% increase from baseline in skin hydration, compared to a 14.3 % increase with the placebo cream, representing a statistically significant improvement for the Active Cream over that of the placebo cream (Figure 6A).

After 28 days of application, the Active Cream presented a 1.1% increase in skin luminosity after 28 days, compared to a 1.5% decrease in skin luminosity with the use of the placebo cream. This increase in luminosity is statistically different from the results observed with the placebo cream (Figure 6B).

RESEARCH HIGHLIGHTS

In vitro research on cold-pressed, black seed oil standardized to 3% thymoquinone (BSO) had previously elucidated a variety of mechanisms centered around its antioxidant and anti-inflammatory activities and promoting the revitalization of mitochondrial biogenesis. This clinical trial utilizing a topical Active Cream with 3% B'utyQuin validates the mechanisms established with in vitro research by demonstrating the beneficial effects observed in the skin of trial participants. The most significant finding of

FIGURE 5A

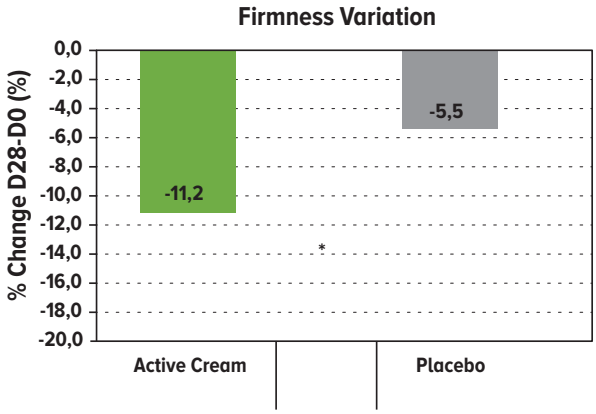


FIGURE 5B

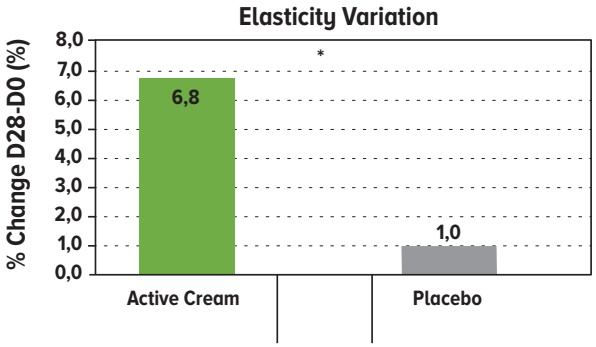


FIGURE 6A

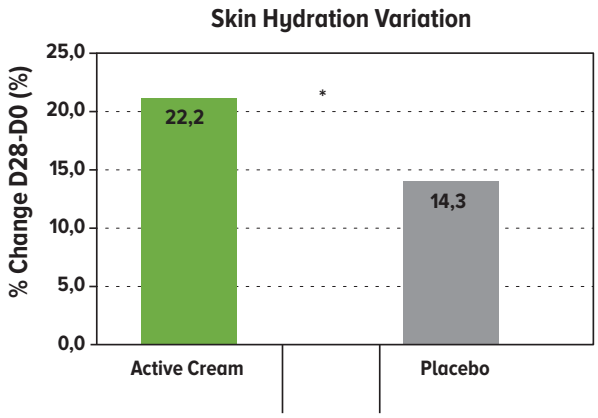
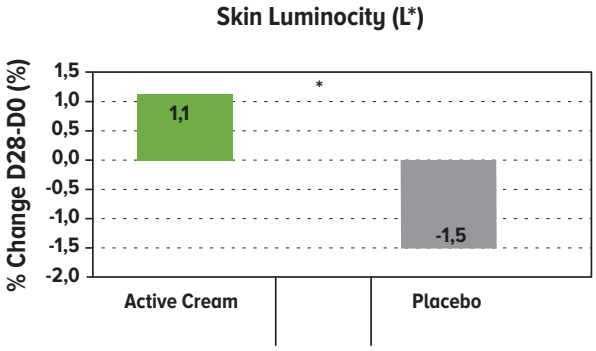


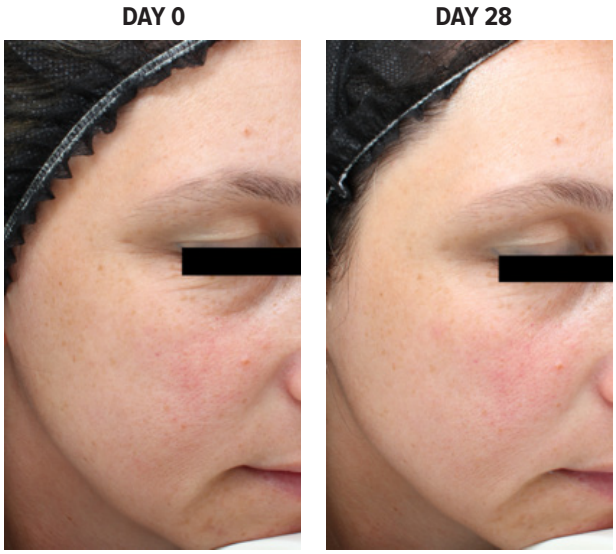
FIGURE 6B



our clinical trial is the determination that topical application of a cream containing 3% B'utyQuin specifically improves multiple characteristics of aging skin – hydration, elasticity, firmness, and luminosity – in a statistically significant, superior fashion to that of a placebo cream. These results support the mechanisms related to the mitochondrial biogenesis and revitalization effects observed in vitro for BSO as well as its antioxidant and anti-inflammatory benefits. (Figure 7).

FIGURE 7

Effect of cream containing 3% B'utyQuin on general skin appearance over a 28-day study.



CONCLUSION

Nigella sativa black seed oil has been used since the days of the Egyptian pharaohs and Cleopatra as a medicinal and beauty treatment. It was featured in the Bible and Koran as the "curative black cumin." Over the past 60 years, more than 1000 clinical studies have been conducted to identify and support its many health and cosmetic benefits. Standardized (3% thymoquinone and less than 2% free fatty acids), cold-pressed, non-GMO black seed oil has been studied in vitro to identify its mechanisms related to mitochondrial biogenesis and revitalization.

A topical Active Cream's (with 3% B'utyQuin) ability to positively impact several characteristics of aging human skin has been revealed! This clinical trial demonstrates that topical application of the Active Cream to a variety of healthy skin types over a 28-day period yields statistically significant improvements in skin hydration, elasticity, firmness ,and luminosity when compared to a placebo cream applied in the same fashion and for the same duration. The Active Cream was found to be superior in all dermatologist-measured aspects and resulted in a superior "flawless appearance" of the skin compared to placebo. The B'utyQuin was also found to be very safe, causing no adverse skin reactions over the course of the clinical trial. We conclude that the topical use of B'utyQuin in cosmetic formulations is a safe, compatible, and effective tool for improving the characteristics of aging skin in healthy subjects.

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