How to de-stress your skin?

With Asyntra® CBD-Alt
Consequences of Stress on Skin

Stress
- Psychosocial stress
  - Anxiety
  - Depression
- Internal stress

Internal Environment
- Cortisol
- Impact on immune system
- Inflammation
- Scratch skin
Consequences of Stress on Skin

Psychosocial stress
- Anxiety
- Depression

Internal stress

MECHANISM OF ACTION

Consequences of Stress on Skin

- Increased oil production in skin glands
- Clogged pores
- Acne breakouts
- Sensitive skin, redness

Stress

Psychosocial stress
- Anxiety
- Depression

Internal stress

Internal Environment

- Cortisol
- Impact on immune system
- Inflammation
- Scratch skin

Consequences on the Skin
**EndoCannabinoid System (ECS)**

**Mechanism Of Action**

**Endocannabinoid System**
Maintains homeostasis & provides stability

- **Endocannabinoids**
  - Anandamide (AEA)
  - SEE DETAIL

- **Transportation**
  - Fatty Acid Binding Protein-5 (FABP-5)
  - SEE DETAIL

- **Degradation**
  - Fatty Acid Amide Hydrolase (FAAH)
  - SEE DETAIL

- **Receptors**
  - CB1R & CB2R
  - SEE DETAIL

**Anandandamide (AEA)**

Ananda: Sanskrit word which means "joy, bliss, delight"

Arachidonoyl Ethanolamide = Anandamide
Targeting Stress through Anandamide

**Functional Level**

- **Anandamide**
  - **TRPV4**
    - Reduction lipid production
  - **TRPV1**
    - Itching
    - Pain
    - Sensitivity
  - **Sebostatic effect**
  - **Antimicrobial**

**Mechanism of Action**

- **CB2**
  - TRPV4
- **CB1**
  - TRPV1
  - AEA

- **Hair Follicle**
  - Sebaceous gland
Anandamide: an Answer to Stress

During stress, Anandamide helps to maintain homeostasis & provides stability through cannabidiol receptor CB1 & CB2.
Anandamide: an Answer to Stress

1. Anandamide & CB Receptor
   - During stress, Anandamide helps to maintain homeostasis & provides stability through cannabidiol receptor CB1 & CB2

2. Transportation by FABP-5
   - Anandamide passes through the cellular membrane without the need for a protein transport
   - It is shuttled through the aqueous environment of the cytoplasm with FABP-5 to endoplasmic reticulum
Anandamide: an Answer to Stress

1. **Anandamide & CB Receptor**
   - During stress, Anandamide helps to maintain homeostasis & provides stability through cannabidiol receptor CB1 & CB2

2. **Transportation by FABP-5**
   - Anandamide passes through the cellular membrane without the need for a protein transport
   - It is shuttled through the aqueous environment of the cytoplasm with FABP-5 to endoplasmic reticulum

3. **Degradation by FAAH**
   - Anandamide is then metabolized by FAAH into arachidonic acid & ethanolamine

**MECHANISM OF ACTION**

- Anandamide
- CB Receptor
- FABP-5
- Arachidonic acid
- Ethanolamine
- FAAH
Mechanism of Action of Asyntra® CBD-Alt

Asyntra® CBD-Alt binds with FABP-5 and FAAH. It will block the transportation of Anandamide and its degradation, therefore it maintains a high level of Anandamide.
Anandamide: Reversing Tired-Looking Face

**Light reflects from within the skin (Luminous & glowing)**

**Yoga State of mind**
NON-STRESSED

**Chotic State of mind**
STRESSED

**Anandamide Bliss Molecule**
Maintains homeostasis to provide optimal environment to function

**Metabolized by FAAH**
Very short lived

**ANANDAMIDE'S PURPOSE** is to maintain homeostasis and make sure that there isn't any overactivity or under production

**Dull complexion & perception of being unhealthy, tired & aging**
Product Definition

Synovea® EL
INCI: Ethyl Linoleate

Sytenol® A
INCI: Bakuchiol

HydraSynol® DOI
INCI: Isosorbide dicaprylate

Asyntra® CBD-Alt
After the screening of numerous molecules like Synovea® EL, Sytenol® A, and the MOA of CBD-Alt (= Synovea® EL + Sytenol® A), we launched Asyntra® CBD-Alt, a combination of CBD-Alt and HydraSynol® DOI to enhance the formulation aesthetics and scale up.
CBD-Alt is a Potent Inhibitor of FAAH Enzyme as Compared to Hemp Oil

**Objective**
To investigate the effect of CBD-Alt on FAAH inhibition as compared to Hemp Oil.

**Protocol**
- 0.01 gram of sample was dissolved in 1 mL DMSO to make stock solution. For the analysis, 1 to 5 serial dilution of the sample using DMSO was made to determine the IC50.
- The analysis was done following FAAH inhibitor screening assay kit (Cayman cat #10005196) protocol.

**CBD-Alt** is 2,653-fold more effective than Hemp Oil.
**CBD-Alt is a Potent Inhibitor of FAAH Enzyme as Compared to CBD**

**Objective**
To investigate the effect of CBD-Alt on FAAH inhibition as compared to CBD.

**Protocol**
- 0.01 gram of sample was dissolved in 1 mL DMSO to make stock solution. For the analysis, 1 to 5 serial dilution of the sample using DMSO was made to determine the IC50.
- The analysis was done following FAAH inhibitor screening assay kit (Cayman cat #10005196) protocol.

**CBD-Alt is 90-fold more effective than Cannabidiol (CBD)**
CBD-Alt Binds Effectively to FAAH Enzyme as Compared to CBD Resulting in Inhibition

CBD-Alt displayed ~9.5-fold stronger binding affinity for FAAH with respect to CBD
CBD-Alt is a Potent Inhibitor of FABP-5 Enzyme as Compared to CBD

**Objective**
To investigate the effect of CBD-Alt on FABP-5 enzyme inhibition as compared to CBD

**Protocol**
- 0.1 gram of sample was dissolved in 1 mL DMSO to make stock solution. For the analysis, 1 to 2 serial dilution of the sample using buffer was made to determine the IC50.
- 50μL of sample and 50μL of 200 ng/mL of FABP-5 (Raybiotech # 268-10276-1) was added per well for the analysis.
- The analysis was done following FABP-5 ELISA Kit (Raybiotech #ELH-FABP5) protocol.

CBD-Alt is ~ 3-fold more effective than CBD.
CBD-Alt suppresses inflammation

CBD-Alt Decreases IL-8 in Skin Epiderm

CBD-Alt can potentially reduce inflammation by decreasing IL-8 level in 3D skin models.
CBD-Alt Decreases Stress Marker: Cortisol

Cortisol is a stress marker

CBD-Alt Decreases cortisol in keratinocytes

CBD-Alt Decreases cortisol In Epiderm model

Increased Stress

Results

Increased Cortisol

CBD Alt can potentially reduce stress by decreasing cortisol amount both in single cell layer and 3D skin models
 Objective
To investigate the effect of CBD-Alt on sebogenesis

 Protocol
- Stem cell from Caucasian explant donor were stimulated to express sebocyte cell lineage. The respective cells were treated with arachidonic acid at 15uM to induce significant increase in lipid levels
- Isotretinoin 10uM was used as the positive control and led to a significant decrease in lipid levels, by about 1.5 fold
- 0.5% of CBD-Alt have been tested
- Compound effect on lipid synthesis (BODIPY intensity) and Normalized fluorescence levels

CBD-Alt strongly inhibited sebogenesis activity (100 fold) after 48h treatment, better inhibition than positive control, isotretinoin
Consumer Studies: Stress

### Clinical study parameters

<table>
<thead>
<tr>
<th>Objective</th>
<th>Evaluate consumer perceived efficacy of the formula containing Asyntra® CBD-Alt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study design</td>
<td>Formula used on the face twice a day for 4 weeks</td>
</tr>
<tr>
<td>Study dates</td>
<td>July – during Covid-19 crisis in the US</td>
</tr>
<tr>
<td>Panelists</td>
<td>70 Volunteers from 30 to 55 who self-assess to suffer from visible stress signs on their skin (dryness, dullness and redness)</td>
</tr>
<tr>
<td>Test article &amp; application frequency</td>
<td>Emulsion with 2% Asyntra® CBD-Alt (without sunscreen), Twice a day</td>
</tr>
<tr>
<td>Time points</td>
<td>Respondent provided feedback 3 times during the study at Day 1, Day 7, and week 4</td>
</tr>
<tr>
<td>Work done by</td>
<td>The Boshra</td>
</tr>
</tbody>
</table>
Consumer test: overall satisfaction and perceived benefits

**Overall**
- Liking: 84%
- Recommendation: 92%
- Stress signs Improvement: 86%

**Benefits**

<table>
<thead>
<tr>
<th>Benefit</th>
<th>Total Stressed Skin Improvement</th>
<th>Mood Enhancing Top 3 Box</th>
<th>Skin Benefits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dryness</td>
<td>93%</td>
<td>Soothing: 84%</td>
<td>Hydrating: 84%</td>
</tr>
<tr>
<td>Dullness</td>
<td>87%</td>
<td>Relaxing: 74%</td>
<td>Refreshing: 83%</td>
</tr>
<tr>
<td>Redness</td>
<td>83%</td>
<td>Calming: 74%</td>
<td>Comforting: 61%</td>
</tr>
</tbody>
</table>

Consumer test: overall satisfaction and perceived benefits

84% Liking
92% Recommendation
86% Stress signs Improvement

Top 2 Box:
- Hydrating: 84%
- Refreshing: 83%
- Comforting: 61%
Improvements on Factors of Stress

Q: Overall how much do you think the cream improved your stress skin symptoms?

86% of respondents perceived skin stress improvement, mainly driven by improvement on dryness, dullness and redness.

**Factors affecting overall stressed skin improvement**
- No change: 14%
- Slight improvement: 34%
- Significant improvement: 52%

**4th Week Improvement**
- Total Improvement (Top 2 Box)
  - Dryness: 93%
  - Dullness: 87%
  - Redness: 83%
  - Tightness: 83%
  - Uneven Texture: 81%
  - Itchiness: 75%
  - Enlarged Pores: 74%
  - Sensitivity: 71%

**Overall Stressed Skin Improvement**

*Base: Respondents who have stress symptoms*
Respondents perceived improvement on dry skin from first day. Significant higher number of respondents felt the improvement at the 1st and 4th weeks of usage.
More and more respondents perceived improvement on dullness with time.

**Q: After using the product, How do you perceive your skin improvement on dullness caused by stress issues?**

**Perceived Improvement on DULLNESS**

**Q: After using the product, How do you perceive your skin improvement on dullness caused by stress issues?**

**Base: Respondents who have stress symptoms**

Significant at 90% confidence level
Perceived Improvement on REDNESS

Q: After using the product, How do you perceive your skin improvement on redness caused by stress issues?

Redness improvement was felt as quick as the first week

Significant at 90% confidence level

Base: Respondents who have stress symptoms
Investigate the effect of CBD-Alt vs CBD on TRPV1

Understand the Molecular signature of CBD-Alt as compared to CBD

Investigate the effect of CBD-Alt vs CBD on ceramide synthase enzyme

Planned In vivo studies

Investigate the effect of Asyntra®CBD-Alt: Effect on acne

Ongoing In vitro studies

Consumer Studies

Ongoing Experiments
Clinical Benefits of Asyntra® CBD-Alt
On Stressed Skin

Asyntra®CBD-Alt

Endogenous and Exogenous Stress Factors

↑ FAPB-5, FAAH, IL-8, Cortisol, ↓ Ceramide
Clinical Benefits of Asyntra® CBD-Alt
On Stressed Skin

<table>
<thead>
<tr>
<th>Endogenous and Exogenous Stress Factors</th>
<th>Asyntra®CBD-Alt</th>
<th>Maintains homeostasis &amp; provides stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑ FAPB-5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>↑ FAAH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>↑ IL-8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>↑ Cortisol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>↓ Ceramide</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Clinical Benefits of Asyntra® CBD-Alt
On Stressed Skin

<table>
<thead>
<tr>
<th>Enhances the half life of Anandamide</th>
<th>Asyntra®CBD-Alt</th>
<th>Maintains homeostasis &amp; provides stability</th>
</tr>
</thead>
</table>

Endogenous and Exogenous Stress Factors

- **FAPB-5**
- **FAAH**
- **IL-8**
- **Cortisol**
- **Ceramide**

- Inhibits Anandamide Degradation
- Reduces Inflammation
- Reduces Stress
- Strengthen Skin Surface
Clinical Benefits of Asyntra® CBD-Alt On Stressed Skin

Enhances the half life of Anandamide
Maintains homeostasis & provides stability

Endogenous and Exogenous Stress Factors

- FAPB-5
- FAAH
- IL-8
- Cortisol
- Ceramide

Inhibits Anandamide Degradation
Reduces Inflammation
Reduces Stress
Strengthen Skin Surface

Improved Skin Barrier
Reduced Skin Sensitivity
Even Skin Tone

BLISSFUL SKIN
(Harness The Proactive Approach)
**Formulation Tips**

**Suggested use level**
- 2.0 to 4.0% w/w

**Formulation choices**
- Skin care, antiaging, soothing/calming oils or creams

**Advises**
- Add Asyntra® CBD-Alt to the formulation after making emulsion with a processing temperature of about 50°C or below. Alternately, Asyntra® CBD-Alt A can be included in the oil phase. Avoid prolonged heating of the oil phase above 75°C
- Miscible in a wide variety of emollients like capric/caprylic triglycerides, C12-15 alkyl benzoates, dicaprylyl ether), vegetable oils (sunflower oil, jojoba oils, olive oil)
- Asyntra® CBD-Alt is compatible in emulsions with CBD powder and Hemp Oil
- As Asyntra® CBD-Alt contains Bakuchiol, a phenolic compound, presence of iron or copper ions must be avoided to eliminate coloration due to the interaction with these metal ions. Addition of a small amount of disodium EDTA (0.1%)
- Airless/opaque packaging is recommended to protect product integrity and stability over time
- Finished product must be acidic:
  - pH<6.5 is recommended
Asyntra CBD-Alt: Skin Benefits

**Stressed Skin**
- Helps to improve stress skin symptoms
- Reduce dryness, dullness & redness
- Effects visible after only 7 days
- 86% of panelists perceived skin stress improvement

**Acne-prone Skin**
- Improves skin texture, pores and pimples
- Down-regulation of sebum production (in vitro)
- Improves Maskne condition
- Reduces pimples and appearances of pores

**With CBD or Hemp oil**
- Hype Up Your Hemp With Asyntra® CBD-Alt
- Well defined mechanism of action
- Better efficacy of CBD/Hemp oil when combined with CBD-Alt
- Trend supported by solid science
# Anti-Aging Lotion with Asyntra® CBD-Alt Formulation#RC-03-131E

<table>
<thead>
<tr>
<th>INCI NAME</th>
<th>TRADE NAME/SUPPLIER</th>
<th>% W/W</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phase A1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water (demineralized)</td>
<td></td>
<td>81.25</td>
</tr>
<tr>
<td>Disodium EDTA</td>
<td>Versene Na/Dow</td>
<td>0.10</td>
</tr>
<tr>
<td>Glycerin</td>
<td>Glycerine 99%/Ruger</td>
<td>3.00</td>
</tr>
<tr>
<td>Butylene Glycol</td>
<td>Butylene Glycol/Ruger</td>
<td>3.00</td>
</tr>
<tr>
<td>Panthenol</td>
<td>Ritapan DL 50%/Rita</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>Phase A2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acrylates/C10-30 Alkyl Acrylate Crosspolymer</td>
<td>Carbopol Ultrez 21/Lubrizol</td>
<td>0.15</td>
</tr>
<tr>
<td><strong>Phase B</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triethanolamine</td>
<td>Triethanolamine 99%/Ruger</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>Phase C</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isosorbide Dicaprylate &amp; Bakuchiol &amp; Ethyl Linoleate</td>
<td>Asyntra® CBD-Alt/Sytheon</td>
<td>2.00</td>
</tr>
<tr>
<td>Caprylic/Capric Triglycerides</td>
<td>Myritol 318/BASF</td>
<td>2.00</td>
</tr>
<tr>
<td>Isostearyl Alcohol &amp; Butylene Glycol Cocos &amp; Ethylcellulose</td>
<td>Emulfree CBG/Gattefosse</td>
<td>2.00</td>
</tr>
<tr>
<td>Cyclopentasiloxane &amp; Dimethiconol</td>
<td>Dow Corning 1501 Fluid/Dow Corning</td>
<td>2.50</td>
</tr>
<tr>
<td><strong>Phase D</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxyethylacrylate/Sodium Acryloyldimethyltaurate Copolymer &amp; Squalane &amp; Polysorbate</td>
<td>Simulgel NS/Seppic</td>
<td>2.50</td>
</tr>
<tr>
<td><strong>Phase E</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fragrance</td>
<td>Frag. Rosemary Lemon EE17-26505/Preamier</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>Phase F</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenoxethanol &amp; Ethylhexylglycerin</td>
<td>Euxyl PE 9010/Schuülke</td>
<td>1.00</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>100.00</td>
</tr>
</tbody>
</table>

**Procedure**

Weigh Phase A1 in the main kettle equipped with a homogenizer. Sprinkle in Phase A2. Once dispersed, heat to 60-65°C. Add in Phase B to Phase A. Weigh Phase C in a side kettle and heat to 50°C. Add Phase C to Phase AB and mix for 15-20 minutes. Add Phase D to Phase ABC. Mix for 5 minutes. Switch to side sweep mixing and add Phase E and F. Mix until uniform. Cool to room temperature.

**Notes**

pH value 5.0-6.0; Viscosity: 30,000-35,000mPas (Spindle-C, Speed-10rpm)
Illuminating Under Eye Cream with Asyntra® CBD-Alt and Hemp Seed Oil
Formulation#RC-03-143.01

<table>
<thead>
<tr>
<th>INCI NAME</th>
<th>TRADE NAME/SUPPLIER</th>
<th>% W/W</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (demineralized)</td>
<td>Butylene Glycol/Jeen</td>
<td>57.20</td>
</tr>
<tr>
<td>Butylene Glycol</td>
<td>Butylene Glycol/Acme Hardesty</td>
<td>5.00</td>
</tr>
<tr>
<td>Glycerin</td>
<td>Glycerin/Acme Hardesty</td>
<td>4.00</td>
</tr>
<tr>
<td>Phenoxethanol</td>
<td>Phenoxethanol/Acme-Hardesty</td>
<td>1.00</td>
</tr>
<tr>
<td>Polysorbate 20</td>
<td>Ritabate 20/Rita</td>
<td>1.00</td>
</tr>
<tr>
<td>Water &amp; Sodium Hydroxide</td>
<td>NaOH, 50% Aq. Sln./Sigma Aldrich</td>
<td>0.05</td>
</tr>
<tr>
<td>Disodium EDTA</td>
<td>RonaCare Disodium EDTA/EMD</td>
<td>0.25</td>
</tr>
<tr>
<td>Caffeine</td>
<td>RonaCare Caffeine/EMD</td>
<td>0.10</td>
</tr>
<tr>
<td>Phase B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xanthan Gum</td>
<td>Vanzan NF/Vanderbilt</td>
<td>0.30</td>
</tr>
<tr>
<td>Phase C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butyrospermum Parkii (Shea Butter)</td>
<td>Shebu Refined/Rita</td>
<td>2.00</td>
</tr>
<tr>
<td>Polymethysilsesquioxane</td>
<td>Gransil PSQ/Grant</td>
<td>5.00</td>
</tr>
<tr>
<td>Stearic Acid</td>
<td>Stearic Acid/Protameen</td>
<td>3.00</td>
</tr>
<tr>
<td>Dicapryl Ether</td>
<td>Cetiol 0E/BASF</td>
<td>4.00</td>
</tr>
<tr>
<td>Isosorbide Dicaprylate &amp; Bakuchiol &amp; Ethyl Linoleate</td>
<td>Asyntra® CBD-Alt/Sytheon</td>
<td>2.00</td>
</tr>
<tr>
<td>Cannabis Sativa Seed Oil</td>
<td>Hemp Seed Oil/ICSC</td>
<td>2.00</td>
</tr>
<tr>
<td>Cetearyl Alcohol</td>
<td>Protachem CS-50/Protameen</td>
<td>4.00</td>
</tr>
<tr>
<td>Behenyl Alcohol</td>
<td>Lanette 22/BASF</td>
<td>4.00</td>
</tr>
<tr>
<td>Glyceryl Stearate SE</td>
<td>Protachem GMS-D/Protameen</td>
<td>2.00</td>
</tr>
<tr>
<td>Isostearyl Alcohol &amp; Butylene Glycol Cooce &amp; Ethylcellulose</td>
<td>Emulfree CBD/Gattefosse</td>
<td>2.50</td>
</tr>
<tr>
<td>Tocopherol Acetate</td>
<td>Vitamin E Acetate/Jeen</td>
<td>0.10</td>
</tr>
<tr>
<td>Titanium Dioxide &amp; Mica &amp; Silica</td>
<td>Timiron Splendid Gold/EMD</td>
<td>0.50</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>100.00</td>
</tr>
</tbody>
</table>

Procedure
Weigh Phase A in the main kettle equipped with a homogenizer. Sprinkle in Phase B. Once dispersed, heat to 70-75°C. Weigh Phase C in a side kettle equipped with a stir bar. Heat to 70-75°C. Once both phases are at the proper temperature, add Phase C to Phase AB. Mix for 15-20 minutes. Switch to side sweep mixing and cool to room temperature.

Notes
pH value: 5.0-5.5; Viscosity: 2,000,000-2,500,000mPas (Spindle-TF, S95, Speed-0.3 rpm)
# Soothing Facial Oil with Asyntra® CBD-Alt

**Formulation#RC-03-133.01**

<table>
<thead>
<tr>
<th>INCI NAME</th>
<th>TRADE NAME/SUPPLIER</th>
<th>% W/W</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phase A</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caprylic/Capric Triglyceride</td>
<td>Jeechem CTG/.Jeen</td>
<td>39.40</td>
</tr>
<tr>
<td>Dicaprylyl Ether</td>
<td>Cetiol OE/BASF</td>
<td>40.00</td>
</tr>
<tr>
<td>Cocos Nucifera (Coconut) Oil</td>
<td>Coconut Oil/Protameen</td>
<td>10.00</td>
</tr>
<tr>
<td>Tocopheryl Acetate</td>
<td>Vitamin E Acetate/.Jeen</td>
<td>0.30</td>
</tr>
<tr>
<td>Isosorbide Dicaprylate &amp; Bakuchiol &amp; Ethyl Linoleate</td>
<td>Asyntra® CBD-Alt/Sytheon</td>
<td>4.00</td>
</tr>
<tr>
<td>Lavandula angustifolia (Lavender) Flower Oil</td>
<td>Lavender Oil/.Jeen</td>
<td>0.30</td>
</tr>
<tr>
<td>Helianthus Annuus (Sunflower) Seed Oil</td>
<td>Florasun 90/FloraTech</td>
<td>6.00</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>100.00</td>
</tr>
</tbody>
</table>

**Procedure**

Weigh Phase A in the main kettle equipped with a stir bar. Heat to 40-45°C. Mix until all solids are dissolved.
The endocannabinoid system of the skin. A potential approach for the treatment of skin disorders
Carmen del Río et al - Biochemical Pharmacology Volume 157, November 2018, Pages 122-133

A Personal Retrospective: Elevating Anandamide (AEA) by Targeting Fatty Acid Amide Hydrolase (FAAH) and the Fatty Acid Binding Proteins (FABPs)

How Stress Affects Your Skin?
Florida Dermatology and Skin Cancer
Endocannabinoids
Anandamide (AEA)

AEA is an endocannabinoid, an endogenous molecule in response to stress. It binds to cannabinoid receptors and is involved in regulating physiological and cognitive processes, like regulating activity of immune system, appetite, pain-sensation, mood, and memory.

It’s a short-lived molecule, its half life is less than 5 minutes
Transportation
Fatty Acid Binding Protein-5 (FABP-5)

FABP5 is an intracellular endocannabinoid transporter, which promotes the hydrolysis of AEA into arachidonic acid. This results in an increase of pro-inflammatory response. The FABP5 gene was first identified as being upregulated in psoriasis tissue.
Degradation
Fatty Acid Amide Hydrolase (FAAH)

FAAH is an enzyme that breaks down anandamide

Anandamide $\rightarrow_{\text{FAAH}}$ Arachidonic Acid + Ethanolamine

In collaboration with University of Kwazulu-Natal. (Durban, South Africa)
**EndoCannabinoid System (ECS)**

**Mechanism Of Action**

**Endocannabinoid System**
Maintains homeostasis & provides stability

Endocannabinoids
Anandamide (AEA)

Transportation
Fatty Acid Binding Protein (FABP-5)

Degradation
Fatty Acid Amide Hydrolyase (FAAH)

Receptors
CB1R & CB2R

**Functional Level**

Endocannabinoids

TRPV1

Itching

Pain

Sensitivity

Barrier function

Antimicrobial

CB1R is primarily located in central nervous system, whereas CB2 receptors mostly found in your peripheral nervous system. Binding of EAE to these receptors activates the downstream pathway.
CBD-Alt is a Potent Inhibitor of FAAH Enzyme as Compared to Hemp Oil

FAAH Enzyme Inhibitory Activity

- **CBD-Alt** is 2,653-fold more effective than Hemp Oil

**Objective**
To investigate the effect of CBD-Alt on FAAH inhibition as compared to Hemp Oil

**Protocol**
0.01 gram of sample was dissolved in 1 mL DMSO to make stock solution. For the analysis, 1 to 5 serial dilution of the sample using DMSO was made to determine the IC50. The analysis was done following FAAH inhibitor screening assay kit (Cayman cat #10005196) protocol.

**A fluorescence-based assay was used to detect FAAH activity using a novel substrate: AMC Arachidonoyl Amide**

**FAAH enzyme hydrolyze the substrate, resulting in the liberation of the highly fluorescent 7-amino 4-methyl coumarin (AMC) that can be monitored at an excitation wavelength of 355 nm and emission wavelength at 460 nm**

**Efficacy**
CBD-Alt is 2,653-fold more effective than Hemp Oil
Objective
To investigate the effect of CBD-Alt on FAAH inhibition as compared to CBD

Protocol
0.01 gram of sample was dissolved in 1 mL DMSO to make stock solution. For the analysis, 1 to 5 serial dilution of the sample using DMSO was made to determine the IC50.

The analysis was done following FAAH inhibitor screening assay kit (Cayman cat #10005196) protocol.

CBD-Alt is a Potent Inhibitor of FAAH Enzyme as Compared to CBD

CBD-Alt is 90-fold more effective than Cannabidiol (CBD)

A fluorescence-based assay was used to detect FAAH activity using a novel substrate: AMC Arachidonoyl Amide.

FAAH enzyme hydrolyze the substrate, resulting in the liberation of the highly fluorescent 7-amino 4-methyl coumarin (AMC) that can be monitored at an excitation wavelength of 355 nm and emission wavelength at 460 nm.
CBD-Alt Binds Effectively to FAAH Enzyme as Compared to CBD Resulting in Inhibition

**Objective**
- CBD-Alt and CBD were investigated in In-silico experiments on FAAH enzyme
- Lower the binding energy the better affinity for FAAH enzyme

**Results**
CBD-Alt displayed ~9.5 stronger binding affinity for FAAH with respect to CBD

**Conclusion**
Both components of CBD-Alt tends to bind effectively to FAAH and may potentially result in active site crowding

In collaboration with University of Kwazulu-Natal. (Durban, South Africa)
CBD-Alt suppresses inflammation

CBD-Alt Decreases IL-8 in Skin Epiderm

Reconstituted human epidermis tissues were equilibrated with media containing hydrocortisone for 4hrs followed by treatment with respective control and respective ingredients CBD and CBD-Alt (Sytenol A + Synovea EL)

After 48hrs of incubation, IL-8 output in the conditioned medium was measured by sandwich ELISA using antibody pair from invitrogen and tissue viability was assessed by MTT kit.
CBD-Alt Decreases Stress Marker: Cortisol

Cortisol is a stress marker. CBD-Alt decreases cortisol in keratinocytes and epiderm model.

Results:
- The cells were treated with TNFα @ 100ng/ml to induce cortisol.
- Following the induction, the cells were treated with respective controls, CBD and CBD-Alt.
- The cells were analyzed for cytotoxicity through MTT assay.
- The media was analyzed for Cortisol through Elisa method.

Protocol for cell model:

- The cells were treated with TNFα @ 100ng/ml to induce cortisol.
- Following the induction, the cells were treated with respective controls, CBD and CBD-Alt.
- The cells were analyzed for cytotoxicity through MTT assay.
- The media was analyzed for Cortisol through Elisa method.

% Reduction in Cortisol Content

- 74% increase
- 64% decrease

P<0.05
CBD-Alt Decreases Stress Marker: Cortisol

Cortisol is a stress marker

CBD-Alt Decreases cortisol in keratinocytes

CBD-Alt Decreases cortisol in Epiderm model

Protocol for Epiderm model

- Reconstituted human epidermis tissues were equilibrated with media containing hydrocortisone for 4hrs followed by treatment with respective control and respective ingredients CBD and CBD-Alt (Sytenol A + Synovea EL)

- After 48hrs of incubation, cortisol output in the conditioned medium was measured with cortisol parameter assay kit and tissue viability was assessed by MTT kit

Results

Increased Stress

Increased Cortisol