An Investigation of Cannabidiol-Rich Hemp Extract and the Stress Response in Male and Female Rats

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AN INVESTIGATION OF CANNABIDIOL-RICH HEMP EXTRACT AND THE STRESS RESPONSE IN MALE AND FEMALE RATS

by

RACHEL ROLLER

BA, University of Colorado Boulder, 2016

A thesis submitted to the
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Department of Integrative Physiology
2018
This thesis entitled:

An investigation of cannabidiol-rich hemp extract and the stress response in male and female rats

Written by Rachel Roller

Has been approved for the Department of Integrative Physiology

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Monika Fleshner, PhD

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Robert Spencer, PhD

Date _________________

The final copy of this thesis has been examined by the signatories, and we find that both the content and the form meet acceptable presentation standards of scholarly work in the above mentioned discipline.

IACUC protocol #2512
In recent years, hemp-derived cannabidiol supplements have become popular among people seeking relief from stress and inflammation. While there is evidence that pure cannabidiol has some stress-protective and anti-inflammatory effects, no studies have yet investigated its effects on stress-induced inflammation, and few have investigated cannabidiol-rich hemp extracts, despite evidence suggesting that such extracts may have greater clinical application than pure cannabidiol. The current study was designed to investigate the effects of ad libitum consumption of cannabidiol-rich hemp extract for one week on the response to acute stress (100 inescapable tail shocks) in male and female rats. We examined basic physiological features of the stress response including spleen, thymus, and adrenal weights, as well as plasma corticosterone and blood glucose. We also assessed plasma concentrations of the inflammatory proteins CINC-1, IL-1β, IL-6, and IL-10. As expected, tail shock decreased spleen weight and increased plasma corticosterone, blood glucose, and levels of CINC-1, IL-1β, IL-6, and IL-10. Hemp extract did not impact these responses; however, it reduced body weight gain, spleen weight, and thymus weight, and increased plasma corticosterone, regardless of stressor exposure. Interestingly, females consumed more hemp extract than males. It remains unclear whether the observed effects reflect a true pharmacological effect of the hemp extract, as opposed to a conditioned taste and/or odor aversion. If so, this could have important implications for human supplementation with cannabidiol-rich hemp extracts.
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CONTENTS

CHAPTER

I. INTRODUCTION ............................................................................................................. 1

II. MATERIALS AND METHODS
   i. Experimental design ...................................................................................................... 4
   ii. Animals ........................................................................................................................ 4
   iii. Drug administration .................................................................................................... 4
   iv. Stressor ........................................................................................................................ 6
   v. Tissue collection .......................................................................................................... 6
   vi. Corticosterone and inflammatory protein assays ......................................................... 6
   vii. Statistical analysis ...................................................................................................... 7

III. RESULTS
   i. Cereal consumption ...................................................................................................... 8
   ii. Body weights ............................................................................................................... 10
   iii. Organ weights ............................................................................................................ 11
   iv. Plasma corticosterone and blood glucose .................................................................... 12
   v. Plasma inflammatory protein levels ........................................................................... 13
   vi. Relationship between plasma corticosterone and inflammatory proteins ................. 14
   vii. An examination of rats that did not consume the hemp extract ................................. 15

IV. DISCUSSION ................................................................................................................... 17

REFERENCES ...................................................................................................................... 21
TABLES

Table

1. Composition of the cannabidiol-rich hemp extract ........................................... 5
2. Group sample sizes .................................................................................................. 9
FIGURES

Figure

1. Cereal consumption ................................................................................................................. 9

2. Body weights ............................................................................................................................... 10

3. Organ weights .............................................................................................................................. 11

4. Plasma corticosterone and blood glucose ................................................................................... 12

5. Plasma inflammatory proteins ................................................................................................... 13

6. Relationship between plasma corticosterone and inflammatory proteins ......................... 14

7. All unstressed rats, including those that consumed <5 doses of hemp extract ........ 16

8. Unstressed male rats that consumed only the first dose of hemp extract ...................... 16
CHAPTER I: INTRODUCTION

Within the last few years, an entire industry has developed around hemp-derived cannabidiol supplements. These products have attracted great interest, in part due to evidence that cannabidiol (CBD) appears to have a wide range of potential therapeutic applications 1: It has been suggested to have antiepileptic 2-4, anxiolytic 5-7, anti-inflammatory 8, sleep promoting 2,9, analgesic 10, and even anti-cancer 11 properties.

Most companies selling hemp-derived cannabidiol supplements list stress relief among their purported benefits, and stress is one of the most commonly cited reasons for use of cannabis and cannabis-derived products. In surveys of medical cannabis users, up to 50% report using it for stress/anxiety 12 13. It is therefore important to understand the effects that these supplements may have on the response to stress.

Pure CBD has been reported to have some stress-protective effects. Several studies have demonstrated that CBD attenuates acute stress-induced anxiety-like behavior and some measures of autonomic arousal in rodents 14-16. Repeated treatment with CBD has also been reported to prevent anxiogenic effects of chronic unpredictable stress in mice 17. These effects are prevented by administration of 5-HT1A receptor antagonist WAY100635, suggesting that CBD exerts its anxiolytic effects via activation of 5-HT1A receptors.

Less is known about how CBD affects the physiological response to stress. Some basic physiological features of the stress response, including organ weight changes and elevation of corticosterone, have yet to be investigated. Stress can also cause systemic inflammation, which in the absence of a pathogen can be detrimental 18-20. CBD can have anti-inflammatory effects in addition to its stress-protective effects. For example, it has been shown to reduce levels of pro-inflammatory cytokines in animal models of asthma 21,
lung injury, cerebral malaria, pneumococcal meningitis, and multiple sclerosis. Thus, there is reason to believe that it might also modulate the stress-evoked inflammatory response. It is important to test this hypothesis as some hemp-derived cannabidiol supplements are advertised as anti-inflammatory.

While the evidence for CBD is promising, few studies have investigated the effects of oral administration, or of cannabidiol-rich hemp extracts. Most studies report on the effects of pure CBD which has been microinjected into a targeted area of the brain or administered intraperitoneally, yet almost all CBD products on the market are available as hemp extracts, and designed to be taken orally in either capsule or liquid form. It is important to understand the effects of cannabidiol-rich hemp extracts as they are used in real life.

Furthermore, there is some thought within the scientific community that cannabis extracts may have greater therapeutic benefits than cannabidiol alone. In fact, pure CBD is limited in its clinical practicality at present because it seems to exhibit a biphasic or inverted U-shaped dose-response curve. For instance, 30nmol of CBD attenuates stress-induced autonomic and behavioral changes, but 15 and 60nmol have no effect. Three hundred milligrams of CBD is anxiolytic in humans completing a public speaking task, but not 100 or 900mg. A study on inflammation and nociception provided evidence that there is a clear correlation between dose and response for a cannabis extract, in contrast to the bell-shaped dose-response curve exhibited by pure CBD. The authors suggest that other minor cannabinoids and non-cannabinoid compounds may synergize with cannabidiol to produce therapeutic effects—this has been called the “entourage effect.” For example, the terpenes linalool and limonene often present in cannabis
extracts have been shown to possess anxiolytic properties that could contribute to the potential stress-protective properties of a cannabidiol-rich hemp extract.

We designed this study to investigate whether one week of daily administration of a cannabidiol-rich hemp extract similar to a product currently on the market for human use could modulate the physiological response to an acute stressor (100 intermittent inescapable tail shocks) in male and female rats. We measured the weights of the spleen, thymus, and adrenals; blood glucose; and plasma corticosterone and inflammatory protein concentrations. The weights of the spleen and thymus are known to decrease with acute and chronic stress, respectively; the adrenals hypertrophy with chronic stress. Organ weight is also one of the most sensitive drug toxicity indicators. Previous work has established that a variety of stressors, including tail shock, also elevate corticosterone, blood glucose, and the inflammatory proteins CINC-1, IL-1β, IL-6, and IL-10. We hypothesized that the cannabidiol-rich hemp extract might modulate these stress responses.
CHAPTER II: MATERIALS AND METHODS

Experimental design

Male and female rats were randomly assigned to receive either cannabidiol-rich hemp extract at a dose of 20mg/kg cannabidiol or an equivalent volume of vehicle (coconut oil) every day for one week. After a week of receiving hemp extract/vehicle, rats were assigned to groups that were either exposed to 100 inescapable tail shocks or remained in their home cages as unstressed controls. Sacrifice and tissue collection occurred immediately following stressor termination.

Animals

Adult (PND69-PND80) male and female Sprague Dawley rats (Envigo) were individually housed in a temperature- (22 degrees Celsius) and humidity-controlled environment on a 12/12 hour light/dark cycle. Animals had ad libitum access to food and water. Rats were weighed once two days prior to the start of hemp extract/vehicle administration, and again immediately prior to stressor exposure and sacrifice. All experimental protocols were approved by the University of Colorado Animal Care and Use Committee, and care was taken to minimize animal discomfort during all procedures.

Drug administration

Cannabidiol-rich hemp extract was supplied by CBDRx/Functional Remedies (Boulder, CO). Table 1 details the composition of the hemp extract. Cannabinoid profile was determined by Botanacor (Denver, CO) using high-performance liquid chromatography; terpene profile was determined by ProVerde Laboratories (Milford, MA) using head-space gas chromatography. Rats in the hemp extract group received hemp extract at a dose of 20mg/kg cannabidiol. Rats in the vehicle group received an equivalent volume of coconut
oil. The appropriate volume of hemp extract or coconut oil was applied to the frosted side of a plain- (days 1, 2, and 5) or chocolate- (days 3, 4, 6, and 7) flavored sugary cereal and placed in the animal’s cage for ad libitum consumption. Administration occurred between 1400 and 1600 each day. Prior to administration each day, an experimenter recorded whether the cereal given the previous day had been consumed. A piece of cereal was counted as “consumed” if at least half of the frosted side had been consumed. Starting on day 6, after consumption of hemp extract-treated cereal had notably declined, a thin layer of peanut butter was applied to the drug-/ vehicle-coated, frosted side of the cereal in an effort to increase palatability and encourage consumption.

<table>
<thead>
<tr>
<th>Cannabinoid</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cannabidiol (CBD)</td>
<td>46.8 mg/g</td>
</tr>
<tr>
<td>Tetrahydrocannabinol (THC)</td>
<td>2 mg/g</td>
</tr>
<tr>
<td>Cannabidiolic acid (CBDA)</td>
<td>0 mg/g</td>
</tr>
<tr>
<td>Tetrahydrocannabinolic acid (THCA)</td>
<td>0 mg/g</td>
</tr>
<tr>
<td>Cannabinol (CBN)</td>
<td>0 mg/g</td>
</tr>
<tr>
<td>Cannabigerol (CBG)</td>
<td>0 mg/g</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Terpene</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-caryophyllene</td>
<td>193 ppm</td>
</tr>
<tr>
<td>Myrcene</td>
<td>59 ppm</td>
</tr>
<tr>
<td>Humulene</td>
<td>37 ppm</td>
</tr>
<tr>
<td>A-bisabolol</td>
<td>34 ppm</td>
</tr>
<tr>
<td>Guaiol</td>
<td>25 ppm</td>
</tr>
<tr>
<td>Limonene</td>
<td>23 ppm</td>
</tr>
<tr>
<td>Terpineol</td>
<td>11 ppm</td>
</tr>
<tr>
<td>Caryophyllene oxide</td>
<td>10 ppm</td>
</tr>
<tr>
<td>Borneol</td>
<td>6 ppm</td>
</tr>
<tr>
<td>Isopulegol</td>
<td>5 ppm</td>
</tr>
<tr>
<td>Nerolidol-trans</td>
<td>5 ppm</td>
</tr>
<tr>
<td>Linalool</td>
<td>4 ppm</td>
</tr>
</tbody>
</table>

Table 1. Composition of the cannabidiol-rich hemp extract. Cannabinoid profile was determined using high-performance liquid chromatography; terpene profile was determined using head-space gas chromatography.
**Stressor**

Rats in the acute stress group were subjected to 100 inescapable tail shocks. This stressor was chosen because of its robust activation of the stress response and the stress-induced inflammatory response \(^{40,41}\). Briefly, rats were restrained in plexiglass tubes with their tails protruding out of the tube. Electrodes were placed over the tail and 100 5-second, 1-1.5mA shocks were administered intermittently by an automated shock system (Precision Calculated Animal Shocker, Colbourne Instruments) over approximately 90 minutes.

**Tissue collection**

Rats were sacrificed by rapid decapitation immediately after stressor termination. Trunk blood was collected into EDTA tubes and blood glucose measurements were taken using a glucometer (Contour Next). Blood was then spun at 3000rpm for 10 minutes and divided into 200uL aliquots. Spleen, thymus, and adrenals were removed, weighed, collected into polypropylene tubes, and snap frozen in liquid nitrogen. All tissues were stored at -80 degrees Celsius.

**Corticosterone and inflammatory protein assays**

Plasma corticosterone was measured using a commercial corticosterone enzyme immunoassay kit (Arbor Assays). Plasma was diluted 1:50.

Plasma CINC-1, IL-1\(\beta\), IL-6 and IL-10 were measured using commercial sandwich ELISAs (R&D systems). Plasma was diluted 1:25 for the CINC-1 ELISA. Plasma was run neat for the IL-1\(\beta\), IL-6, and IL-10 ELISAs.
Statistical analysis

All statistical analyses were performed using StatView. Total cereal consumption was analyzed by 2x2 ANOVA (drug x sex). Only rats that consumed at least 5 pieces of hemp extract-treated cereal were included in subsequent analyses. Body weight at the time of sacrifice was analyzed by 2x2 ANOVA (drug x sex); body weight gain was calculated as (weight at time of sacrifice minus weight before beginning drug administration) divided by weight before beginning drug administration, and then analyzed in the same manner. All organ weights and blood/plasma measures were analyzed by 2x2x2 ANOVA (drug x sex x stress). Post hoc analyses were done using Fisher's PLSD. Correlations between plasma corticosterone and plasma cytokines, and between doses consumed and plasma corticosterone, were made by simple regression.
CHAPTER III: RESULTS

Cereal consumption

All rats in the vehicle group consumed every piece of coconut oil-treated cereal they received (cereal consumption; Figure 1). While all rats in the hemp extract group consumed at least the first piece of hemp extract-treated cereal they received, their consumption of the hemp extract-treated cereal tended to decline over time, although this decline appeared to slow or reverse on days when chocolate-flavored cereal and or/peanut butter were used (days 3, 4, 6 and 7). Overall, rats consumed significantly less hemp extract-treated cereal compared to vehicle-treated cereal (F(1,65)=54.774, p<0.0001). There was also a significant drug x sex interaction (F(1,65)=9.303, p=0.0033). Post hoc analysis revealed that within the hemp extract group, males consumed significantly fewer doses than females (p=0.0001). For all other outcome measures, only rats that consumed at least 5 doses of hemp extract were included in the analysis. Thus, for all following analyses, group sample sizes are as presented in Table 2 unless otherwise stated.
Figure 1. Consumption of hemp extract-/vehicle-treated cereal (A) in total and (B) over the course of the experiment. A piece of cereal was counted as “consumed” if at least half of the frosted side had been consumed after 24 hours. All male and female rats assigned to vehicle consumed every piece of coconut oil-treated cereal they received. Data are presented as mean ± SEM; *p<0.05 compared to vehicle.

<table>
<thead>
<tr>
<th>Group</th>
<th>Male (n)</th>
<th>Female (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle + No stress</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Vehicle + Inescapable shock</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Hemp extract + No stress</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Hemp extract + Inescapable shock</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 2. Group sample sizes.
Body weights

All rats gained weight over the course of the experiment (body weights; Figure 2), but hemp extract-treated rats gained significantly less weight compared to vehicle-treated rats (F(1,49)=14.113, p=0.0005). This effect was stronger in females (drug x sex interaction: F(1,49)=5.974, p=0.0182). However, body weights were not significantly different between hemp extract- and vehicle-treated rats at the time of sacrifice (F(1,49)=3.199, p=0.0799). Female rats gained a significantly smaller proportion of their initial body weight overall compared to males (F(1,49)=20.496, p<0.0001), and males were significantly heavier than females at the time of sacrifice (F(1,49)=658.983, p<0.0001).

Figure 2. (A) Body weight gain over the course of the experiment and (B) body weights at time of sacrifice. Data are presented as mean ± SEM; *p<0.05 compared to vehicle, $p<0.05$ compared to males.
**Organ weights**

Figure 3 depicts the weights of the spleen, thymus, and adrenals at the time of sacrifice. Inescapable shock produced a decrease in spleen weight regardless of drug or sex (F(1,45)=16.357, p=0.0002). Hemp extract produced a decrease in thymus weight regardless of stress condition or sex (F(1,44)=14.267, p=0.0005). The weight of the adrenals was not affected by either stress or hemp extract. Female rats had significantly smaller spleens (F(1,45)=69.616, p<0.0001) and thymuses (F(1,44)=78.278, p<0.0001), but larger adrenals (F(1,45)=51.236, p<0.0001).

![Spleen weight](image)

![Thymus weight](image)

![Weight of adrenals](image)

Figure 3. Spleen, thymus, and adrenal weights at time of sacrifice. Data are presented as mean ± SEM; *p<0.05 compared to vehicle, #p<0.05 compared to no stress, $p<0.05$ compared to males.
**Plasma corticosterone and blood glucose**

As depicted in Figure 4, inescapable shock produced an increase in plasma corticosterone ($F(1,45)=50.752$, $p<0.0001$) and blood glucose ($F(1,45)=6.591$, $p=0.0136$). Hemp extract also produced an increase in plasma corticosterone regardless of stress condition ($F(1,45)=6.603$, $p=0.0136$) but had no effect on blood glucose ($F(1,45)=0.166$, $p=0.6855$). Females had higher overall plasma corticosterone levels compared to males ($F(1,45)=7.445$, $p=0.0090$).

![Figure 4. (A) Plasma corticosterone and (B) blood glucose. Data are presented as mean ± SEM; *p<0.05 compared to vehicle, #p<0.05 compared to no stress, $p<0.05$ compared to males.](image)
Plasma inflammatory proteins

Figure 5 depicts plasma inflammatory protein concentrations. Inescapable shock increased levels of the pro-inflammatory proteins CINC-1 (F(1,45)=75.797, p<0.0001), IL-1β (F(1,45)=35.551, p<0.0001) and IL-6 (F(1,45)=26.166, p<0.0001); it also increased levels of the anti-inflammatory cytokine IL-10 (F(1,45)=10.948, p=0.0019). Hemp extract had no effect on plasma inflammatory protein concentrations, and there were no differences between males and females.

Figure 5. Plasma (A) CINC-1, (B) IL-1β, (C) IL-6, and (D) IL-10. Data are presented as mean ± SEM; #p<0.05 compared to no stress.
Relationship between plasma corticosterone and inflammatory proteins

Plasma corticosterone levels were correlated with levels of plasma CINC-1 (R=0.728, p<0.0001; R=0.543, p=0.0006), IL-1β (R=0.598, p<0.0001; R=0.383, p=0.0212), IL-6 (R=0.675, p<0.0001; R=0.522, p=0.0011), and IL-10 (R=0.500, p<0.0001; R=0.389, p=0.0190), both when all rats are included and when only stressed rats are included, respectively (relationship between plasma corticosterone and inflammatory proteins; Figure 6).

Figure 6. Relationship between plasma corticosterone and plasma (A) CINC-1, (B) IL-1β, (C) IL-6, and (D) IL-10. Black regression lines indicate relationship when all rats are included; red regression lines indicate relationship within just stressed rats.
An examination of rats that did not consume the hemp extract

To investigate the possibility that the results presented above constitute a stress response to the administration of the hemp extract rather than a pharmacological effect, we compared all unstressed rats that received hemp extract (including those that consumed less than 5 doses) to unstressed vehicle-treated rats (Figure 7). Among these rats, hemp extract decreased weight gain \((F(1,29)=18.460, p=0.0002)\), spleen weight \((F(1,29)=7.330, p=0.0112)\), thymus weight \((F(1,28)=30.773, p<0.0001)\), and plasma IL-6 \((F(1,29)=4.700, p=0.0385)\). It also produced an increase in plasma corticosterone \((F(1,29)=10.997, p=0.0025)\), which was not correlated with the number of doses consumed in either males \((R=0.158, p=0.6622)\) or females \((R=0.201, p=0.6335)\). Next, we examined four male rats that consumed only the first dose of hemp extract they received (Figure 8). The decreases in thymus weight \((F(1,9)=6.026, p=0.0365)\) and plasma IL-6 \((F(1,9)=12.928, p=0.0058)\) are still observed, although the other effects are no longer significant in just these four rats.
Figure 7. (A-D) Body weight gain, spleen weight, thymus weight, plasma corticosterone and (F) plasma IL-6 in all unstressed rats, including those that did not consume at least 5 doses of hemp extract. (E) Relationship between number of doses consumed and plasma corticosterone in unstressed rats that received hemp extract. Blue regression line indicates relationship in males; pink regression line indicates relationship in females. *p<0.05 compared to vehicle, $p<0.05$ compared to males.

Figure 8. (A) Thymus weight and (B) plasma IL-6 in unstressed male rats that consumed only the first dose of hemp extract they received. Data are presented as ± SEM; *p<0.05 compared to vehicle.
CHAPTER IV: DISCUSSION

This study was the first to administer cannabidiol-rich hemp extract to animals for ad libitum consumption. In doing so, we intended to mimic a human supplementation regimen as closely as possible. However, the rats seemed to develop a taste aversion toward the hemp extract over time, especially males. Rats have consistently been reported to exhibit taste avoidance and place aversion to THC, and recent evidence indicates that this is not affected by co-administration of CBD. One study found that this taste aversion was greater in females, which is in contrast to the results of the current study. The greater inclination of females to consume the hemp extract-coated sugary cereal in this study may in part be explained by observations that palatability-driven feeding is greater in female rats, especially for sweet foods. Failure of some rats to consume at least 5 doses of the hemp extract was a limitation of this study, because it resulted in heterogeneity in group sizes and decreased the statistical power of our analyses.

Among rats that consumed at least 5 doses, however, exposure to 100 inescapable tail shocks produced the expected acute stress responses, reducing spleen weight and increasing plasma corticosterone and blood glucose. It also induced an inflammatory response, as evidenced by elevated levels of CINC-1, IL-1β, IL-6, and IL-10. Daily administration of cannabidiol-rich hemp extract for one week did not attenuate these acute stress responses; rather, it produced several main effects resembling chronic stress, including reduced weight gain, reduced thymus size, and increased plasma corticosterone. The decreased weight gain was more pronounced in female rats, which provides some indication that the effects of the hemp extract were more potent in females.
Others have reported that the effects of cannabinoids are more potent in females. For example, both THC and the synthetic cannabinoid CP 55,940 increase anxiety-like behavior in the elevated plus test in female rats, but not in male rats. Female rodents are more sensitive to the effects of cannabinoids on tests of nociception, motor activity, and reinforcing efficacy, and they may be especially sensitive to the biphasic effects of different doses. These differences may arise from differential pharmacokinetics, hormonal influences, and sexual dimorphism in the endocannabinoid system. While there has been little research on sex differences in the pharmacokinetics or effects of cannabidiol specifically, limited evidence from this study suggests that it may indeed be metabolized differently between males and females: Protein precipitation yielded detectable levels of the CBD metabolite 7-COOH-CBD in plasma samples from two female rats that consistently consumed the hemp extract, but not from two male rats that consistently consumed the extract.

While at first glance the results we observed appear to be physiological evidence of hemp extract-induced anxiogenesis that was perhaps more pronounced in females, we must also consider that they may not reflect a pharmacological effect of the extract, but rather, a stress response to the administration of it. We investigated this possibility by first comparing all unstressed rats that received hemp extract (including those that did not consume at least 5 doses) to unstressed vehicle-treated rats. The hemp extract-treated rats exhibited reduced weight gain, reduced spleen size, reduced thymus size, and reduced plasma IL-6 (likely attributable to the increase in corticosterone), as well as increased plasma corticosterone which was not correlated with the number of doses consumed. We then examined four male rats that consumed only the first dose of hemp extract that they
were administered, and observed the same decreases in thymus size and plasma IL-6, although the other effects were no longer significant.

The results are difficult to interpret for several reasons. We acknowledge that thymic involution, which occurs with chronic stress, is unlikely to have resulted from pharmacological action of the hemp extract in rats that consumed only one dose, and more likely indicates that the administration of the hemp extract was stressful to these rats. If this is true, it was almost certainly the odor or taste that was stressful, as in all other respects administration of the hemp extract was identical to administration of the vehicle. The extract did have an odor much like whole-plant cannabis, which the rats may have found aversive, and conditioned taste aversion paradigms have been shown to produce elevated corticosterone in rats \(^5^6\). However, it may be that only the rats that refused to consume the hemp extract found the odor and/or taste aversive and stressful. Many of the rats, especially the females, consumed every dose, which may indicate that they did not find it aversive or stressful. The effects we observed in the rats that were included in the analysis may well reflect a pharmacological effect of the extract, a conditioned taste and/or odor aversion, or a combination of both.

It is entirely plausible that pharmacological action of the hemp extract could have contributed to the effects we observed. CBD is known to produce differential effects based on dose. In many cases, a desired effect is produced at one dose and no effect, or even the opposite effect, is produced at other doses. For example, low doses of CBD appear to promote arousal, while high doses seem to produce sedation \(^9\). Others have reported behavioral evidence of anxiogenic rather than anxiolytic effects of CBD under some circumstances. For example, although 30nmol intra-prefrontal CBD reduced anxiety-like
behavior in rats subjected to restraint stress, it increased anxiety-like behavior in unstressed rats 16. Intra-infralimbic CBD also increased freezing in the contextual fear conditioning test. Like anxiolytic effects of CBD, this anxiogenic effect was also prevented by administration of 5-HT1A receptor antagonist WAY100635 57.

Pharmacological action of tetrahydrocannabinol (THC) may also have contributed to the effects we observed. Although the extract contained 23 times less THC than CBD, rats in the hemp extract group were administered 0.85mg/kg THC each day, and THC has been shown to produce anxiogenesis 46,58,59 as well as decrease food intake in rats 60. The effects we observed could have been produced by any of the components in the extract, or even by many components acting synergistically.

Moving forward, we plan to optimize the administration of the hemp extract by completely concealing the odor and increasing the palatability to achieve consistent consumption over an extended period of time. Alternatively, we might choose to administer the extract by oral gavage. Then we will repeat this study in unstressed animals to determine whether the effects we observed are still present and thus whether they constitute true pharmacological effects. If so, this could have important implications for human supplementation with hemp extracts. Further research would be needed to assess behavioral effects, as well as the effects of different extract compositions and dosing regimens.
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