A cannabigerol-rich Cannabis sativa extract, devoid of Δ9-tetrahydrocannabinol, elicits hyperphagia in rats

Daniel I Brierley*, b, James Samuelsa, Marnie Duncanc, Benjamin J Whalleyb, Claire M Williamsa †

a. School of Psychology and Clinical Language Sciences, University of Reading
b. School of Chemistry, Food & Nutritional Sciences, and Pharmacy, University of Reading
c. GW Research Ltd, United Kingdom

† Corresponding author:

Professor Claire M Williams, School of Psychology and Clinical Language Sciences, University of Reading, Harry Pitt Building, Early Gate, Reading, RG6 7BE United Kingdom
Tel.: +44 118 378 7540; fax: +44 118 378 6715
Email address: claire.williams@reading.ac.uk

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Abstract

Objective:

Non-psychoactive phytocannabinoids (pCBs) from Cannabis sativa may represent novel therapeutic options for cachexia due to their pleiotropic pharmacological activities, including appetite stimulation. We have recently shown that purified cannabigerol (CBG) is a novel appetite stimulant in rats. As standardised extracts from Cannabis chemotypes dominant in one pCB (botanical drug substances (BDS)) often show greater efficacy and/or potency than purified pCBs, we investigated the effects of a CBG-rich BDS, devoid of psychoactive Δ⁹-THC, on feeding behaviour.

Methods:

Following a 2 hour pre-feed satiation procedure, 16 male Lister-hooded rats were administered CBG-BDS (at 30-240 mg/kg) or vehicle. Food intake, meal pattern microstructure and locomotor activity were recorded over 2 hours.

Results:

Total food intake was increased by 120 and 240mg/kg CBG-BDS vs vehicle (1.53g and 1.36g, respectively, vs 0.56g; p<0.05 and p<0.01). Latency to feeding onset was dose-dependently decreased by all doses (p<0.05-0.01), and 120 and 240mg/kg doses increased both the number of meals consumed (p<0.01) and cumulative size of the first 2 meals (p<0.05 and p<.0.01). No significant effect was observed on ambulatory activity or rearing behaviour.

Conclusions:

CBG-BDS is a novel appetite stimulant, which may have greater potency than purified CBG, despite the absence of Δ⁹-THC in the extract.
Keywords

Appetite; Cannabigerol; Cannabis; Feeding; Hyperphagia; Phytocannabinoid

Abbreviations

2-AG: 2-arachidonoylglycerol

Δ⁹-THC: Δ⁹-tetrahydrocannabinol

AEA: anandamide (arachidonylethanolamide)

BDS: botanical drug substance

CBC: cannabichromene

CBDV: cannabidivarin

CBG: cannabigerol

CBGA: cannabigerol acid (or: propyl analogue of cannabigerol)

CBGV: cannabigivarin

FAAH: fatty acid amide hydrolase

NAAA: N-acylethanolamine acid amide hydrolase

pCB: phytocannabinoid

PEA: palmitoylethanolamide
Introduction

There is an urgent unmet clinical need for well-tolerated pharmacotherapeutics for cancer- and chemotherapy-induced cachexia. Phytocannabinoids (pCBs) from Cannabis sativa may represent viable candidates for this indication, due to their pleiotropic pharmacological activities, including modulation of feeding behaviour, metabolic homeostasis and inflammation (Brodie et al. 2015).

While the appetite-stimulating properties of C. sativa have historically been attributed to the psychoactive pCB ∆9-tetrahydrocannabinol (∆9-THC), we have previously shown that C. sativa extracts containing little or no ∆9-THC still stimulate appetite (Farrimond et al. 2011), and that purified pCBs other than ∆9-THC can modulate feeding behaviours (Farrimond et al. 2012). Recent studies have investigated isolated non-psychoactive pCBs (with known anti-inflammatory and/or anti-tumour activities) for their ability to stimulate feeding, and thus their potential as novel cachexia treatments. One such pCB is cannabigerol (CBG), which attenuates inflammatory bowel disease and colon carcinogenesis in vivo (Borrelli et al. 2013; Borrelli et al. 2014) and has in vitro affinities for molecular targets involved in feeding and metabolic regulation (Cascio et al. 2010; De Petrocellis et al. 2011). Using our well-established pre-feed satiation paradigm, we have recently shown that purified CBG stimulates multiple components of feeding behaviour, without detrimental motoric side-effects (Brierley et al. 2016a).

These previous data (reproduced here in Table 1 for reference), demonstrated that purified CBG (120-240 mg/kg) increased total food intake over a 2 hour test. CBG-induced hyperphagia was predominantly due to increased appetitive behaviours, evidenced by increased frequency of feeding, rather than affects on meal sizes or durations.

While testing the purified forms of pCBs is the rational first step in determining their pharmacological activities, in vitro and in vivo studies have shown that their botanical
drug substance (BDS) form may have greater efficacy and/or potency (De Petrocellis et al. 2011; Hill et al. 2013). Such BDSs (standardised extracts from chemotypes in which a particular pCB is dominant (de Meijer and Hammond 2005)), may exert differential effects to purified pCBs due to polypharmacology with the other low-abundance pCBs and/or terpenoids present, or via altered pharmacokinetics (Wagner and Ulrich-Merzenich 2009). The present study was thus conducted to investigate the effects of a CBG-rich BDS (devoid of $\Delta^9$-THC) on feeding behaviours, using an identical pre-feed paradigm and dose range as that in our study of purified CBG.

**Methods**

The effects of CBG-BDS on feeding behaviour were investigated using our pre-feed satiation paradigm, as fully detailed in previous reports (Brierley et al. 2016a; Brierley et al. 2016b). All experiments were performed in accordance with UK Home Office regulations [Animals (Scientific Procedures) Act 1986].

**Drugs:**

CBG-BDS was supplied by GW Research (Salisbury, UK), containing 72.2% w/w CBG, trace additional phytocannabinoids (CBGV: 0.4%; CBGA: 0.3%; CBC: 0.7%) and a non-pCB fraction including terpenoids and residual plant matter. Notably, this BDS contained no $\Delta^9$-THC. CBG-BDS or sesame seed oil vehicle were orally administered to 16 Lister-hooded rats (Harlan, UK; 200-225g on delivery), using a within-subjects design. Animals thus received doses of 0, 30, 60, 120 and 240mg/kg (absolute mass of CBG-BDS) according to a pseudo-random, counterbalanced Latin square protocol, with a ≥48 hour washout period.

**Procedure:**
At dark photoperiod onset, animals began a 2 hour pre-feed procedure, during which they had access to highly palatable wet-mash feed. Animals were habituated to this procedure until stable pre-feed consumption levels were observed over 4 consecutive habituation days, determined by a non-significant effect of day ($F_{3, 63} = 0.5603, p = 0.644$).

On test days, animals completed the pre-feed procedure and were immediately administered CBG-BDS or vehicle and returned to home cages for 1 hour drug assimilation, during which food was unavailable. They were then placed into custom-designed feeder cages (270mm x 405mm) for the 2 hour test, during which food consumption and locomotor activity were automatically recorded. Food intake monitors (TSE Systems, Germany) provided data on the time, duration and size of each feeding bout, which were combined into ‘meals’, defined as bouts consuming ≥0.5 g and separated by ≥900s. Two levels of infrared activity monitors (Ugo Basile, Italy) were arrayed alongside the feeder cages, such that ambulatory locomotor activity was quantified by horizontal beam breaks in a plane 20mm above the cage base, and rearing behaviour by vertical breaks in a plane 120mm high.

Data Analysis:

Data were analysed to provide measures of appetitive and consummatory behaviours, using the parameters of latency to first meal and meal number (appetitive) and meal size and duration (consummatory). Ambulatory activity and rearing were quantified using horizontal and vertical infrared beam breaks. Data were analysed by one-way repeated-measures ANOVA, with significant overall effects followed by planned comparisons of all dose groups vs vehicle. Non-parametric data were analysed by Friedman’s ANOVA and Wilcoxon’s signed rank comparisons. Results were considered significant if $p < 0.05$. 
Results

Consistent with previously reported effects of purified CBG, CBG-BDS significantly increased total food intake during the test (Fig. 1A; $F_{2.2,33.2}=3.841$, $p=0.028$). Total intake was increased following administration of CBG-BDS at 120mg/kg ($F_{1.15}=8.230$, $p=0.012$) and 240mg/kg ($F_{1.15}=11.097$, $p=0.005$), with animals consuming 1.53g ($±0.39$) and 1.36g ($±0.39$), respectively, compared to 0.56g ($±0.26$) vehicle intakes.

Increased intake was predominantly driven by stimulation of appetitive feeding, evidenced by the dose-dependently decreased latency to feeding onset (Figs. 1B and 2; $X^2_{44}=10.4221$, $p=0.034$). All doses of CBG-BDS significantly decreased this latency, with maximal effects observed at 120mg/kg ($Z=-2.805$, $p=0.005$), which advanced feeding onset by approximately 40 minutes. Frequency of feeding was also increased, demonstrated by significantly increased number of meals (Fig. 1C; $F_{4,60}=3.761$, $p=0.009$). In contrast, while an increase in the cumulative size of the first two meals was observed (Table 1; $F_{2.1,32.7}=3.353$, $p=0.044$), the duration of meals, another measure of consummatory behaviour, was not significantly affected, including the cumulative duration of the first two meals ($F_{1.8,26.4}=2.575$, $p=0.101$) or of all meals combined ($F_{1.9,27.7}=3.099$, $p=0.065$). Corroborating the previously observed lack of detrimental motoric side-effects of purified CBG, CBG-BDS had no effect on either ambulatory activity ($F_{4,60}=1.894$, $p=0.123$) nor rearing ($F_{4,60}=0.876$, $p=0.484$) over the 2 hour test (Table 2).

Discussion

CBG-BDS, at doses matched to our study of purified CBG, had similar effects on feeding patterns, despite the effective doses of CBG itself being approximately 30% lower. Overall, animals administered CBG-BDS began feeding sooner, consumed more
meals and consumed more within these meals. However, subtle differences were evident indicating that while CBG-BDS has similar efficacy in this paradigm, it has apparently greater potency than purified CBG in stimulating feeding behaviours. Total intake over the test duration was maximally increased by ~1g following doses of 120mg/kg, a three-fold increase vs vehicle. Purified CBG elicited a similar maximal increase of ~1g, however this only represented a two-fold increase and was seen following 240 mg/kg doses. Appetitive feeding behaviour, measured by decreased latency to feeding onset, was dose-dependently stimulated by all doses of CBG-BDS, with a maximal reduction at 120mg/kg of ~40 minutes. In contrast, purified CBG only significantly advanced feeding onset at 240mg/kg, by ~30 minutes. Both the number of meals and cumulative size of the first two meals were approximately doubled by both 120 and 240 mg/kg CBG-BDS, in this case demonstrating a consistent pattern of feeding stimulation to purified CBG. It is thus apparent that CBG-BDS is similarly efficacious to purified CBG at stimulating feeding behaviours, but as the maximal effects were seen following doses of 120 mg/kg, it may be more potent, and demonstrates a ceiling effect not seen following purified CBG.

Although determining the mechanism of action for this hyperphagia was beyond the scope of these studies, we have previously speculated on putative mechanisms based on the published *in vitro* affinities and activities of CBG (Brierley et al. 2016a). In light of the apparent greater potency of CBG-BDS, such speculation can be extended based on the differential affinities and activities reported in comparative *in vitro* studies of the purified and BDS forms (De Petrocellis et al. 2011). While both have little affinity or activity at cannabinoid 1 or 2 receptors, they have similar efficacy as inhibitors of anandamide (AEA) reuptake, and may thus elicit hyperphagia via upregulation of orexigenic endocannabinoid tone. CBG-BDS has four-fold greater potency as an inhibitor of monoacylglycerol lipase (De Petrocellis et al. 2011), the hydrolytic enzyme
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for 2-arachidonoylglycerol (2-AG). Given that 2-AG also elicits hyperphagia (Kirkham et al. 2002), it is possible that the increased potency of CBG-BDS is due to concurrent elevation of 2-AG and AEA. The apparent ceiling effect of CBG-BDS at 120 mg/kg, not observed for purified CBG, also points to the potential involvement of another mechanism, involving the endocannabinoid-degrading enzyme N-acyl ethanolamine acid amid hydrolase (NAAA). While neither forms of CBG have appreciable activity as fatty acid amid hydrolase (FAAH) inhibitors, CBG-BDS alone is a potent inhibitor of NAAA, which would result in a selective inhibition of palmitoyl ethanolamine (PEA) hydrolysis over AEA. Given that PEA attenuates hyperphagia (Mattace Raso et al. 2014), it is plausible that at CBG-BDS doses >120mg/kg, PEA is elevated to physiologically-relevant levels, attenuating CBG-induced hyperphagia mediated by other mechanisms. While neither the minor pCBs nor terpenoids present in CBG-BDS have known appetite-stimulating properties per se, they may improve the bioavailability of CBG and hence contribute to the apparent greater potency of the BDS via pharmacokinetic effects (Wagner and Ulrich-Merzenich 2009). Indeed, a recent study of the anticonvulsant effects of cannabidivarin-BDS demonstrated that a pCB-free BDS was without intrinsic effect, but apparently slightly increased efficacy of the purified pCB, supporting such a pharmacokinetic effect (Hill et al. 2013). While no direct pharmacokinetic comparison of purified CBG and CBG-BDS has been published to date, it should be noted that purified forms of several major pCBs have shown differential brain concentrations dependent on route of administration, with CBG reaching higher concentrations via the intraperitoneal route, in contrast to cannabidiol and CBDV for which the oral route was more effective (Deiana et al. 2012). Further studies investigating the effects of intraperitoneal purified CBG and CBG-BDS on feeding behaviours may thus be warranted to determine which form, dose level and route of administration may have the greatest translational potential for cachexia.
Conclusion

Here we report for the first time that a CBG-rich BDS, devoid of Δ⁹-THC or other pCBs with known hyperphagic activity, stimulates appetite in pre-satiated rats. CBG-BDS appears to have similar efficacy but greater potency than purified CBG, and warrants investigation as a potential novel treatment for cachexia.
References


Kirkham TC, Williams CM, Fezza F, Di Marzo V (2002). Endocannabinoid levels in rat


Figure 1. Total food intake (A) and meal pattern microstructure parameters of latency to feeding onset (B) and number of meals consumed (C). Data presented as group mean ± SEM, analysed by one-way repeated measures ANOVA (latency by Friedman’s ANOVA) and planned comparisons of all dose groups vs vehicle. * $p < 0.05$, ** $p < 0.01$. 
Figure 2. Graphical summary of group mean meal pattern microstructure parameters for meals 1 and 2. Boxes are positioned along x-axis according to meal latencies, box widths are scaled to meal durations and meal sizes are given above. Where individual animals did not consume a second meal, minimum (size and duration) or maximum (latency) values were imputed. Asterisks indicate significantly decreased latencies compared to vehicle, * $p < 0.05$, ** $p < 0.01$. 
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Table 1. Hourly food consumption and meal pattern analysis data. Data presented as group mean ± SEM, analysed by one-way repeated measures ANOVA and planned comparisons of all dose groups vs vehicle. All groups n = 16, * p < 0.05, ** p < 0.01. † Data for purified CBG has been previously published (Brierley et al. 2016a), and is reproduced here for comparison with CBG-BDS.