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Report on a Randomized, Double-Blinded, Placebo-Controlled Crossover Clinical Trial Assessing the Effects of Powdered Soulera® Taken by Oral Ingestion on Daily Cortisol Production

Summary

- **Study design: randomized, double-blinded, placebo-controlled crossover study in which each participant served as their own control**
- **Conducted by an independent FDA-licensed contract research organization in Florida**
- **Participants: 50 adults, both male and female**
- **Salivary cortisol was measured for all samples using enzyme-linked immunosorbant assays (ELISA)**
- **Results: (i) For cortisol production throughout the entire waking day, placebo increased cortisol by 7.9%, [P = 0.62125 (not statistically significant)] while Soulera® reduced cortisol by 40.2% [P = 0.00000003 (highly statistically significant)], a net reduction of 48.1%**
- **Compared to placebo treatment, Soulera® reduced cortisol by a net 30.9% during the Cortisol Awakening Response (CAR) in the first hour after awakening**
- **Compared to placebo treatment, Soulera® reduced cortisol by a net 46.9% during the remainder of the waking day between 8 AM and 10 PM**
- **Adverse events reported: none of consequence**
- **Conclusion: Soulera® is an effective and safe product for reducing cortisol to mitigate the effects of stress**

Study Purpose

To test the hypothesis that compared to a placebo, the oral ingestion of Soulera® would reduce salivary cortisol levels in order to mitigate the effects of stress

Study Design

The study was a double-blinded, placebo-controlled randomized crossover study. It included healthy males and females between the ages of 25 and 65 years resident in Florida.

Inclusion Criteria

The following conditions were to be met for a participant to be included in the study:

1. Participants were to be healthy men and women aged 25 to 65 years.
2. Women were not to have been pregnant or become pregnant during the duration of the study.

3. No participant was to have any health concerns that would confound the study as determined by the study physicians.

Exclusion Criteria

If any of the following exclusion criteria were met, a participant would not have been eligible for participation in the study:

1. The person had a history of sensitivity to any of the ingredients used in the study.
2. The person was a pregnant or lactating female. If the potential participant is a post-menarchial female, a pregnancy test (urine or serum) was performed within 24 hours prior to the study drug administration and confirmed negative in order for that potential participant to be enrolled.
3. History of psychiatric illness or chronic stress or anxiety disorders.
4. Hypertension, cardiovascular disease, liver or kidney disease or other health concerns that the study physicians thought might confound the study.
5. Cognition impairment or anyone not able to give informed consent.
6. Any routine prescription medication or nutraceutical intake.
7. Previous participation in a clinical research trial within 30 days prior to randomization
8. Ongoing abuse of illicit substances, alcohol or actively smoking marijuana.
9. The potential participant was actively engaged in the use of herbal medicine or yoga.

The Food and Drug Administration assigned clinical trial number NCT01299402 to this protocol. It was reviewed and all ethical considerations were approved by an independent Institutional Review Board. All participants gave informed consent in order to be enrolled.

Study Execution

LeraPharm Inc. supplied powdered Soulera® and placebo in sachets for reconstitution with water prior to consumption. The Soulera® and placebo powders were matched for appearance, aroma, and flavor.

After randomization and a physical examination by the study physicians, each participant was given instructions about how to provide saliva samples and the timing of sampling so that cortisol could be measured.

Upon entry into the study, each participant was given a one-month supply of either the placebo or Soulera®, and instructed to take the product at the same time each morning for the entire month before returning to the clinic for evaluation. Participants were instructed to conduct their days according to their usual habits and lifestyle so that the effects of the placebo and Soulera® would be observed under naturalistic settings and conditions. Both before and after taking placebo or Soulera®, the participants provided saliva for cortisol testing in separate vials at the time of awakening, 30 minutes after

awakening, 45 minutes after awakening 60 minutes after awakening, and then at 2 PM, 6 PM and 10 PM. Saliva tubes were returned to the research facility and forwarded to a laboratory for cortisol testing of duplicate samples by enzyme-linked immunosorbant assay (ELISA).

After the first month, each participant underwent a one month washout period during which they received neither Soulera® nor placebo. At the end of that month, each participant was given the treatment they did not receive in their first month. Saliva samples were obtained from this second treatment month, and cortisol determinations were assayed using duplicate samples by ELISA.

At all stages of the clinical trial, participants were asked to report any adverse events they experienced to the trial physicians.

Results

No adverse events of any consequence were reported by any participant.

Sufficient saliva samples for cortisol assessment at all phases of the trial were obtained from 52 participants.

Cortisol output for the day can be demonstrated by plotting a graph of cortisol concentration as determined by ELISA on the y-axis of the graph versus the time of day on the x-axis. Cortisol output can be measured by calculating the area-under-curve (AUC) of the resulting plot. This calculation was performed using the “trapezoid method” for each time increment. In this way, it was possible to calculate the total daily cortisol output for the day, and further calculate cortisol output during the Cortisol Awakening Response (“CAR”, an indicator of a person’s response to chronic stress) during the first hour after awakening, and also the cortisol output during the rest of the day after the CAR, an indicator of a person’s response to acute stress.

Four plots were prepared for each participant. They included plots of salivary cortisol both before and after administration of the placebo, and both before and after use of Soulera®.

After breaking the blinding code in order to determine which participant received which treatment at which time, results were assessed and evaluated for statistical significance by means of the two-tailed Student’s t-test for matched data points. Results in which the probability value was equal to or less than 5% ($P = / < .05$) were considered to have achieved “statistical significance”.

Data were pooled for the entire population of 52 participants under four categories in order to determine significance. These categories were: (i) before placebo, (ii) after placebo, (iii) before Soulera®, (iv) after Soulera®.

For all categories, the AUC calculations formed a normal distribution pattern, justifying the use of the Student's t-test as a means of assessment. Two participants, however, produced values that were several standard deviations outside the normal range, and these data were therefore eliminated from further consideration. The following results were obtained for the remaining 50 participants.

Cortisol Production for the Entire Waking Day (7 AM to 10 PM)

Before Placebo

Mean AUC = 28.78969
Standard Deviation = 16.97565
Standard Error = 2.82928

After Placebo

Mean AUC = 31.08063
Standard Deviation = 21.88497
Standard Error = 3.64749

P = 0.62126

Average Cortisol Increase = 7.9%

Before Soulera®

Mean AUC = 48.46166
Standard Deviation = 25.00794
Standard Error = 3.60959

After Soulera®

Mean AUC = 28.96677
Standard Deviation = 12.34542
Standard Error = 1.78191

P = .00000003

Average Cortisol Decrease = 40.2%

Cortisol Production During the CAR (7 AM to 8 AM)

Before Placebo

Mean AUC = 4.52811
Standard Deviation = 3.67624
Standard Error = 0.60437

After Placebo

Mean AUC = 4.39643
Standard Deviation = 3.77974
Standard Error = 0.62139

P = 0.7425

Average Cortisol Reduction = 2.9%

Before Soulera®

Mean AUC = 7.14327
Standard Deviation = 10.54516
Standard Error = 1.50645

After Soulera®

Mean AUC = 4.80109
Standard Deviation = 7.03371
Standard Error = 1.00482

P = .19896

Average Cortisol Reduction = 33.8%

Cortisol Production During the Remainder of the Waking Day (8 AM to 10 PM)

Before Placebo

Mean AUC = 30.68162
Standard Deviation = 26.85677
Standard Error = 4.41523

After Placebo

Mean AUC = 30.55946
Standard Deviation = 29.72982
Standard Error = 4.4875

P = 0.98553

Average Cortisol Reduction = 0.4%

Before Soulera®

Mean AUC = 53.34571
Standard Deviation = 82.5747
Standard Error = 11.79639

After Soulera®

Mean AUC = 28.15607
Standard Deviation = 26.27514
Standard Error = 3.75359

P = 0.04462

Average Cortisol Reduction = 47.3%

Discussion

An “adverse event” was defined as any health-related occurrence during the study possibly attributable to the consumption of the placebo or Soulera® that was not trivial and transient, and which resulted in a visit to a doctor or hospital with treatment prescribed, a missed day or more of work due to the resulting effect, or voluntary withdrawal from the study because of discomfort with the ingestible product. No such events were reported by any of the participants, indicating Soulera® is safe for daily consumption.

Cortisol production in most humans follows a diurnal (ie, daily) pattern. Cortisol production by the adrenal glands begins to increase 2 – 3 hours before awakening and peaks between 30 and 45 minutes after awakening. This Cortisol Awakening Response (CAR) is superimposed on top of the rest of the days’ cortisol production pattern, and is under the control of the Hypothalamic-Pituitary-Adrenal axis. Current thinking among the researchers in this field is that the CAR is an indicator of a person’s response to chronic stress. After the daily CAR, cortisol production gradually declines and reaches its lowest level early in the morning while a person is still asleep. Production after the CAR each day is under the control of the Sympathetic Adrenal System (SAS) and is a measure of “stress reactivity”, a person’s response to the acute stressors of everyday life.

Although there is great variability of the diurnal pattern from person to person, each individual person has a daily pattern that varies only slightly through time. As a person ages and experiences the cumulative effects of a lifetime of stress and illness, there is a tendency for the CAR to become blunted and the slope of the decline of cortisol production later in the day to become flatter. Accordingly, scientists have not defined what a “normal” cortisol reading or daily profile is for an individual, despite many years of intense study.

Salivary cortisol is highly representative of the cortisol concentration in all body fluids and tissues, making its measurement a suitable, inobtrusive method of looking at a person’s cortisol status.

In this study, it was determined that that for the entire waking day, the administration of the placebo increased cortisol production by an average of 7.9% across the entire population of participants. This increase was not unanticipated because submitting to cortisol testing is, in itself, stressful. The difference before the placebo and after the placebo was not statistically significant. By comparison, the administration of Soulera® during the time increments resulted in a cortisol reduction of 40.2% for the entire population. With $P = .00000003$, this finding is highly statistically significant. The net reduction of cortisol for the population for the entire day was 48.1%.

This study was planned after performing an initial study using a smaller population of participants. The power analysis after that first study suggested statistical significance would be achieved if a larger study enrolled 70 persons. The results of this study were so strong that it was possible to achieve significance with a population of 50 persons, excluding the 2 outliers described above.

The CAR is a brief phenomenon each day. In this study Soulera® reduced cortisol by an average of 33.8%. Here, $P = 0.19896$, which approaches significance without achieving it. The use of the placebo reduced cortisol by 2.9%, with $P = 0.7425$ (not significant). The net cortisol reduction of 30.9% observed with Soulera®, however, is highly worthwhile when using the product to assist with dealing with chronic stress. The AUC determined for each member of the population for the duration of the CAR is only a small fraction of the total AUC for the entire day. Thus, measurements of the efficacy of a treatment of this subtle phenomenon must be very sensitive. It is likely a study of this phenomenon using a larger population of participants would demonstrate statistical significance.

After cortisol production subsides post-CAR during the remainder of the waking day, this study found that the administration of the placebo reduced cortisol by an average of 0.4% across the population ($P = 0.98553$, not significant). On the other hand, Soulera® caused a reduction of 47.3% ($P = 0.04462$, which is statistically significant). This is a net reduction of 46.9% through the use of Soulera®.

Since the early 1990’s, an extensive body of literature published in peer-reviewed scientific and medical journals has shown the association between elevated cortisol levels

and the negative health consequences of stress. This study provides strong evidence that Soulera® is a safe and efficacious product for reducing daily cortisol output for the purpose of mitigating the effects of stress.