Inflammatory Bowel Diseases

The use of cannabinoids in colitis: a systematic review and meta-analysis

--Manuscript Draft--

Manuscript Number:	IBD-D-17-00866R1
Article Type:	Review Article - Basic Science
Keywords:	cannabinoids; colitis, meta-analysis
Corresponding Author:	Daniel G Couch University of Nottingham School of Medicine Derby, N/A UNITED KINGDOM
First Author:	Daniel G Couch
Order of Authors:	Daniel G Couch
	Henry Maudslay
	Brett Doleman
	Jon N, Lund
	Saoirse E O'Sullivan
Manuscript Region of Origin:	UNITED KINGDOM
Abstract:	Background:Clinical trials investigating the use of cannabinoid drugs for the treatment of intestinal inflammation are anticipated secondary to preclinical literature demonstrating efficacy in reducing inflammation. Methods:We systematically reviewed publications on the benefit of drugs targeting the endo-cannabinoid system in intestinal inflammation. We collated studies examining outcomes for meta-analysis from EMBASE, MEDLINE and Pubmed until March 2017. Quality was assessed according to mSTAIR and SRYCLE score. Results:From 2008 papers, 51 publications examining the effect of cannabinoid compounds on murine colitis, and two clinical studies were identified. 24 compounds were assessed across 71 endpoints. Cannabidiol, a phytocannabinoid, was the most investigated drug. Macroscopic colitis severity (disease activity index - DAI) and myeloperoxidase activity (MPO) were assessed throughout publications and were meta-analysed using random effects models. Cannabinoids reduced DAI in comparison with vehicle; SMD -1.36, 95% CI -1.62 to-1.09, I2=61%). FAAH inhibitor URB597 had the largest effect size (SMD-4.43, 95% CI-6.32,-2.55), followed by the synthetic drug AM1241 (SMD -3.11, 95% CI -5.01, -1.22) and the endocannabinoid anandamide (SMD-3.03, 95% CI -4.89,-1.17, I2 not assessed). Cannabinoids reduced MPO in rodents compared to vehicle; SMD -1.26, 95% CI-1.54 to -0.97, I2=48.1%. Cannabigerol had the largest effect size (SMD -6.20, 95%CI-9.90, -2.50), followed by the synthetic CB1 agonist ACEA(SMD -3.15, 95%CI-4.75, -1.55) and synthetic CB1/2 agonist WIN55,212-2(SMD-1.74, 95%CI-2.81, -0.67, I2=57%). We found no evidence of reporting bias. No significant difference was found between the prophylactic and therapeutic use of cannabinoid drugs. Conclusions:There is abundant pre-clinical literature demonstrating the anti-inflammatory effects of cannabinoid drugs in inflammation of the gut. Larger randomised controlled-trials are warranted.

The use of cannabinoids in colitis: a systematic review and meta-analysis

DG Couch, H Maudslay, B Doleman, J N Lund, SE O'Sullivan

School of Medicine, Royal Derby Hospital, University of Nottingham, Nottingham, United Kingdom

Correspondence: Daniel Couch, School of Medicine, Royal Derby Hospital, University of Nottingham, Derby. DE22 3DT UK

E-mail: Couch27@gmail.com

Phone: 01332724701

No disclosures. No funding was recived for this study.

Author contributions:

Conception and design of the study; Couch DG, Lund J, O'Sullivan SE conceived and designed the study

D Couch and H Maudslay collected data.

D Couch, H Maudslay, B Doleman, J Lund and S O'Sullivan analysed data.

D Couch, J Lund and S O'Sullivan were responsible for overall content of the article.

Drafting or revision of the manuscript; all

Approval of the final version of the manuscript: all

Keywords: cannabinoid inflammation gut intestine colitis

Abstract

Background:Clinical trials investigating the use of cannabinoid drugs for the treatment of intestinal inflammation are anticipated secondary to preclinical literature demonstrating efficacy in reducing inflammation.

Methods:We systematically reviewed publications on the benefit of drugs targeting the endo-cannabinoid system in intestinal inflammation. We collated studies examining outcomes for meta-analysis from EMBASE, MEDLINE and Pubmed until March 2017. Quality was assessed according to mSTAIR and SRYCLE score.

Results:From 2008 papers, 51 publications examining the effect of cannabinoid compounds on murine colitis, and two clinical studies were identified. 24 compounds were assessed across 71 endpoints. Cannabidiol, a phytocannabinoid, was the most investigated drug. Macroscopic colitis severity (disease activity index - DAI) and myeloperoxidase activity (MPO) were assessed throughout publications and were meta-analysed using random effects models. Cannabinoids reduced DAI in comparison with vehicle; SMD -1.36, 95% CI -1.62 to-1.09, I^2=61%). FAAH inhibitor URB597 had the largest effect size (SMD-4.43, 95% CI-6.32,-2.55), followed by the synthetic drug AM1241 (SMD –3.11, 95% CI -5.01, -1.22) and the endocannabinoid anandamide (SMD-3.03, 95% CI -4.89,-1.17, I^2 not assessed). Cannabinoids reduced MPO in rodents compared to vehicle; SMD -1.26, 95% CI-1.54 to -0.97, I^2=48.1%. Cannabigerol had the largest effect size (SMD -6.20, 95%CI-9.90, -2.50), followed by the synthetic CB_1 agonist ACEA(SMD -3.15, 95%CI-4.75, -1.55) and synthetic $CB_{1/2}$ agonist WIN55,212-2(SMD-1.74, 95%CI-2.81, -0.67, I^2=57%). We found no evidence of reporting bias. No significant difference was found between the prophylactic and therapeutic use of cannabinoid drugs.

Conclusions:There is abundant pre-clinical literature demonstrating the anti-inflammatory effects of cannabinoid drugs in inflammation of the gut. Larger randomised controlled-trials are warranted.

Table of abbreviations

PPAR - Peroxisome Proliferator Activating Receptor

TRPV1 - Transient receptor potential vanilloid 1

AEA - Anandamide

2-AG - 2-arachidonoyl glycerol

PEA - Palmitoylethanolamide

DNBS - Dinitrobenzene sulphonic acid

OM - Oil of mustard

TNBS - Trinitrobenzene sulphonic acid

DSS - Dextran sulphate sodium

CO - Croton oil

THC - Δ^9-Tetrahydrocannabinol

CBD - Cannabidiol

Ab-CBD - Abnormal cannabidiol

CBG - Cannabigerol

CBN - Cannabinol

MMJ - Medicinal cannabis

MPO - Myeloperoxidase

DAI - Disease activity index

IL-10 - Interleukin-10

SMD - Standard mean difference

CI - Confidence interval

I.c. - Intracolonic

p.o.- Oral

i.v. - Intravenous

p.r. - Per rectum

s.c. - Subcutaneous

Introduction

Inflammatory bowel disease (IBD) affects 200 per 100,000 adults in the United States and 400 per 100,000 in the UK (1,2). Major subtypes consist of Crohns disease and ulcerative colitis. A definitive clinical treatment for these chronic relapsing diseases remains elusive, as currently no therapy exists to reverse the clinical pathology without a risk of significant side effects. 5-ASA agents, corticosteroids, anti-TNFα antibodies and other immunomodulatory drugs have all been shown to induce significant remission in IBD, but are associated with bone marrow suppression, opportunistic infection, infusion reactions and malignancy secondary to immunosuppression (3–5).

The endocannabinoid system (ECS), consisting of multiple receptors and endogenous ligands, controls multiple homeostatic processes including gastrointestinal motility, hunger, perception of pain and immunity (6–10). The receptors of the ECS consist of the classical CB_1 and CB_2 receptors, but also the orphan GPR55 receptor, peroxisome proliferator-activated receptors (PPARs) and transient receptor potential receptor vanilloid (TRPV) receptors. These targets are all found on the cells of gut mucosa, submucosa, enteric nervous and immune systems. Endocannabinoids, such as anandamide (AEA) and 2-arachiodoylglycerol (2-AG), are intercellular lipid signalling molecules derived on demand from membrane precursors (11). They are metabolised by fatty acid amide hydrolase (FAAH) as well as N-acyl ethanolamine-hydrolysing acid amidase (NAAA) in the case of AEA, and monoacylglycerol lipase (MAGL) in the case of 2-AG (12–14). Palmitoylethanolamide (PEA), also metabolised by NAAA, has been shown to activate PPARα and may increase local concentrations of AEA or the affinity of AEA to the CB_1 receptor and is therefore included as an atypical cannabinoid (15,16). Phytocannabinoids include Δ-^{9} tetrahydrocannabinol (THC), cannabidiol (CBD), cannabigerol (CBG), cannibichromene (CBC) and up to 60 others and are isolated from C*annabis Sativa* (11). THC and CBD have found place in clinical practice in the treatment of childhood epilepsy and muscular spasticity in multiple sclerosis (17,18). A growing collection of synthetic cannabinoid agonists have been derived possessing selective high affinity for the CB_1, CB_2, GPR55 and TRPV1 receptors, and have been investigated pre-clinically for roles in gut motility, satiety and immunity (8).

Under inflammatory conditions CB_1, CB_2 and both PPARα and PPARγ expression increases on the submucosa and on adjacent immune cells, whereas GPR55 and TRPV1 expression decreases on the mucosa, but increases on enteric nervous tissue (19–21). Levels of AEA, 2-AG and PEA are upregulated *in vitro*, and also in animal *in vivo* and human *ex-vivo* models of intestinal inflammation (22–24). Early experimentation in murine models demonstrated cannabinoids prevent the onset of experimental murine colitis or reduced its severity (25). Since these initial findings, many reports, including clinical trials, have now investigated the effect of cannabinoid ligands, or the effect of blockade of their metabolising enzymes, on inflammation of the gut.

There is a significant amount of promising preclinical evidence for the use of cannabinoid agents in the treatment of colitis. Within this study we aimed to gather all preclinical and clinical evidence for the use of these drugs in colitis, and where possible, perform meta-analyses across studies in order to assess the efficacy of cannabinoids for further clinical trials. Where possible clinically relevant experimental endpoints were assessed.

Methods

Search Strategy

All studies evaluating the effect of cannabinoid drugs on inflammation of the colon were searched from March 1980 until March 2017 by two independent researchers in Medline, EMBASE and Pubmed. Keywords included cannabidiol, tetrahydrocannabinol, anandamide, 2-AG, cannibichromene, cannabigerol, cannabinoid, cannabis sativa, colon, intestine, gut, inflammation, Crohns, ulcerative and colitis. Names of synthetic cannabinoid agents were also included. References from included studies were searched by hand. Pre-specified inclusion and exclusion criteria were used to prevent bias. Experiments must have been be performed in the context of administration of cannabinoid drugs to inflammatory states of the colon in humans or animals, either experimental or due to endogenous disease (Crohns disease or ulcerative colitis). *In vitro* studies or studies not examining the effect of cannabinoids in intestinal inflammation specifically, or studies using cannabinoid antagonists as a primary agent were excluded. A PRISMA checklist is included in the appendix.

Data Acquisition

The mode of colitis induction in preclinical studies was recorded in addition to the timing of cannabinoid application. For the purposes of meta-analysis, data on the macroscopic or histological disease scores (disease activity index – DAI) and myeloperoxidase (MPO) activity were collected. If the exact number of animals was not available, the lowest number of animals within the range given were used for the experimental group, and the highest number used for the control/vehicle group. Where studies reported the effects of more than one cannabinoid sharing a single control group for comparison, control group numbers were equally distributed between comparisons to avoid unit of analysis issues. WebPlotDigitiser (version 3.11) was used to extract values from figures in published articles where no data values were given in the text.

Quality

Quality of included studies were assessed by two independent researchers to quantify risk of bias according to the six-point criteria developed by the Cochrane Collaboration risk of bias tool (26). In order to assess the quality of preclinical studies, the STAIR and Arrive preclinical assessment tools were adapted (27,28). Each of the below were awarded one point: randomisation, assessor blinding, results replicated in a second species, dose-response experiments, results replicated in a second model of colitis, n=5 or greater in each group, the use of clinically relevant endpoint to assess response of colitis, definitive statement of animal numbers in each group, a statement regarding the housing of animals and a statement describing the location and timing of animal experimentation (i.e. in animal housing or a separate cage, time of day etc), giving a highest possible score of 10.

Data analysis

Where possible, data were grouped into DAI and MPO activity, and subdivided by species and compound. Data from each group were analysed as forest plots using Cochrane Review Manager Software (Review Manager 5.3, Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014), and as funnel plots using Stat (Stat Corp. 2009 Stat Statistical Software: Release 11. College Station, TX, USA). Funnel plot asymmetry was tested using Egger's linear regression test. A P value of <0.05 was considered statistically significant. As differing studies measured MPO activity and DAI using various scales, we present effect estimates as standardized mean differences (SMD) with 95% confidence intervals (CI). We used the following SMD values to assess results for clinical significance: < -0.5 small clinical significance, -0.5 to -0.8 moderate clinical significance and >-0.8 high clinical significance. Due to clinical heterogeneity between the various studies, a random-effects model was used. We assessed statistical heterogeneity using the I^2 statistic, with >50% regarded as evidence of statistical heterogeneity. We assessed the quality of evidence using the previously validated SYRCLE criteria, with studies graded out of 10 (29). Studies were weighted by sample size and statistical significance was set at a minimum of p<0.05.

Results

Search results and study characteristics

The search strategy returned 2008 results from which 199 relevant publications were identified. From these, 53 publications comprising 106 experiments examining 35 compounds met the inclusion criteria (figure 1, table 1 and 2). Thirty four studies were included in the meta-analysis.

Forty-three publications studied the effects of cannabinoids on experimental murine colitis, 5 in rats, and 3 in both mice and rats. Two clinical trials examined the effect of a cannabinoid (THC and CBD) in Crohns disease. Within animal publications, 43 used caustic agents (Di-nitrobenzine sulphonic acid (DNBS), trinitrobenzene sulphonic acid (TNBS), oil of mustard (OM), dextran sulphate sodium (DSS) and croton oil (CO)) to induce colitis, 6 used intravenous or topical lipopolysaccharide, 2 induced colonic inflammation using surgical arterial ligation or puncture of the colon and 1 induced colitis with interleukin-10 (IL-10) knock-down and DSS (figure 2A). Across all publications, including clinical trials, 71 endpoints were examined to evaluate the effect of cannabinoid drugs on colitis. Forty-nine publications (89 experiments) examined more than one endpoint. Of these endpoints MPO and DAI were the most consistently used (34 and 26 studies respectively), and were therefore selected for meta-analysis. Incidence of endpoints is given in figure 2B.

The effect of 7 phytocannabinoids were studied across 18 publications; cannabinol (CBN), CBD, THC, CBC, CBG, medicinal cannabis (MMJ) and abnormal CBD (Ab-CBD). 4 endocannabinoids were studied across 11 publications (PEA, ultramicronized PEA (uPEA), Arachidonyl-2'-chloroethylamide (ACEA) and AEA), 15 synthetic cannabinoid agonists were studied across 22 publications (AM841, Adelmidrol , HU210, CP55,940, WIN55,212-2, AM1241, JHW015, JWH133, βCaryophyllene, O-1602, HU308, αβ amyrin CID 16020046 compound 26 and SAB378), and 9 compounds targeting the catabolism or transport of endogenous cannabinoids were studied across 13 publications (ARN2508, PF-3845, compound 39, JZL184, AA5HT, VDM11, URB597, AM9053, AM3506). These compounds are delineated by class in table 1. The degree of positivity or negativity of the outcomes of these studies are displayed in figure 2C. Twenty-three studies investigated underlying receptor mechanisms using knock-out (KO) animals or receptor antagonists.

Of the 105 experiments comparing cannabinoids with vehicle or placebo, 67 (63.8%) favoured cannabinoids, 34 (32.3%) reported no difference, and 4 (3.8%) favoured vehicle. Mice were used in 89 experiments (68.5% of which favoured cannabinoids), rats in 14 (71.4% favoured cannabinoids), in 4 experiments both mice and rats were used showing no difference between cannabinoids and vehicle. In the two clinical trials, no difference in primary outcome was found between the use of THC cigarettes or oral CBD and placebo. 11 of 14 publications (78.6%) using synthetic CB_2 receptor agonists favoured cannabinoid use over vehicle, and a further 11 of 13 (84.6%) favoured using FAAH inhibitors over vehicle. The outcome of all cannabinoids across publications is given in figure 2C.

Two clinical trials examining the effect of CBD and THC in Crohns disease were found. Naftali et al. (2013) conducted a placebo controlled study in Crohns disease patients, comparing THC 115mg inhaled alone with placebo. Disease activity was compared between groups by means of validated questionnaire (Crohns disease activity index – CDAI) after 8 weeks of treatment. A non-significant reduction in clinical disease remission as defined by the authors was found at the end of the study period, however a secondary endpoint of reduction in overall activity scores was found between groups (p=0.028). In a second study, Naftali et al. (2017) compared oral CBD 10 mg p.o. twice daily with placebo in Crohns disease, using CDAI in an identical fashion. No reduction in disease activity was detected between groups. In both studies the authors measured changes in serum C-reactive protein (CRP), within both experimental and placebo groups CRP levels were below 5 units per ml at the end of the study periods. Clinically, CRP levels greater than 5 units per ml are considered indicative of inflammatory disease. Within both studies the combination of CBD and THC within a single study were not assessed.

Of the 104 experiments where timing of drug administration of drug was stated, 37 administered cannabinoids therapeutically, of which 62.2% favoured cannabinoid treatment. 19 experiments administered cannabinoids prophylactically, of which 52.6% favoured cannabinoid treatment. 48 experiments administered cannabinoids both prophylactically and therapeutically, of which 75% favoured cannabinoid treatment versus vehicle.

Meta-Analysis

34 studies reported the same endpoints of disease activity index or myeloperoxidase activity allowing for meta-analysis. Of the remaining studies heterogeneity of endpoints prevented further meta-analysis.

Crohns Disease Activity Index (CDAI).

The use of two phytocannabinoids, THC or CBD, in two human studies were meta-analysed. Phytocannabinoid use decreased severity scores in comparison with placebo (mean difference (MD) -74.97, 95% CI –229, 0.79, I^2=75%. Figure 3). THC alone had a significant effect on reducing CDAI (MD-154.00, 95% CI -2.68.57, -44.43), whereas CBD alone did not (MD +4.00 95% CI -1.5.39, +113.39).

Disease Activity Index (DAI)

Thirty-four publications examined the effects of 25 cannabinoid drugs across 68 experiments, within mouse and rat models (total n = 948, n = 519 experimental vs 429 in control groups). Cannabinoid drugs reduced DAI in comparison with vehicle; SMD -1.36, 95% CI -1.62 to-1.09, I^2=61% (figure 4, table 3). On subgroup analysis there was significant difference between drug subtypes (P<0.001). DAI was significantly reduced in mice (SMD -1.49, 95% CI -1.77 to -1.22; I^2=61%). Seven experiments within one publication examined the effects of cannabinoids on rat colitis (THC and CBD, both conducted in a dose response manner), but did not reach significance at any concentration; SMD -0.29, 95% CI -0.77 to 0.20, I^2=0%. SMD and confidence intervals for individual drugs on DAI are given in table 3.

The largest effect size in DAI reduction was caused by an enzyme inhibitor: the FAAH inhibitor URB597 (SMD-4.43, 95% CI-6.32,-2.55). The largest effect size of DAI reduction by an endocannabinoid was AEA (SMD-3.03, 95% CI -4.89,-1.17), the largest effect size of DAI reduction by a phytocannabinoid was CBD (SMD -0.56, 95% CI-0.97, -0.16, I^2= 29%), and the largest synthetic cannabinoid effect size on DAI was AM1241 (SMD –3.11, 95% CI -5.01, -1.22). SMD and confidence intervals of individual drugs on DAI are given in table 4. Eighteen of twenty-five drugs had a large effect size, one had a moderate effect size, and six had no significant effect on DAI.

Myeloperoxidase Activity (MPO)

Twenty-six publications investigated the effects of 21 cannabinoid drugs on MPO activity throughout 57 individual experiments (total n = 757, n = 419 in experimental vs 338 in control groups). Cannabinoid drugs reduced MPO in comparison with vehicle; SMD -1.26, 95% CI-1.54 to -0.97, I^2=48.1% (figure 5, table 4). Overall, there was significant heterogeneity between studies and there was significant subgroup difference (I^2=48.1%, P<0.008). MPO was significantly reduced in mice and rats (SMD -1.28, 95% CI -1.59 to -0.98 I^2=61% and -1.06, 95% CI -1.99 to -0.13, I^2=56% respectively).

The largest effect size in MPO reduction was caused by the phytocannabinoid CBG (SMD -6.20, 95% CI -9.90 to -2.50, I^2 not assessed). The largest effect size by an endocannabinoid was PEA (SMD -2.74, 95% CI -4.42, -1.06, I^2=85%), the largest synthetic cannabinoid effect size on MPO was caused by ACEA (SMD -3.15, 95% CI -4.75, -1.55, I^2 not assessed), and the largest effect size of any enzyme or transport inhibitor was AA5HT (SMD -2.27, 95% CI -4.05, -0.49, I^2 not assessed). SMD and confidence intervals of individual drugs on MPO activity are given in table 4. Thirteen of 21 cannabinoid drugs had a large clinical effect, the remaining of which had no significant effect on MPO.

Time of administration

From the 50 publications examining the effect of cannabinoids on murine colitis, 28 studies administered cannabinoid agents either simultaneously with colitis onset, or prophylactically. 17 administered drugs between 15 minutes and 7 days after the onset of colitis. Additionally 7 studies compared the benefit of prophylactic cannabinoid use to therapeutic, but did not find any difference in efficacy. To investigate if timing of drug treatment affected DAI or MPO we compared study size-weighted effect sizes (dependent variable) with time of administration (covariate) using meta-regression. We found that timing of drug administration weakly predicted effect size in reducing DAI and MPO, although this was of borderline statistical significance (P=0.09 R^2=11% and P=0.055 R^2=41% respectively, figure 6 A and B).

Quality and risk of bias

Of the 53 papers, 21 used randomisation in their design, 7 reported blinding of assessment, 5 replicated their results in a second species, and 14 replicated their findings in a second model of colitis. 50 reported n≥5 in control and experimental groups. 15 publications reported specific numbers within groups. All papers reported a clinically relevant endpoint. Median study quality modified STAIR score was 5 out of 10 (mean 4.9, SD 2.29). Using meta-regression, higher quality scores predicted greater reductions in MPO activity (P=0.043 R^2=65%, figure 6 D), but not in DAI (P=0.98 R^2= 35%, Figure 6 C).

The SYRCLE risk of bias score for each endpoint showed a trend to larger reduction in DAI in studies with a larger risk of bias (P=0.084 R^2=69%, figure 6 E), but not MPO (P=0.345 R^2=8%, figure 6F).

Publication Bias

Funnel plots comparing MPO activity and DAI were constructed and analysed statistically for bias. The presence of publication bias was not found in either group (MPO; Egger's statistic P=0.570, figure 7A; DAI; Egger's statistic P=0.274, figure 7B).

Discussion

The aim of this study was to determine the efficacy of cannabinoid drugs in reducing gut inflammation to aid the design of further clinical studies. We found 53 studies that examined this effect using endocannabinoids, phytocannabinoids, synthetic cannabinoids, and enzyme and reuptake inhibitors across multiple models of murine and human colitis. In both qualitative assessment and meta-analysis, these controlled studies demonstrate that the use of cannabinoid drugs are beneficial in reducing colonic inflammation in rats and mice, with unclear effects in human subjects.

In animal studies, cannabinoids were shown to reduce inflammation both qualitatively, and at meta-analysis. Across experiments included in this review CB_2 agonists, FAAH inhibitors and CBD were the most widely studied and showed the greatest therapeutic benefit across all endpoints. Subgroup analyses suggested that CBG caused the greatest reduction in MPO activity scores followed by synthetic CB_1 agonist ACEA. However both agents were only studied within a single publication. In the MPO analysis the most studied drug was CBD, with 157 animals across 7 publications, demonstrating a significant effect on MPO activity reduction. Similarly, within DAI analysis CBD was again the most studied single drug including 181 animal across 6 publications. Although CBD demonstrated a significant effect on DAI reduction, the largest reduction in DAI was caused by the FAAH antagonist URB597, studied in one publication. There was statistical heterogeneity in both MPO and DAI analyses, which was partially accounted for by subgroup differences. At meta-regression, factors leading to subgroup differences were quality, timing and risk of bias.

Receptor targets were explored in 23 publications using receptor-specific agonists or antagonists, and receptor knock-down. In murine colitis, agonism of the CB_1 or CB_2 receptor brought about reduction in inflammation, and at subgroup analysis use of the synthetic CB_1/CB_2 agonists acting demonstrated the greatest reduction in disease scores and MPO activity. In addition, agonism of the PPARα, GPR55 and GPR18 receptors also reduced inflammation of the colon. The wide variation in the measured inflammatory endpoints across these studies prevented further meta-analysis. Interestingly the use of the peripherally restricted synthetic agonist SAB378, which agonises both CB_1 and CB_2 receptors, had no significant effect on either MPO activity or DAI. This is in contrast to *ex vivo* explant human colonic data, which demonstrated that cannabinoid agonism with AEA or CBD was beneficial in colonic mucosal inflammation, which were peripherally restricted by definition of the explant model (30,31). Izzo et al. (9) found through receptor antagonism that the effect of CBN in preventing hypermobility caused by croton oil was mediated by CB_1, but not CB_2. PEA was investigated by Capasso et al. (20,32) using two models of inflammation-induced hypermotility. Using receptor antagonists in both experiments Capasso et al. found that PEA, in an OM model, acted through CB_1 but not CB_2 or PPARα, but in a CO model PEA was still effective, but did not act through CB_1 or CB_2. This suggests that the mechanism by which PEA acts as an anti-inflammatory agent was not mediated by a single receptor, but by receptor co-dependence. ACEA was investigated for receptor mechanism in two publications, both of which found ACEA dependent on CB_1. None of the reviewed studies investigated a mechanism of action for AEA in gut inflammation, however one *ex vivo* human study from Harvey et al. found that AEA prevented increased cytokine production in experimentally inflamed human mucosa was dependent on CB_2, although the authors did not report antagonism of any other receptor (31).

The specific mechanism by which manipulation of the cannabinoid system affects inflammation is not clear. Esposito et al. (33) demonstrated that PEA brought about anti-inflammatory effects on enteric glial cells acting at toll-like receptor 4, suggesting that rather than acting at an epithelial mucosal level, acts at either at innate immune colonies or the enteric nervous system. This hypothesis as recently been evidence by a study demonstrating that both CBD and PEA do not act on the immune response of epithelial cells, but are likely to require the presence of these other cells types, acting through down regulation of NF-κβ (34), but is challenged by Cluny et al, demonstrating that peripherally restricted cannabinoids have a diminished effect on inflammation. Nevertheless it is clear that the mechanism of action of cannabinoids does not simply lie at the epithelial level, but is likely to reside within the gut-brain axis.

From the clinical literature we found two randomised placebo-controlled studies examining the effect of phytocannabinoids in humans. Our analysis found no overall effect of THC or CBD on disease scores, however there was large statistical and clinical heterogeneity between these studies. We found from meta-analysis that inhaled THC did have a beneficial effect on CDAI at 8 weeks, whereas CBD did not. There may be several reasons for this heterogeneity, firstly in all groups, small cohort sizes were used which may have overestimated positive or negative effects in both studies, making meaningful conclusions regarding the use of CBD or THC in inflammatory bowel disease difficult. Secondly, within the Naftali et al. (2017) study, very low doses of CBD were utilized compared to the use of CBD in other clinical trials, which commonly used 600mg twice daily (35). A recent trial in drug-resistant epilepsy used 20mg.kg^{-1} daily for 4 weeks, with a small number of participants experiencing side effects such as vomiting and diarrhoea (36). It is likely that in adult males such 10mg doses had no clinical effect on Crohns disease as insufficient plasma concentrations may have been reached due to the poor bioavailability of oral CBD. A major flaw within the Naftali et al. 2013 trial is that sham cigarettes contained cannabis sativa flowers in which active cannabinoids had been removed. However, it is unlikely that other compounds present in cannabis (such as terpenes) which are known to have an anti-inflammatory effect had also been removed, which may have introduced positive bias into the study (37). However, despite these drawbacks, the Naftali et al. 2013 trial demonstrated a significant reduction in pain and the use of steroid therapy, with increased sleep and satisfaction levels with THC use compared to placebo. Although not included in this analysis, a study from Storr et al. (38) demonstrated that although cannabis use provided symptomatic relief from Crohns disease, the risk of salvage surgery was increased within 6 months of use (odds ratio = 5.03, 95% confidence interval = 1.45-17.46). However these findings have not yet been supported from randomised, blinding controlled trials. We may suggest, therefore, that phytocannabinoid use may be a future therapy in intestinal inflammation, although before firm conclusions are drawn, further clinical studies examining their effects be conducted at higher, therapeutic dosages with adequately powered cohort sizes. As MMJ use in inflammatory bowel disease has been justified because of its effects on appetite and diarrhoea, studies may be designed to examine these quality of life-affecting endpoints directly.

We found that most of the existing cannabinoid-gut research focusses on the therapeutic potential of CBD. This is unsurprising as CBD is currently used clinically, is well tolerated, and has shown consistently positive results. Nine studies found a positive, dose dependent effect on local inflammatory cytokine expression, COX2 activation, MPO activity, enteric glial cell activation and caspase-3 production, with associated improvements in macroscopic and histologic grades of inflammation (39–46). One study also showed that intraperitoneal CBD administration decreased oxidative-stress scores of peripheral lung and brain tissue following intestinal inflammation (47), adding to the existing evidence that CBD maintains the gut barrier during inflammation (48). Despite being the most-studied drug, the mechanism by which CBD acts was not made clear by this review. One study by De Fillipis et al (44), found that hyper-motility caused by LPS administration in mice was reduced by CBD through a CB_1 dependent mechanism. Similarly, Capasso et al. in 2008 found that CBD prevented croton oil-induced hypermotility via CB_1. *In vitro*, de Fillipis et al. in 2011 demonstrated that in human explant tissue S100B levels, as a marker of glial cell activation was decreased by CBD in a PPARγ dependent mechanism (although other antagonists were not investigated) (49).

The timing of cannabinoid administration correlated with reduction in effect on colitis activity, although did not reach statistical significance. There was a correlation between time of drug administration and effect size in both DAI and MPO reduction, with earlier administration of cannabinoids drugs producing a greater effect size, suggesting that in clinical trials cannabinoids may be used prophylactically and therapeutically. There is promise therefore that compounds targeting the endocannabinoid system may be able to not only prevent colonic inflammation, but treat established intestinal inflammatory conditions. As it is not clear if cannabinoids are more effective when treating new-onset or established intestinal inflammation, further study designs should investigate this endpoint specifically.

One important potential area for research is the combination of cannabinoid drugs with existing treatments for inflammatory bowel disease. In clinical practice it is common to treat patients with acute severe Crohns and ulcerative colitis with combination of agents, such as antibiotic, anti-TNFα, and corticosteroid therapy. One study compared the efficacy of CBD and THC with that of sulphasalazine, a 5-ASA, a drug commonly used in clinical practice (45). Although in this study CBD and THC efficacy were comparable to that of sulphasalazine, the authors did not examine for the potential additive or subtractive effect of these agents in the context of colitis.

The findings of this study are limited by several factors typically seen in meta-analyses and systematic reviews. We found significant heterogeneity between sub-groups in both DAI and MPO analyses, and suggested that 11% and 41% of this was due to the difference in time of administration in terms of changes in DAI and MPO respectively. Additionally we found a high risk of bias study design, and median study quality to be relatively low. Meta-regression demonstrated these factors significantly correlated with study outcomes. Although we did not analyse for differences between scoring systems and mode of colitis, these factors may have also contributed to heterogeneity and influenced outcome. We sought to overcome this variability between scoring systems with random effects analysis. Additionally within this review we have examined the effect of cannabinoid drugs *en mass*, which may have affected the overall outcome of meta-analyses. It is possible that some articles may have not been identified in initial searches, or conference abstracts missed from the search period. Lastly, where control groups were compared to multiple experimental groups within the same set of experiments variance and SMD may be exaggerated, leading to further bias.

In conclusion, we have shown in this systematic review and meta-analysis that cannabinoid drugs are beneficial in treating experimentally-induced murine models of colitis. These positive findings support the development of further human clinical trials. Current literature converges on CBD, and in order to avoid research bias the effect of all cannabinoid drugs, including the large number of currently un-investigated phytocannabinoid drugs, should also be investigated.

References

1. Kappelman MD, Rifas–Shiman SL, Kleinman K, Ollendorf D, Bousvaros A, Grand RJ, et al. The Prevalence and Geographic Distribution of Crohn's Disease and Ulcerative Colitis in the United States. Clin Gastroenterol Hepatol [Internet]. 2007 Dec;5(12):1424–9.

2. Rubin GP, Hungin AP, Kelly PJ, Ling J. Inflammatory bowel disease: epidemiology and management in an English general practice population. Aliment Pharmacol Ther [Internet]. 2000 Dec;14(12):1553–9.

3. Sandborn WJ, Rutgeerts P, Feagan BG, Reinisch W, Olson A, Johanns J, et al. Colectomy Rate Comparison After Treatment of Ulcerative Colitis With Placebo or Infliximab. Gastroenterology [Internet]. 137:1250–60.

4. Sutherland LR, Martin F, Bailey RJ, Fedorak RN, Poleski M, Dallaire C, et al. A Randomized, Placebo-Controlled, Double-Blind Trial of Mesalamine in the Maintenance of Remission of Crohn's Disease. Gastroenterology [Internet]. 1997;112:1069–77.

5. Herrinton LJ, Liu L, Weng X, Lewis JD, Hutfless S, Allison JE. Role of Thiopurine and Anti-TNF Therapy in Lymphoma in Inflammatory Bowel Disease. Am J Gastroenterol [Internet]. 2011 Dec 25;106(12):2146–53.

6. Alhamoruni A, Wright KL, Larvin M, O'Sullivan SE. Cannabinoids mediate opposing effects on inflammation-induced intestinal permeability. Br J Pharmacol. 2012;165:2598–610.

7. Storr M a., Sharkey K a. The endocannabinoid system and gut-brain signalling. Curr Opin Pharmacol. 2007;7:575–82.

8. Lee Y, Jo J, Chung HY, Pothoulakis C, Im E. Endocannabinoids in the gut. Am J Physiol Gastrointest Liver Physiol [Internet]. 2016 Aug 18;ajpgi.00294.2015.

9. Izzo a a, Fezza F, Capasso R, Bisogno T, Pinto L, Iuvone T, et al. Cannabinoid CB1-receptor mediated regulation of gastrointestinal motility in mice in a model of intestinal inflammation. Br J Pharmacol. 2001;134:563–70.

10. Di Marzo V, Izzo a a. Endocannabinoid overactivity and intestinal inflammation. Gut. 2006;55:1373–6.

11. Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, et al. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science [Internet]. 1992 Dec 18;258(5090):1946–9.

12. Freund TF, Katona I, Piomelli D. Role of endogenous cannabinoids in synaptic signaling. Physiol Rev [Internet]. 2003 Jul;83(3):1017–66.

13. Muccioli GG. Endocannabinoid biosynthesis and inactivation, from simple to complex. Vol. 15, Drug Discovery Today. 2010. p. 474–83.

14. Blankman JL, Simon GM, Cravatt BF. A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. Chem Biol [Internet]. 2007 Dec;14(12):1347–56.

15. O'Sullivan SE. Cannabinoids go nuclear: evidence for activation of peroxisome proliferator-activated receptors. Br J Pharmacol [Internet]. 2007 Nov;152(5):576–82.

16. Smart D, Jonsson K-O, Vandevoorde S, Lambert DM, Fowler CJ. "Entourage" effects of N-acyl ethanolamines at human vanilloid receptors. Comparison of effects upon anandamide-induced vanilloid receptor activation and upon anandamide metabolism. Br J Pharmacol [Internet]. 2002 Jun;136(3):452–8.

17. Leocani L, Nuara A, Houdayer E, Schiavetti I, Del Carro U, Amadio S, et al. Sativex(®) and clinical-neurophysiological measures of spasticity in progressive multiple sclerosis. J Neurol [Internet]. 2015 Nov;262(11):2520–7.

18. Reddy DS, Golub VM. The Pharmacological Basis of Cannabis Therapy for Epilepsy. J Pharmacol Exp Ther [Internet]. 2016 Mar 1;357(1):45–55.

19. Suárez J, Romero-Zerbo Y, Márquez L, Rivera P, Iglesias M, Bermúdez-Silva FJ, et al. Ulcerative colitis impairs the acylethanolamide-based anti-inflammatory system reversal by 5-aminosalicylic acid and glucocorticoids. PLoS One [Internet]. 2012 Jan;7(5):e37729.

20. Capasso R, Orlando P, Pagano E, Aveta T, Buono L, Borrelli F, et al. Palmitoylethanolamide normalizes intestinal motility in a model of post-inflammatory accelerated transit: involvement of CB_1 receptors and TRPV1 channels. Br J Pharmacol [Internet]. 2014 Sep;171(17):4026–37.

21. Akbar A, Yiangou Y, Facer P, Brydon WG, Walters JRF, Anand P, et al. Expression of the TRPV1 receptor differs in quiescent inflammatory bowel disease with or without abdominal pain. Gut [Internet]. 2010 Jun 1;59(6):767–74.

22. D'Argenio G, Valenti M, Scaglione G, Cosenza V, Sorrentini I, Di Marzo V. Up-regulation of anandamide levels as an endogenous mechanism and a pharmacological strategy to limit colon inflammation. FASEB J. 2006;20:568–70.

23. D'Argenio G, Petrosino S, Gianfrani C, Valenti M, Scaglione G, Grandone I, et al. Overactivity of the intestinal endocannabinoid system in celiac disease and in methotrexate-treated rats. J Mol Med. 2007;85:523–30.

24. Karwad MA, Macpherson T, Wang B, Theophilidou E, Sarmad S, Barrett DA, et al. Oleoylethanolamine and palmitoylethanolamine modulate intestinal permeability in vitro via TRPV1 and PPARα. FASEB J [Internet]. 2016 Sep 13;fj.201500132.

25. Massa F, Marsicano G, Hermann H, Cannich A, Monory K, Cravatt BF, et al. The endogenous cannabinoid system protects against colonic inflammation. J Clin Invest [Internet]. 2004 Apr 15;113(8):1202–9.

26. Higgins JPT, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ [Internet]. 2011;343.

27. Fisher M, Feuerstein G, Howells DW, Hurn PD, Kent TA, Savitz SI, et al. Update of the stroke therapy academic industry roundtable preclinical recommendations. Stroke [Internet]. 2009 Jun;40(6):2244–50.

28. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. PLOS Biol [Internet]. 2010;8(6).

29. Hooijmans CR, Rovers MM, de Vries RBM, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE's risk of bias tool for animal studies. BMC Med Res Methodol [Internet]. 2014 Mar 26;14:43.

30. Harvey BS, Sia TC, Wattchow D a., Smid SD. Interleukin 17A evoked mucosal damage is attenuated by cannabidiol and anandamide in a human colonic explant model. Cytokine [Internet]. 2014;65(2):236–44.

31. Nicotra LL, Vu M, Harvey BS, Smid SD. Prostaglandin ethanolamides attenuate damage in a human explant colitis model. Prostaglandins Other Lipid Mediat [Internet]. 2013;100–101:22–9.

32. Capasso R, Izzo AA, Fezza F, Pinto A, Capasso F, Mascolo N, et al. Inhibitory effect of palmitoylethanolamide on gastrointestinal motility in mice. Br J Pharmacol [Internet]. 2001 Nov;134(5):945–50.

33. Esposito G, Capoccia E, Turco F, Palumbo I, Lu J, Steardo A, et al. Palmitoylethanolamide improves colon inflammation through an enteric glia/toll like receptor 4-dependent PPAR-α activation. Gut [Internet]. 2014;1300–12.

34. Couch DG, Tasker C, Theophilidou E, Lund JN, O'Sullivan SE. Cannabidiol and palmitoylethanolamide are anti-inflammatory in the acutely inflamed human colon. Clin Sci [Internet]. 2017 Nov 1;131(21):2611–26.

35. Naftali T, Mechulam R, Marii A, Gabay G, Stein A, Bronshtain M, et al. Low-Dose Cannabidiol Is Safe but Not Effective in the Treatment for Crohn's Disease, a Randomized Controlled Trial. Dig Dis Sci [Internet]. 2017 Mar 27;

36. Devinsky O, Cross JH, Laux L, Marsh E, Miller I, Nabbout R, et al. Trial of Cannabidiol for Drug-Resistant Seizures in the Dravet Syndrome. N Engl J Med [Internet]. 2017 May 25;376(21):2011–20.

37. de las Heras B, Hortelano S. Molecular basis of the anti-inflammatory effects of terpenoids. Inflamm Allergy Drug Targets [Internet]. 2009 Mar;8(1):28–39.

38. Storr M, Devlin S, Kaplan GG, Panaccione R, Andrews CN. Cannabis Use Provides Symptom Relief in Patients with Inflammatory Bowel Disease but Is Associated with Worse Disease Prognosis in Patients with Crohn's Disease. Inflamm Bowel Dis [Internet]. 2014 Mar;20(3):472–80.

39. Pagano E, Capasso R, Piscitelli F, Romano B, Parisi OA, Finizio S, et al. An Orally Active Cannabis Extract with High Content in Cannabidiol attenuates Chemically-induced Intestinal Inflammation and Hypermotility in the Mouse. Front Pharmacol [Internet]. 2016 Oct 4;7:341.

40. Krohn RM, Parsons SA, Fichna J, Patel KD, Yates RM, Sharkey KA, et al. Abnormal cannabidiol attenuates experimental colitis in mice, promotes wound healing and inhibits neutrophil recruitment. J Inflamm (Lond) [Internet]. 2016 Dec 14;13(1):21.

41. Harvey BS, Nicotra LL, Vu M, Smid SD. Cannabinoid CB2 receptor activation attenuates cytokine-evoked mucosal damage in a human colonic explant model without changing epithelial permeability. Cytokine. 2013;63:209–17.

42. Schicho R, Storr M. Topical and systemic cannabidiol improves trinitrobenzene sulfonic acid colitis in mice. Pharmacology [Internet]. 2012 Jan;89(3–4):149–55.

43. Lin X-H, Yuece B, Li Y-Y, Feng Y-J, Feng J-Y, Yu L-Y, et al. A novel CB receptor GPR55 and its ligands are involved in regulation of gut movement in rodents. Neurogastroenterol Motil [Internet]. 2011 Sep;23(9):862-e342.

44. de Filippis D, Iuvone T, D'amico A, Esposito G, Steardo L, Herman AG, et al. Effect of cannabidiol on sepsis-induced motility disturbances in mice: involvement of CB receptors and fatty acid amide hydrolase. Neurogastroenterol Motil [Internet]. 2008 Aug;20(8):919–27.

45. Jamontt JM, Molleman A, Pertwee RG, Parsons ME. The effects of delta 9-tetrahydrocannabinol and cannabidiol alone and in combination on damage, inflammation and in vitro motility disturbances in rat colitis. Br J Pharmacol. 2010;160(November 2009):712–23.

46. Borrelli F, Aviello G, Romano B, Orlando P, Capasso R, Maiello F, et al. Cannabidiol, a safe and non-psychotropic ingredient of the marijuana plant Cannabis sativa, is protective in a murine model of colitis. J Mol Med. 2009;87:1111–21.

47. Cassol-Jr OJ, Comim CM, Silva BR, Hermani F V., Constantino LS, Felisberto F, et al. Treatment with cannabidiol reverses oxidative stress parameters, cognitive impairment and mortality in rats submitted to sepsis by cecal ligation and puncture. Brain Res [Internet]. 2010 Aug 12;1348:128–38.

48. Alhamoruni A, Lee C, Wright KL, Larvin M, O'Sullivan SE. Pharmacological effects of cannabinoids on the Caco-2 cell culture model of intestinal permeability. J Pharmacol Exp Ther. 2010;335(1):92–102.

49. de Filippis D, Esposito G, Cirillo C, Cipriano M, de Winter BY, Scuderi C, et al. Cannabidiol reduces intestinal inflammation through the control of neuroimmune axis. PLoS One [Internet]. 2011;6(12):1–8.

50. Mathison R, Ho W, Pittman QJ, Davison JS, Sharkey K a. Effects of cannabinoid receptor-2 activation on accelerated gastrointestinal transit in lipopolysaccharide-treated rats. Br J Pharmacol. 2004;142:1247–54.

51. Kimball ES, Schneider CR, Wallace NH, Hornby PJ. Agonists of cannabinoid receptor 1 and 2 inhibit experimental colitis induced by oil of mustard and by dextran sulfate sodium. Am J Physiol Gastrointest Liver Physiol. 2006;291:G364–71.

52. Capasso R, Borrelli F, Aviello G, Romano B, Scalisi C, Capasso F, et al. Cannabidiol, extracted from Cannabis sativa, selectively inhibits inflammatory hypermotility in mice. Br J Pharmacol. 2008;154:1001–8.

53. Engel M a., Kellermann C a., Rau T, Burnat G, Hahn EG, Konturek PC. Ulcerative colitis in AKR mice is attenuated by intraperitoneally administered anandamide. J Physiol Pharmacol. 2008;59(Cd):673–89.

54. Storr MA, Keenan CM, Emmerdinger D, Zhang H, Yüce B, Sibaev A, et al. Targeting endocannabinoid degradation protects against experimental colitis in mice: involvement of CB1 and CB2 receptors. J Mol Med (Berl) [Internet]. 2008 Aug;86(8):925–36.

55. li y. -y., li y. -n., ni j. -b., chen c. -j., lv s., chai s. -y., et al. Involvement of cannabinoid-1 and cannabinoid-2 receptors in septic ileus. Neurogastroenterol Motil [Internet]. 2010 Mar 1;22(3):350-e88.

56. Storr M a., Keenan CM, Zhang H, Patel KD, Makriyannis A, Sharkey K a. Activation of the cannabinoid 2 receptor (CB2) protects against experimental colitis. Inflamm Bowel Dis. 2009;15(11):1678–85.

57. Cluny NL, Keenan CM, Duncan M, Fox A, Lutz B, Sharkey K a. (SAB378), a Peripherally Restricted Cannabinoid CB 1 / CB 2 Receptor Agonist , Inhibits Gastrointestinal Motility but Has No Effect on Experimental Colitis in Mice. 2010;334(3):973–80.

58. Kimball ES, Wallace NH, Schneider CR, D 'andrea MR, Hornby PJ, Storr M. Small intestinal cannabinoid receptor changes following a single colonic insult with oil of mustard in mice. Front Pharmacol [Internet]. 2010;1(132):1–8.

59. Alhouayek M, Lambert DM, Delzenne NM, Cani PD, Muccioli GG. Increasing endogenous 2-arachidonoylglycerol levels counteracts colitis and related systemic inflammation. FASEB J. 2011;25:2711–21.

60. Andrzejak V, Muccioli GG, Body-Malapel M, El Bakali J, Djouina M, Renault N, et al. New FAAH inhibitors based on 3-carboxamido-5-aryl-isoxazole scaffold that protect against experimental colitis. Bioorg Med Chem [Internet]. 2011 Jun 15;19(12):3777–86.

61. Bento AF, Marcon R, Dutra RC, Claudino RF, Cola M, Leite DFP, et al. β-Caryophyllene inhibits dextran sulfate sodium-induced colitis in mice through CB2 receptor activation and PPARγ pathway. Am J Pathol [Internet]. 2011 Mar;178(3):1153–66.

62. Schicho R, Bashashati M, Bawa M, McHugh D, Saur D, Hu H-M, et al. The atypical cannabinoid O-1602 protects against experimental colitis and inhibits neutrophil recruitment. Inflamm Bowel Dis [Internet]. 2011 Aug;17(8):1651–64.

63. Bashashati M, Storr M a., Nikas SP, Wood JT, Godlewski G, Liu J, et al. Inhibiting fatty acid amide hydrolase normalizes endotoxin-induced enhanced gastrointestinal motility in mice. Br J Pharmacol. 2012;165:1556–71.

64. Izzo AA, Capasso R, Aviello G, Borrelli F, Romano B, Piscitelli F, et al. Inhibitory effect of cannabichromene, a major non-psychotropic cannabinoid extracted from Cannabis sativa, on inflammation-induced hypermotility in mice. Br J Pharmacol [Internet]. 2012 Jun;166(4):1444–60.

65. Lehmann C, Kianian M, Zhou J, Küster I, Kuschnereit R, Whynot S, et al. Cannabinoid receptor 2 activation reduces intestinal leukocyte recruitment and systemic inflammatory mediator release in acute experimental sepsis. Crit Care [Internet]. 2012 Dec 12;16(2):R47.

66. Singh UP, Singh NP, Singh B, Price RL, Nagarkatti M, Nagarkatti PS. Cannabinoid receptor-2 (CB2) agonist ameliorates colitis in IL-10(-/-) mice by attenuating the activation of T cells and promoting their apoptosis. Toxicol Appl Pharmacol [Internet]. 2012 Jan 15;258(2):256–67.

67. Borrelli F, Fasolino I, Romano B, Capasso R, Maiello F, Coppola D, et al. Beneficial effect of the non-psychotropic plant cannabinoid cannabigerol on experimental inflammatory bowel disease. Biochem Pharmacol [Internet]. 2013;85(9):1306–16.

68. Li YY, Yuece B, Cao HM, Lin HX, Lv S, Chen JC, et al. Inhibition of p38/Mk2 signaling pathway improves the anti-inflammatory effect of WIN55 on mouse experimental colitis. Lab Investig [Internet]. 2013 Mar 4;93(3):322–33.

69. Matos I, Bento AF, Marcon R, Claudino RF, Calixto JB. Preventive and therapeutic oral administration of the pentacyclic triterpene α,β-amyrin ameliorates dextran sulfate sodium-induced colitis in mice: the relevance of cannabinoid system. Mol Immunol [Internet]. 2013 Jul;54(3–4):482–92.

70. Naftali T, Bar-Lev Schleider L, Dotan I, Lansky EP, Sklerovsky Benjaminov F, Konikoff FM. Cannabis induces a clinical response in patients with Crohn's disease: a prospective placebo-controlled study. Clin Gastroenterol Hepatol [Internet]. 2013 Oct;11(10):1276–1280.e1.

71. Romano B, Borrelli F, Fasolino I, Capasso R, Piscitelli F, Cascio M, et al. The cannabinoid TRPA1 agonist cannabichromene inhibits nitric oxide production in macrophages and ameliorates murine colitis. Br J Pharmacol [Internet]. 2013 May;169(1):213–29.

72. Wallace JL, Flannigan KL, McKnight W, Wang L, Ferraz JGP, Tuitt D. Pro-resolution, protective and anti-nociceptive effects of a cannabis extract in the rat gastrointestinal tract. J Physiol Pharmacol [Internet]. 2013 Apr;64(2):167–75.

73. Borrelli F, Romano B, Petrosino S, Pagano E, Capasso R, Coppola D, et al. Palmitoylethanolamide, a naturally occurring lipid, is an orally effective intestinal anti-inflammatory agent. Br J Pharmacol [Internet]. 2015;172:142–58.

74. Fichna J, Sałaga M, Stuart J, Saur D, Sobczak M, Zatorski H, et al. Selective inhibition of FAAH produces antidiarrheal and antinociceptive effect mediated by endocannabinoids and cannabinoid-like fatty acid amides. Neurogastroenterol Motil [Internet]. 2014 Apr;26(4):470–81.

75. Sałaga M, Mokrowiecka A, Zakrzewski PK, Cygankiewicz A, Leishman E, Sobczak M, et al. Experimental colitis in mice is attenuated by changes in the levels of endocannabinoid metabolites induced by selective inhibition of fatty acid amide hydrolase (FAAH). J Crohns Colitis [Internet]. 2014 Sep 1;8(9):998–1009.

76. Sardinha J, Kelly MEM, Zhou J, Lehmann C. Experimental cannabinoid 2 receptor-mediated immune modulation in sepsis. Mediators Inflamm [Internet]. 2014;2014:978678.

77. Alhouayek M, Bottemanne P, Subramanian K V, Lambert DM, Makriyannis A, Cani PD, et al. N-Acylethanolamine-hydrolyzing acid amidase inhibition increases colon N-palmitoylethanolamine levels and counteracts murine colitis. FASEB J [Internet]. 2015 Feb;29(2):650–61.

78. El Bakali J, Muccioli GG, Body-Malapel M, Djouina M, Klupsch F, Ghinet A, et al. Conformational Restriction Leading to a Selective CB2 Cannabinoid Receptor Agonist Orally Active Against Colitis. ACS Med Chem Lett [Internet]. 2015 Feb 12;6(2):198–203.

79. Impellizzeri D, Di Paola R, Cordaro M, Gugliandolo E, Casili G, Morittu VM, et al. Adelmidrol, a palmitoylethanolamide analogue, as a new pharmacological treatment for the management of acute and chronic inflammation. Biochem Pharmacol [Internet]. 2016 Nov 1;119:27–41.

80. Sasso O, Migliore M, Habrant D, Armirotti A, Albani C, Summa M, et al. Multitarget fatty acid amide hydrolase/cyclooxygenase blockade suppresses intestinal inflammation and protects against nonsteroidal anti-inflammatory drug-dependent gastrointestinal damage. FASEB J [Internet]. 2015 Jun;29(6):2616–27.

81. Stančić A, Jandl K, Hasenöhrl C, Reichmann F, Marsche G, Schuligoi R, et al. The GPR55 antagonist CID16020046 protects against intestinal inflammation. Neurogastroenterol Motil. 2015;27(10).

82. Cordaro M, Impellizzeri D, Gugliandolo E, Siracusa R, Crupi R, Esposito E, et al. Adelmidrol, a Palmitoylethanolamide Analogue, as a New Pharmacological Treatment for the Management of Inflammatory Bowel Disease. Mol Pharmacol [Internet]. 2016 Oct 3;90(5):549–61.

83. Feng Y-J, Li Y-Y, Lin X-H, Li K, Cao M-H. Anti-inflammatory effect of cannabinoid agonist WIN55, 212 on mouse experimental colitis is related to inhibition of p38MAPK. World J Gastroenterol [Internet]. 2016 Nov 21;22(43):9515–24.

84. Ke P, Shao B-Z, Xu Z-Q, Wei W, Han B-Z, Chen X-W, et al. Activation of Cannabinoid Receptor 2 Ameliorates DSS-Induced Colitis through Inhibiting NLRP3 Inflammasome in Macrophages. Allen IC, editor. PLoS One [Internet]. 2016 Sep 9;11(9):e0155076.

85. Sarnelli G, D'Alessandro A, Iuvone T, Capoccia E, Gigli S, Pesce M, et al. Palmitoylethanolamide Modulates Inflammation-Associated Vascular Endothelial Growth Factor (VEGF) Signaling via the Akt/mTOR Pathway in a Selective Peroxisome Proliferator-Activated Receptor Alpha (PPAR-α)-Dependent Manner. PLoS One [Internet]. 2016;11(5):e0156198.

86. Lin S, Li Y, Shen L, Zhang R, Yang L, Li M, et al. The Anti-Inflammatory Effect and Intestinal Barrier Protection of HU210 Differentially Depend on TLR4 Signaling in Dextran Sulfate Sodium-Induced Murine Colitis. Dig Dis Sci [Internet]. 2017 Feb 19;62(2):372–86.

87. Shamran H, Singh NP, Zumbrun EE, Murphy A, Taub DD, Mishra MK, et al. Fatty acid amide hydrolase (FAAH) blockade ameliorates experimental colitis by altering microRNA expression and suppressing inflammation. Brain Behav Immun [Internet]. 2017 Jan;59:10–20.

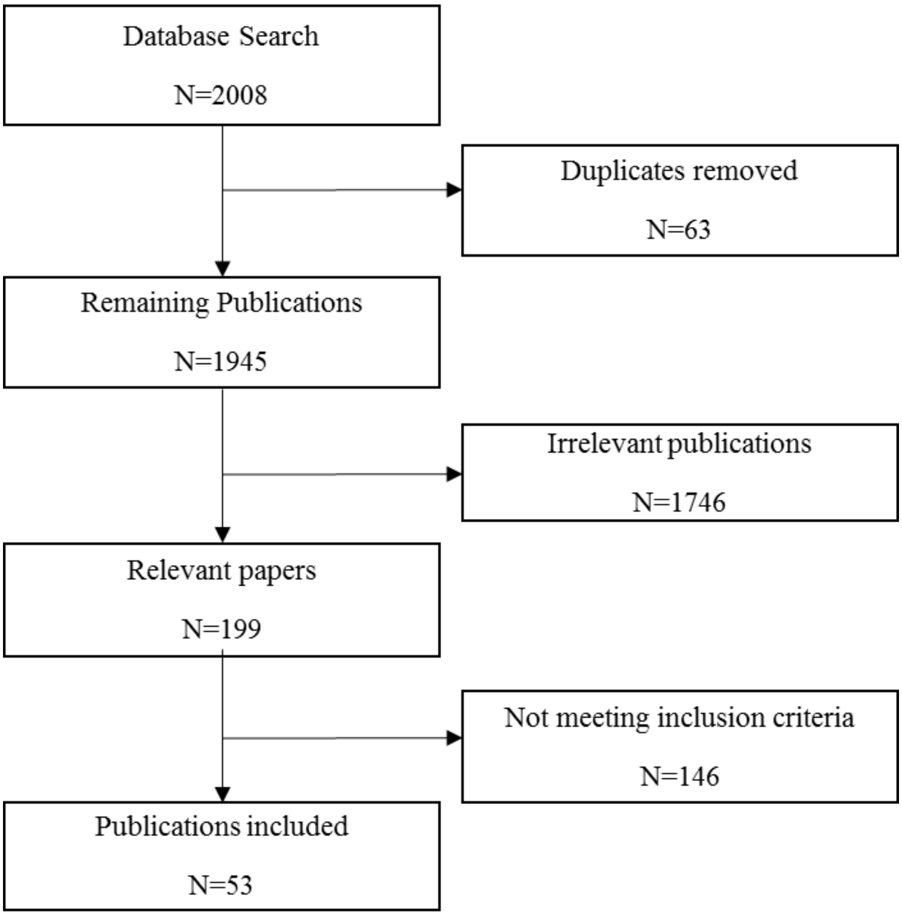


Figure 1. Record identification process

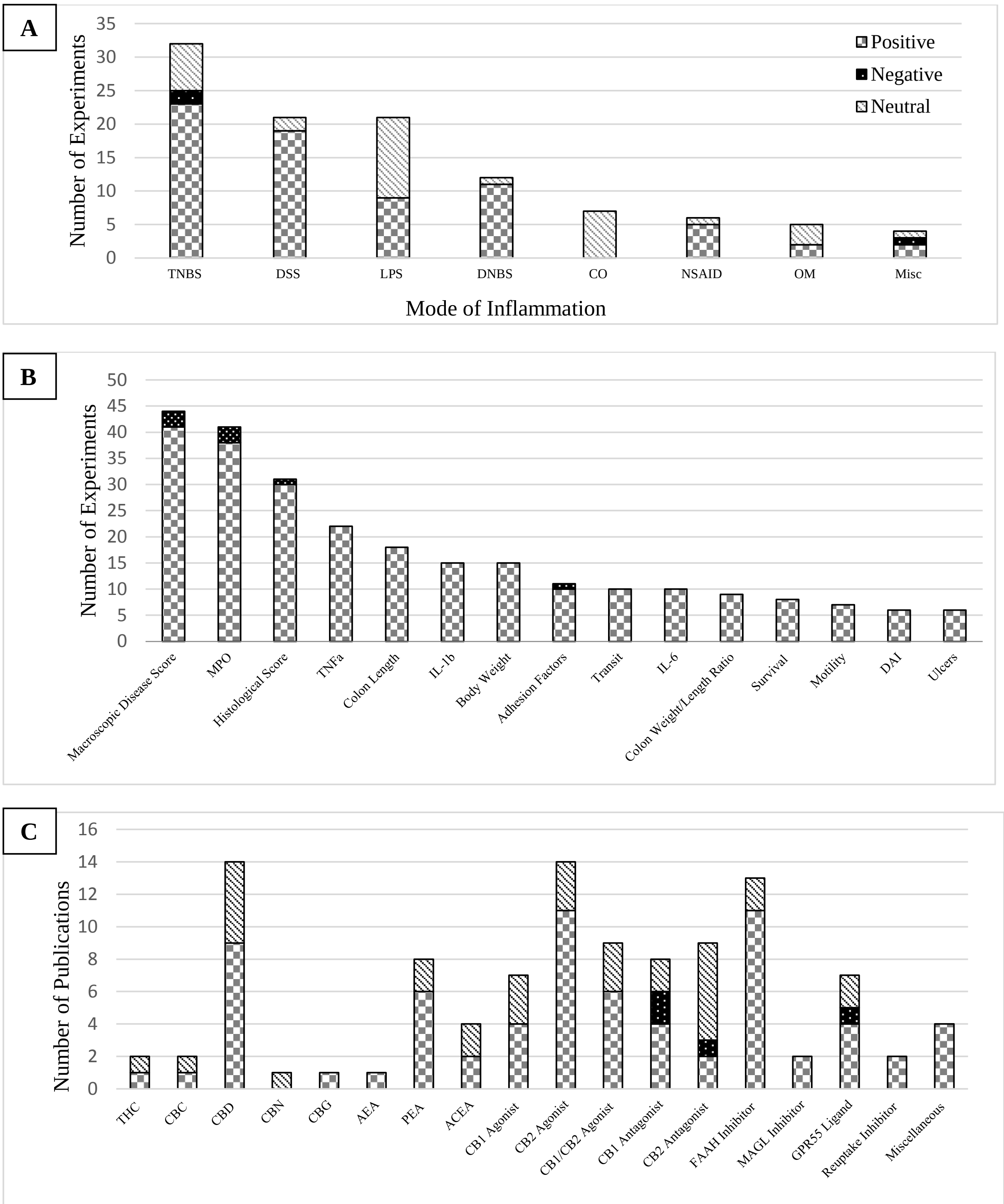


Figure 2. Positive, negative and neutral outcomes of cannabinoid treatment across modes of inflammation (**A**). Incidence of endpoints across all experiments comparing cannabinoid treatment with control (**B**). The effect of cannabinoid drugs compared to control across all endpoints expressed as primary drug investigated (**C**).

	Cannabinoids			Placebo				Mean Difference
Study or Subgroup	**Mean**	**SD**	**Total**	**Mean**	**SD**	**Total**	**Weight**	**IV, Random, 95% CI**
Naftali 2003 THC 115mg INH, 8 wks	152	109	11	306	143	10	50.0%	-154.00 [-263.57, -44.43]
Naftali 2017 CBD 10mg p.o. BD 8 weeks	220	122	10	216	121	9	50.0%	4.00 [-105.39, 113.39]
Total (95% CI)			**21**			**19**	**100.0%**	**-74.97 [-229.81, 79.87]**

Heterogeneity: Tau² = 9361.92; Chi² = 4.00, df = 1 (P = 0.05); I² = 75%

Test for overall effect: Z = 0.95 (P = 0.34)

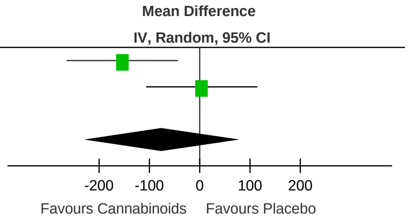
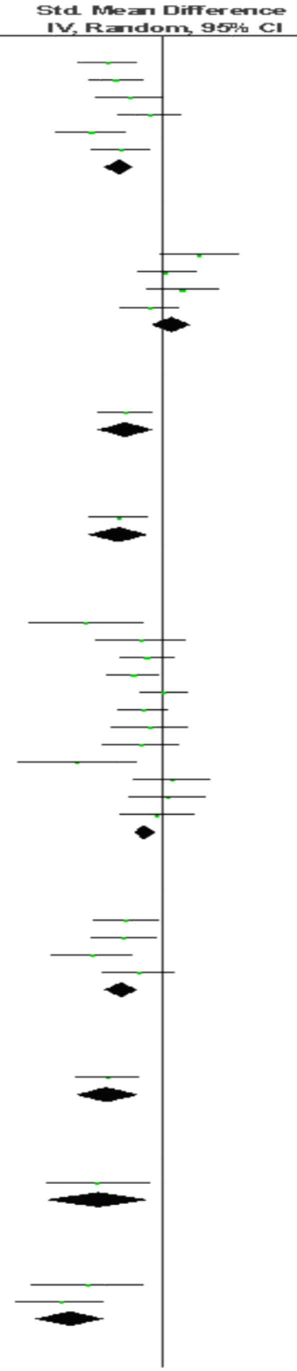
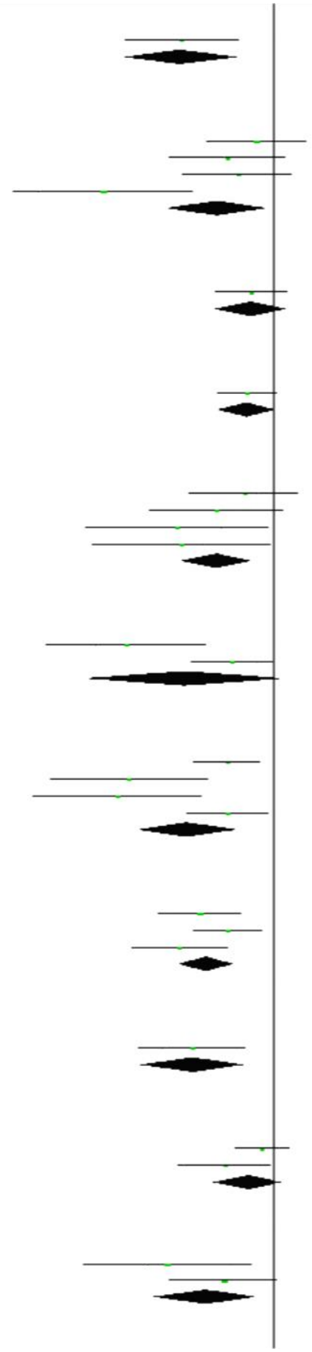


Figure 3. Forest plot of the effects of cannabinoid treatment on Crohns Disease, assessed by reduction in CDAI in human studies.

Study or Subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
1.1.1 PEA								
Borrelli 2014 PEA 1mg/kg p.o. DNBS (t)	50.1	4.5033	12	77.3	19.7454	12	1.9%	-1.83 [-2.81, -0.85]
Borrelli 2014 PEA 1mg/kg i.p. DNBS (t)	48.5	24.2487	12	84.94	20.7846	12	2.0%	-1.56 [-2.49, -0.62]
Esposito 2014 PEA 2mg/kg i.p. DSS (t)	1.12	0.6325	10	1.8	0.4249	5	1.7%	-1.11 [-2.28, 0.06]
Esposito 2014 PEA 10mg/kg i.p. DSS (t)	0.72	2.846	10	1.8	0.4249	5	1.8%	-0.43 [-1.52, 0.66]
Impellizzeri 2015 PEA 10mg/kg i.p. DNBS (t)	40.66	31.3065	10	100	13.0286	10	1.7%	-2.37 [-3.57, -1.17]
Alhouayek 2015 PEA 10mg/kg i.p. DSS (p)	78.3	15.8114	10	100	13.5978	10	1.9%	-1.41 [-2.41, -0.41]
Subtotal (95% CI)			**64**			**54**	**11.1%**	**-1.45 [-1.94, -0.96]**
Heterogeneity: Tau² = 0.09; Chi² = 6.62, df = 5 (P = 0.25); I² = 25%								
Test for overall effect: Z = 5.74 (P < 0.00001)								
1.1.2 SAB378								
Cluny 2010 SAB378 0.1mg/kg i.p. DSS (t)	9.53	1.3294	8	7.2	2.5456	4	1.6%	1.21 [-0.13, 2.54]
Cluny 2010 SAB378 1mg/kg i.p. DSS (t)	7.5	2.2627	8	7.2	2.5456	8	1.9%	0.12 [-0.86, 1.10]
Cluny 2010 SAB378 0.1mg/kg i.p. TNBS (p)	7.45	1.5274	8	6.2	2.2627	4	1.7%	0.65 [-0.59, 1.89]
Cluny 2010 SAB378 1mg/kg i.p.TNBS (p)	5.12	2.5456	8	6.2	2.2627	8	1.9%	-0.42 [-1.42, 0.57]
Subtotal (95% CI)			**32**			**24**	**7.1%**	**0.28 [-0.38, 0.94]**
Heterogeneity: Tau² = 0.13; Chi² = 4.19, df = 3 (P = 0.24); I² = 28%								
Test for overall effect: Z = 0.83 (P = 0.41)								
1.1.3 JZL184								
Alhouayek 2011 JZL184 16mg/kg i.p. TNBS (p)	2.7	2.3216	11	5.13	1.3266	11	2.0%	-1.24 [-2.16, -0.31]
Subtotal (95% CI)			**11**			**11**	**2.0%**	**-1.24 [-2.16, -0.31]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 2.61 (P = 0.009)								
1.1.4 Compound 39								
Andrzejak 2011 Compound 39 5mg/kg i.p. TNBS (p)	2.2	0.9487	10	4.2	1.5811	10	1.9%	-1.47 [-2.48, -0.46]
Subtotal (95% CI)			**10**			**10**	**1.9%**	**-1.47 [-2.48, -0.46]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 2.84 (P = 0.004)								
1.1.5 CBD								
Borrelli 2009 CBD 10mg/kg i.p. DNBS (p)	2.26	0.7155	5	4.2	0.6485	5	1.1%	-2.57 [-4.47, -0.66]
Jamontt 2010 CBD 10mg/kg i.p. TNBS (p)	2.9	3.3166	11	5.51	2.8523	2	1.4%	-0.74 [-2.29, 0.80]
Krohn 2016 abCBD 5mg/kg i.p. TNBS (t)	4.05	2.3085	10	5.38	2.5298	10	2.0%	-0.53 [-1.42, 0.37]
Schicho 2012 CBD 10mg.kg i.p. TNBS (p)	4.3	0.995	11	5.9	1.99	11	2.0%	-0.98 [-1.87, -0.08]
Schicho 2012 CBD 20mg/kg p.o. TNBS (p)	5.57	0.995	11	5.5	1.8905	11	2.1%	0.04 [-0.79, 0.88]
Schicho 2012 CBD 20mg/kg p.r. TNBS (p)	6.15	1.3266	11	7.07	1.3266	11	2.1%	-0.67 [-1.53, 0.20]
Borrelli 2009 CBD 1mg/kg i.p. DNBS (p)	3.9	0.6485	5	4.2	0.6485	5	1.6%	-0.42 [-1.68, 0.84]
Borrelli 2009 CBD 2.5mg/kg i.p. DNBS (p)	3.55	0.9391	5	4.2	0.6485	5	1.6%	-0.73 [-2.03, 0.58]
Borrelli 2009 CBD 5mg/kg i.p. DNBS (p)	2.13	0.6708	5	4.2	0.6485	5	1.0%	-2.83 [-4.85, -0.81]
Jamontt 2010 CBD 5mg/kg i.p. TNBS (p)	6.7	5.3066	11	5.15	2.8523	3	1.6%	0.29 [-0.99, 1.57]
Jamontt 2010 CBD 15mg/kg i.p. TNBS (p)	5.52	1.99	11	5.15	2.8523	3	1.6%	0.16 [-1.12, 1.44]
Jamontt 2010 CBD 20mg/kg i.p. TNBS (p)	4.65	4.6433	11	5.51	2.8523	3	1.6%	-0.18 [-1.46, 1.10]
Subtotal (95% CI)			**107**			**74**	**19.9%**	**-0.56 [-0.97, -0.16]**
Heterogeneity: Tau² = 0.14; Chi² = 15.43, df = 11 (P = 0.16); I² = 29%								
Test for overall effect: Z = 2.75 (P = 0.006)								
1.1.6 WIN 522,212								
Cluny 2010 WIN 52212-2 2mg/kg i.p. DSS (t)	4.2	1.9799	8	7.2	2.5456	8	1.8%	-1.24 [-2.34, -0.15]
Cluny 2010 WIN 52212-2 2mg/kg i.p. TNBS (t)	3.7	1.1314	8	6.2	2.2627	8	1.8%	-1.32 [-2.43, -0.21]
Feng 2016 WIN55,212 5mg/kg i.p. DSS (t)	5.05	2.2627	8	9.77	1.4142	8	1.6%	-2.37 [-3.73, -1.00]
Li 2013 WIN55212-2 5mg/kg i.p. DSS (t)	2.29	0.9798	6	3.5	1.7146	6	1.7%	-0.80 [-2.00, 0.40]
Subtotal (95% CI)			**30**			**30**	**6.9%**	**-1.37 [-1.96, -0.78]**
Heterogeneity: Tau² = 0.00; Chi² = 2.98, df = 3 (P = 0.39); I² = 0%								
Test for overall effect: Z = 4.55 (P < 0.00001)								
1.1.7 Adelmidrol								
Cordaro 2016 Ademidrol 10mg/kg p.o DNBS (t)	4.3	1.8974	10	7.2	0.9487	10	1.8%	-1.85 [-2.94, -0.77]
Subtotal (95% CI)			**10**			**10**	**1.8%**	**-1.85 [-2.94, -0.77]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 3.34 (P = 0.0008)								
1.1.8 AA5HT								
D'Argenio 2006 AA5HT10mg/kg s.c DNBS (t)	1.51	0.8497	5	4.2	1.3416	5	1.2%	-2.16 [-3.90, -0.43]
Subtotal (95% CI)			**5**			**5**	**1.2%**	**-2.16 [-3.90, -0.43]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 2.44 (P = 0.01)								
1.1.9 VDM115								
D'Argenio 2006 VDM11 5mg/kg s.c. DNBS (t)	0.7054	1.1628	5	4.2	1.3416	5	1.1%	-2.51 [-4.40, -0.63]
Storr 2008 VDM11 5mg/kg i.p. TNBS (p)	3.37	1.0752	10	8.09	1.5495	10	1.5%	-3.39 [-4.85, -1.93]
Subtotal (95% CI)			**15**			**15**	**2.6%**	**-3.06 [-4.21, -1.90]**
Heterogeneity: Tau² = 0.00; Chi² = 0.52, df = 1 (P = 0.47); I² = 0%								
Test for overall effect: Z = 5.19 (P < 0.00001)								



1.1.10 AEA								
Engel 2008 AEA 5mg/kg i.p. TNBS (p)	2.6	1.7146	6	8.64	1.9596	6	1.1%	-3.03 [-4.89, -1.17]
Subtotal (95% CI)			**6**			**6**	**1.1%**	**-3.03 [-4.89, -1.17]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 3.19 (P = 0.001)								
1.1.11 AM841								
Fichna 2014 AM841 0.01mg/kg i.p. DSS (p)	7.55	0.8083	6	8.08	0.9798	2	1.3%	-0.55 [-2.19, 1.10]
Fichna 2014 AM841 0.1mg/kg i.p. DSS (p)	5.03	1.8861	6	8.08	0.9798	2	1.1%	-1.50 [-3.40, 0.40]
Fichna 2014 AM841 1mg/kg i.p. DSS (p)	5.3	2.2045	6	8.08	0.9798	2	1.2%	-1.18 [-2.97, 0.62]
Fichna 2014 AM841 1mg/kg i.p. TNBS (p)	4.04	0.2939	6	6.04	0.3674	6	0.6%	-5.55 [-8.49, -2.61]
Subtotal (95% CI)			**24**			**12**	**4.2%**	**-1.87 [-3.57, -0.17]**
Heterogeneity: Tau² = 1.93; Chi² = 8.72, df = 3 (P = 0.03); I² = 66%								
Test for overall effect: Z = 2.16 (P = 0.03)								
1.1.12 HU308								
Ke 2016 HU308 1mg/kg i.p. DSS (t)	2.3	1.9596	6	3.6	1.2247	6	1.7%	-0.73 [-1.92, 0.45]
Subtotal (95% CI)			**6**			**6**	**1.7%**	**-0.73 [-1.92, 0.45]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 1.21 (P = 0.23)								
1.1.13 ACEA								
Kimball 2006 ACEA 10 mg/kg i.p.OM (p)	64	33	9	100	45	9	1.9%	-0.87 [-1.85, 0.11]
Subtotal (95% CI)			**9**			**9**	**1.9%**	**-0.87 [-1.85, 0.11]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 1.74 (P = 0.08)								
1.1.14 αβ Amyrin								
Matos 2013 αβAmyrin 1mg/kg p.o. DSS (p)	3.7	1.3416	5	5.11	0.44	2	1.2%	-0.98 [-2.79, 0.84]
Matos 2013 αβAmyrin 3mg/kg p.o. DSS (p)	2.4	1.3416	5	5.11	0.44	2	0.9%	-1.88 [-4.09, 0.34]
Matos 2013 αβAmyrin 10mg/kg p.o. DSS (p)	1.29	1.118	5	5.11	0.44	2	0.6%	-3.16 [-6.15, -0.17]
Matos 2013 αβAmyrin 10mg/kg p.o. DSS (t)	0.75	1.3416	5	5.11	0.44	2	0.6%	-3.02 [-5.92, -0.12]
Subtotal (95% CI)			**20**			**8**	**3.3%**	**-1.88 [-3.05, -0.72]**
Heterogeneity: Tau² = 0.00; Chi² = 2.25, df = 3 (P = 0.52); I² = 0%								
Test for overall effect: Z = 3.17 (P = 0.002)								
1.1.15 HU210								
Lin 2017 HU210 0.05mg/kg i.p. DSS (p)	2.5	0.2449	6	3.6	0.1715	6	0.7%	-4.80 [-7.41, -2.20]
Massa 2004 HU210 0.05mg/kg i.p. DNBS (p)	1.77	0.8944	5	3.77	1.5875	7	1.6%	-1.36 [-2.69, -0.04]
Subtotal (95% CI)			**11**			**13**	**2.3%**	**-2.89 [-6.24, 0.46]**
Heterogeneity: Tau² = 4.80; Chi² = 5.32, df = 1 (P = 0.02); I² = 81%								
Test for overall effect: Z = 1.69 (P = 0.09)								
1.1.16 JWH133								
Kimball 2006 JWH133 2.5mg/kg i.p. OM (p)	41	30	9	100	42.4264	8	1.8%	-1.54 [-2.66, -0.42]
Singh 2012 JWH133 2.5mg/kg i.p. DSS (t)	0.99	0.098	6	2.8	0.4899	6	0.7%	-4.73 [-7.30, -2.16]
Singh 2012 JWH133 2.5mg/kg i.p. IL10-/- (t)	2.8	0.7348	6	7.6	0.9798	6	0.7%	-5.12 [-7.86, -2.37]
Storr 2009 JWH133 20mg/kg i.p. TNBS (p)	4.7	2.2045	6	7.6	1.1758	6	1.6%	-1.52 [-2.87, -0.16]
Subtotal (95% CI)			**27**			**26**	**4.8%**	**-2.81 [-4.45, -1.17]**
Heterogeneity: Tau² = 1.86; Chi² = 10.34, df = 3 (P = 0.02); I² = 71%								
Test for overall effect: Z = 3.36 (P = 0.0008)								
1.1.17 PF3845								
Salaga 2014 PF3845 5mg/kg i.p. TNBS (p)	4.79	0.8485	8	7.5	1.2445	8	1.5%	-2.41 [-3.78, -1.03]
Salaga 2014 PF3845 5mg/kg p.o. TNBS (p)	3.44	0.9899	8	5.66	1.6971	8	1.8%	-1.51 [-2.66, -0.36]
Salaga 2014 PF3845 5mg.kg i.c. TNBS (p)	3.55	0.8485	8	7.36	1.4142	8	1.4%	-3.09 [-4.66, -1.51]
Subtotal (95% CI)			**24**			**24**	**4.7%**	**-2.21 [-3.11, -1.31]**
Heterogeneity: Tau² = 0.16; Chi² = 2.68, df = 2 (P = 0.26); I² = 25%								
Test for overall effect: Z = 4.81 (P < 0.00001)								
1.1.18 ARN2508								
Sasso 2015 ARN2508 5mg/kg p.o. TNBS (p)	5.7	0.9798	6	8.05	0.6124	6	1.2%	-2.66 [-4.38, -0.93]
Subtotal (95% CI)			**6**			**6**	**1.2%**	**-2.66 [-4.38, -0.93]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 3.02 (P = 0.002)								
1.1.19 O-1602								
Schicho 2011 O-1602 5mg/kg i.p. DSS (p)	3	11.1	9	6.2	2.4	9	2.0%	-0.38 [-1.31, 0.56]
Schicho 2011 O-1602 5mg/kg i.p. TNBS (p)	2.3	1.5652	5	4.9	1.3416	5	1.4%	-1.61 [-3.15, -0.08]
Subtotal (95% CI)			**14**			**14**	**3.4%**	**-0.84 [-2.01, 0.33]**
Heterogeneity: Tau² = 0.34; Chi² = 1.80, df = 1 (P = 0.18); I² = 45%								
Test for overall effect: Z = 1.41 (P = 0.16)								
1.1.20 CID 16020046								
Stancic 2015 CID16020046 20mg/kg i.p. DSS (p)	6.6	1	4	10.6	1	4	0.7%	-3.48 [-6.24, -0.71]
Stancic 2015 CID16020046 20mg/kg i.p. TNBS (p)	2.1	0.8	4	3.6	0.8	4	1.2%	-1.63 [-3.41, 0.15]
Subtotal (95% CI)			**8**			**8**	**1.9%**	**-2.24 [-3.94, -0.54]**
Heterogeneity: Tau² = 0.30; Chi² = 1.21, df = 1 (P = 0.27); I² = 17%								
Test for overall effect: Z = 2.58 (P = 0.010)								



1.1.21 URB597								
Storr 2008 URB597 5mg/kg i.p. TNBS (p)	2.73	1.062	9	8.08	1.23	9	1.1%	-4.43 [-6.32, -2.55]
Subtotal (95% CI)			**9**			**9**	**1.1%**	**-4.43 [-6.32, -2.55]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 4.62 (P < 0.00001)								
1.1.22 AM1241								
Storr 2009 AM1241 20mg/kg i.p. TNBS (p)	3.26	0.9798	6	7.14	1.2982	6	1.1%	-3.11 [-5.01, -1.22]
Subtotal (95% CI)			**6**			**6**	**1.1%**	**-3.11 [-5.01, -1.22]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 3.22 (P = 0.001)								
1.1.23 MMF								
Wallace 2013 MMF 10mg/kg i.c. DNBS (t)	3.45	5.5426	12	7	3.7335	18	2.2%	-0.76 [-1.52, -0.00]
Subtotal (95% CI)			**12**			**18**	**2.2%**	**-0.76 [-1.52, -0.00]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 1.97 (P = 0.05)								
1.1.24 THC								
Jamontt 2010 THC 5mg/kg i.p. TNBS (p)	6.5	2.3216	11	6.2	1.6583	3	1.6%	0.13 [-1.15, 1.40]
Jamontt 2010 THC 10mg/kg i.p. TNBS (p)	2.9	3.3166	11	6.2	1.6583	4	1.7%	-1.03 [-2.25, 0.19]
Jamontt 2010 THC 20mg/kg i.p. TNBS (p)	4.73	2.3216	11	6.2	1.6583	4	1.7%	-0.63 [-1.81, 0.54]
Subtotal (95% CI)			**33**			**11**	**5.1%**	**-0.53 [-1.24, 0.17]**
Heterogeneity: Tau² = 0.00; Chi² = 1.68, df = 2 (P = 0.43); I² = 0%								
Test for overall effect: Z = 1.48 (P = 0.14)								
1.1.26 βCaryophyllene								
Bento 2011 βCaryophyllene 12.5mg/kg i.p. DSS (p)	7.44	2.3255	5	9.4	1.8559	5	1.6%	-0.84 [-2.17, 0.49]
Bento 2011 βCaryophyllene 25mg/kg i.p. DSS (p)	5.78	2.6386	5	9.4	1.8559	5	1.4%	-1.43 [-2.91, 0.05]
Bento 2011 βCaryophyllene 50mg/kg i.p. DSS (p)	3.77	1.364	5	9.4	1.8559	5	1.0%	-3.12 [-5.27, -0.97]
Bento 2011 βCaryophyllene 50mg/kg i.p. DSS (t)	5.88	1.9677	5	9.4	1.8559	5	1.4%	-1.66 [-3.22, -0.11]
Subtotal (95% CI)			**20**			**20**	**5.4%**	**-1.52 [-2.32, -0.72]**
Heterogeneity: Tau² = 0.04; Chi² = 3.18, df = 3 (P = 0.36); I² = 6%								
Test for overall effect: Z = 3.71 (P = 0.0002)								
Total (95% CI)			**519**			**429**	**100.0%**	**-1.36 [-1.62, -1.09]**
Heterogeneity: Tau² = 0.67; Chi² = 169.13, df = 66 (P < 0.00001); I² = 61%								
Test for overall effect: Z = 10.16 (P < 0.00001)								
Test for subgroup differences: Chi² = 84.23, df = 24 (P < 0.00001), I² = 71.5%								

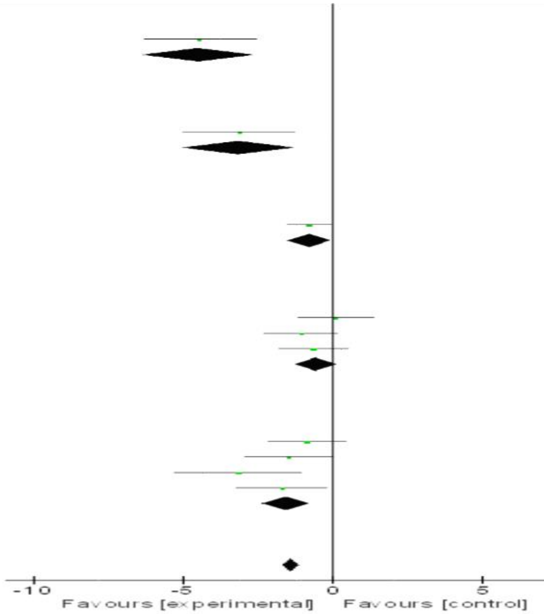
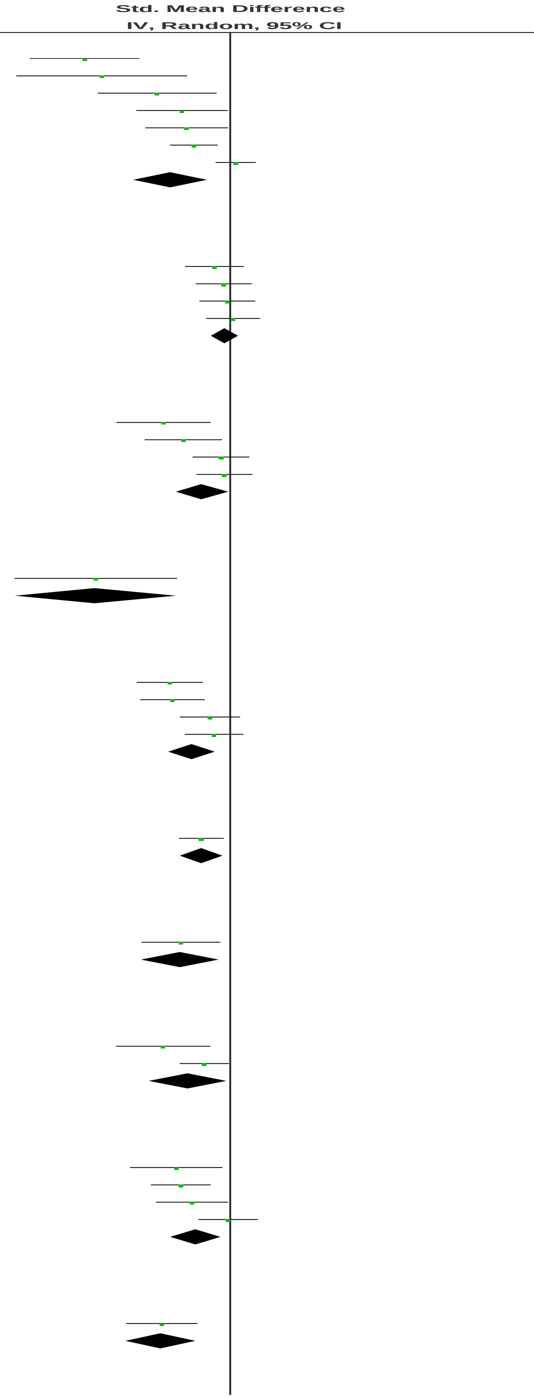
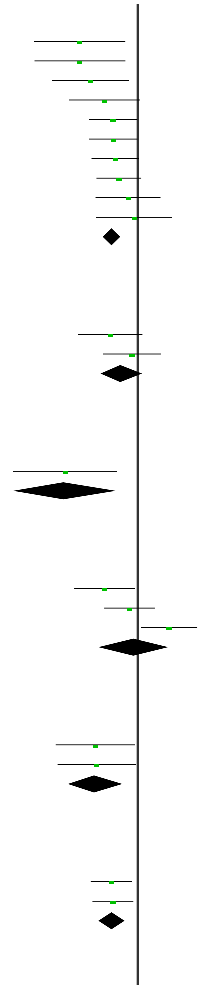


Figure 4. Forest plot of the effects of cannabinoid treatment on Disease Activity Score subdivided by drug type. Time of administration in relation to onset of colitis is given where 'p' represents prophylactic administration, and ’t’ represents therapeutic administration.

Study or Subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight	Std. Mean Difference IV, Random, 95% CI
1.2.1 PEA								
Impellizzeri 2015 PEA 10mg/kg i.p. DNBS (t)	40.91	6.7357	10	100	9.8284	10	0.9%	-6.72 [-9.20, -4.23]
Esposito 2014 PEA 10mg/kg i.p. DSS (t)	7.8	1.4207	6	17.9	1.7321	3	0.5%	-5.92 [-9.82, -2.02]
Borrelli 2014 PEA 1mg/kg p.o. DNBS (t)	34.4	5.6	4	68.69	11.2	4	0.8%	-3.37 [-6.07, -0.67]
Borrelli 2014 PEA 1mg/kg i.p. DNBS (t)	39.19	28.14	4	100	18.4	4	1.2%	-2.22 [-4.29, -0.16]
Esposito 2014 PEA 2mg/kg i.p. DSS (t)	11.9	2.9394	6	17.9	1.7321	3	1.4%	-2.01 [-3.87, -0.15]
Alhouayek 2015 PEA 10mg/kg i.p. DSS (p)	43.14	33.2039	10	100	31.939	10	2.2%	-1.67 [-2.72, -0.62]
Alhouayek 2015 PEA 10mg/kg i.p. TNBS (p)	116.4	84.3379	10	100	26.2469	10	2.5%	0.25 [-0.63, 1.13]
Subtotal (95% CI)			**50**			**44**	**9.5%**	**-2.74 [-4.42, -1.06]**
Heterogeneity: Tau^2 = 3.94; Chi^2 = 39.60, df = 6 (P < 0.00001); I^2 = 85%								
Test for overall effect: Z = 3.20 (P = 0.001)								
1.2.2 SAB378								
Cluny 2010 SAB378 1mg/kg i.p.TNBS (p)	44.5	39.802	5	100	89.6663	5	1.9%	-0.72 [-2.03, 0.58]
Cluny 2010 SAB378 0.1mg/kg i.p. TNBS (p)	77.85	37.7895	5	100	89.66	5	2.0%	-0.29 [-1.54, 0.96]
Cluny 2010 SAB378 1mg/kg i.p. DSS (t)	85.7	56.1253	5	100	127.523	5	2.0%	-0.13 [-1.37, 1.11]
Cluny 2010 SAB378 0.1mg/kg i.p. DSS (t)	116.04	102.1062	8	100	127.52	4	2.0%	0.13 [-1.07, 1.34]
Subtotal (95% CI)			**23**			**19**	**7.9%**	**-0.23 [-0.86, 0.39]**
Heterogeneity: Tau^2 = 0.00; Chi^2 = 0.93, df = 3 (P = 0.82); I^2 = 0%								
Test for overall effect: Z = 0.74 (P = 0.46)								
1.2.3 βCaryophyllene								
Bento 2011 βCaryophyllene 50mg/kg i.p. DSS (p)	0.4438	0.208	5	1.218	0.246	5	1.2%	-3.07 [-5.20, -0.94]
Bento 2011 βCaryophyllene 50mg/kg i.p. DSS (t)	0.68	0.2012	5	1.218	0.246	5	1.5%	-2.16 [-3.90, -0.42]
Bento 2011 βCaryophyllene 12.5mg/kg i.p. DSS (p)	0.69	1.5652	5	1.218	0.246	5	2.0%	-0.43 [-1.69, 0.84]
Bento 2011 βCaryophyllene 25mg/kg i.p. DSS (p)	0.615	2.9069	5	1.218	0.246	5	2.0%	-0.26 [-1.51, 0.98]
Subtotal (95% CI)			**20**			**20**	**6.6%**	**-1.26 [-2.48, -0.05]**
Heterogeneity: Tau^2 = 0.90; Chi^2 = 7.49, df = 3 (P = 0.06); I^2 = 60%								
Test for overall effect: Z = 2.04 (P = 0.04)								
1.2.4 CBG								
Borrelli 2013 CBG 30mg/kg i.p. DNBS (t)	6.279	3.1752	5	68.966	12.522	5	0.5%	-6.20 [-9.90, -2.50]
Subtotal (95% CI)			**5**			**5**	**0.5%**	**-6.20 [-9.90, -2.50]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 3.28 (P = 0.001)								
1.2.5 WIN 55212-2								
Feng 2016 WIN55,212 5mg/kg i.p. DSS (t)	47.2	23.1931	8	185.25	62.2254	8	1.7%	-2.78 [-4.26, -1.30]
Li 2013 WIN55212-2 5mg/kg i.p. DSS (t)	45.3	20.9304	8	184.3	66.468	8	1.8%	-2.67 [-4.11, -1.22]
Cluny 2010 WIN 52212-2 2mg/kg i.p. TNBS (t)	32.8	19.6774	5	100	89.6663	5	1.9%	-0.94 [-2.28, 0.41]
Cluny 2010 WIN 52212-2 2mg/kg i.p. DSS (t)	24.4	20.3482	5	100	128.1267	5	1.9%	-0.74 [-2.05, 0.56]
Subtotal (95% CI)			**26**			**26**	**7.3%**	**-1.74 [-2.81, -0.67]**
Heterogeneity: Tau^2 = 0.68; Chi^2 = 7.05, df = 3 (P = 0.07); I^2 = 57%								
Test for overall effect: Z = 3.20 (P = 0.001)								
1.2.6 Ademidrol								
Cordaro 2016 Ademidrol 10mg/kg p.o DNBS (t)	786	388.9602	10	1,428	528.1004	10	2.3%	-1.33 [-2.31, -0.34]
Subtotal (95% CI)			**10**			**10**	**2.3%**	**-1.33 [-2.31, -0.34]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 2.63 (P = 0.009)								
1.2.9 AA5HT								
D'Argenio 2006 AA5HT10mg/kg s.c DNBS (t)	35.2	19.0066	5	100	31.0813	5	1.4%	-2.27 [-4.05, -0.49]
Subtotal (95% CI)			**5**			**5**	**1.4%**	**-2.27 [-4.05, -0.49]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 2.50 (P = 0.01)								
1.2.10 VDM115								
D'Argenio 2006 VDM11 5mg/kg s.c. DNBS (t)	16	15.4289	5	100	31.0813	5	1.1%	-3.09 [-5.23, -0.96]
Storr 2008 VDM11 5mg/kg i.p. TNBS (p)	17.2	9.051	8	100.1	92.7724	8	2.2%	-1.19 [-2.28, -0.10]
Subtotal (95% CI)			**13**			**13**	**3.3%**	**-1.91 [-3.72, -0.10]**
Heterogeneity: Tau^2 = 1.06; Chi^2 = 2.42, df = 1 (P = 0.12); I^2 = 59%								
Test for overall effect: Z = 2.07 (P = 0.04)								
1.2.11 AM841								
Fichna 2014 AM841 1mg/kg i.p. DSS (p)	16.15	10.748	8	47.45	14.99	2	1.2%	-2.49 [-4.58, -0.40]
Fichna 2014 AM841 1mg/kg i.p. TNBS (p)	18.43	7.3539	8	39.43	9.8995	8	1.9%	-2.28 [-3.61, -0.94]
Fichna 2014 AM841 0.1mg/kg i.p. DSS (p)	27.14	8.8813	8	47.45	14.99	3	1.6%	-1.76 [-3.37, -0.15]
Fichna 2014 AM841 0.01mg/kg i.p. DSS (p)	44.9	27.4357	8	47.45	14.99	3	1.9%	-0.09 [-1.42, 1.24]
Subtotal (95% CI)			**32**			**16**	**6.5%**	**-1.56 [-2.71, -0.41]**
Heterogeneity: Tau^2 = 0.74; Chi^2 = 6.57, df = 3 (P = 0.09); I^2 = 54%								
Test for overall effect: Z = 2.66 (P = 0.008)								
1.2.12 ACEA								
Kimball 2006 ACEA 10 mg/kg i.p.OM (p)	5.7	13.2	9	199	87.3098	7	1.6%	-3.15 [-4.75, -1.55]
Subtotal (95% CI)			**9**			**7**	**1.6%**	**-3.15 [-4.75, -1.55]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 3.86 (P = 0.0001)								



1.2.13 CBD								
Pagano 2016 CBD 30mg/kg i.p. DNBS (t)	4.62	0.5814	5	6.06	0.5143	5	1.4%	-2.37 [-4.19, -0.55]
Pagano 2016 CBD 60mg/kg p.o. DNBS (t)	5.7	1.7889	5	9.03	0.2236	5	1.4%	-2.36 [-4.18, -0.54]
Jamontt 2010 CBD 20mg/kg i.p. TNBS (p)	2.87	1.3266	11	6.05	2.3548	3	1.7%	-1.93 [-3.45, -0.40]
Jamontt 2010 CBD 10mg/kg i.p. TNBS (p)	4.6	0.3317	11	6.05	2.3548	3	1.8%	-1.35 [-2.75, 0.06]
Schicho 2012 CBD 20mg/kg p.r. TNBS (p)	69.4	21.8197	10	105.55	43.9557	10	2.4%	-1.00 [-1.94, -0.06]
Krohn 2016 abCBD 5mg/kg i.p. TNBS (t)	60.03	32.5715	10	100	43.6394	10	2.4%	-0.99 [-1.94, -0.05]
Schicho 2012 CBD 10mg.kg i.p. TNBS (p)	80.5	17.7088	10	106.9	35.1013	10	2.4%	-0.91 [-1.84, 0.02]
Schicho 2012 CBD 20mg/kg p.o. TNBS (p)	70.83	50.7444	11	101.38	18.5731	11	2.5%	-0.77 [-1.64, 0.10]
Jamontt 2010 CBD 15mg/kg i.p. TNBS (p)	4.1	4.9749	11	6.05	2.3548	3	1.9%	-0.39 [-1.68, 0.89]
Jamontt 2010 CBD 5mg/kg i.p. TNBS (p)	5.5	3.6483	11	6.05	2.3548	2	1.7%	-0.14 [-1.65, 1.36]
Subtotal (95% CI)			**95**			**62**	**19.5%**	**-1.03 [-1.40, -0.66]**
Heterogeneity: Tau² = 0.00; Chi² = 8.33, df = 9 (P = 0.50); I² = 0%								
Test for overall effect: Z = 5.47 (P < 0.00001)								
1.2.14 HU210								
Massa 2004 HU210 0.05mg/kg i.p. DNBS (p)	2.9	9.1679	5	100	103.4489	7	2.0%	-1.12 [-2.39, 0.15]
Lin 2017 HU210 0.05mg/kg i.p. DSS (p)	1.74	0.4164	6	3.21	8.0833	6	2.1%	-0.24 [-1.37, 0.90]
Subtotal (95% CI)			**11**			**13**	**4.1%**	**-0.63 [-1.48, 0.23]**
Heterogeneity: Tau² = 0.01; Chi² = 1.02, df = 1 (P = 0.31); I² = 2%								
Test for overall effect: Z = 1.44 (P = 0.15)								
1.2.15 CBC								
Romano 2013 CBC 1mg.kg i.p. DNBS (t)	33.14	7.2001	5	67.64	12.9692	5	1.2%	-2.97 [-5.05, -0.89]
Subtotal (95% CI)			**5**			**5**	**1.2%**	**-2.97 [-5.05, -0.89]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 2.80 (P = 0.005)								
1.2.16 PF3745								
Salaga 2014 PF3845 5mg/kg p.o. TNBS (p)	61.01	29.1297	7	100	24.8701	7	2.0%	-1.35 [-2.55, -0.15]
Salaga 2014 PF3845 5mg.kg i.c. TNBS (p)	86.36	48.1964	8	100	25.7104	8	2.3%	-0.33 [-1.32, 0.66]
Salaga 2014 PF3845 5mg/kg i.p. TNBS (p)	142	35.3553	8	100	25.4558	8	2.2%	1.29 [0.18, 2.40]
Subtotal (95% CI)			**23**			**23**	**6.5%**	**-0.12 [-1.56, 1.32]**
Heterogeneity: Tau² = 1.31; Chi² = 10.40, df = 2 (P = 0.006); I² = 81%								
Test for overall effect: Z = 0.16 (P = 0.87)								
1.2.17 O-1602								
Schicho 2011 O-1602 5mg/kg i.p. TNBS (p)	21.98	33.541	5	100	46.7338	5	1.6%	-1.73 [-3.31, -0.16]
Schicho 2011 O-1602 5mg/kg i.p. DSS (p)	67.06	16.7705	5	100.31	19.0066	5	1.6%	-1.68 [-3.23, -0.12]
Subtotal (95% CI)			**10**			**10**	**3.3%**	**-1.70 [-2.81, -0.60]**
Heterogeneity: Tau² = 0.00; Chi² = 0.00, df = 1 (P = 0.96); I² = 0%								
Test for overall effect: Z = 3.01 (P = 0.003)								
1.2.18 CID16020046								
Stancic 2015 CID16020046 20mg/kg i.p. TNBS (p)	313.5	69.2207	14	435	138.4413	14	2.6%	-1.08 [-1.88, -0.28]
Stancic 2015 CID16020046 20mg/kg i.p. DSS (p)	172	53.1315	14	428.37	344.2325	14	2.6%	-1.01 [-1.80, -0.22]
Subtotal (95% CI)			**28**			**28**	**5.1%**	**-1.04 [-1.61, -0.48]**
Heterogeneity: Tau² = 0.00; Chi² = 0.01, df = 1 (P = 0.91); I² = 0%								
Test for overall effect: Z = 3.63 (P = 0.0003)								



1.2.19 URB597								
Storr 2008 URB597 5mg/kg i.p. TNBS (p)	30.5	6.5054	8	100	92.7724	8	2.2%	-1.00 [-2.06, 0.06]
Subtotal (95% CI)			**8**			**8**	**2.2%**	**-1.00 [-2.06, 0.06]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 1.85 (P = 0.06)								
1.2.20 AM1241								
Storr 2009 AM1241 20mg/kg i.p. TNBS (p)	32.9	49.6407	5	100	74.4611	5	1.9%	-0.96 [-2.31, 0.39]
Subtotal (95% CI)			**5**			**5**	**1.9%**	**-0.96 [-2.31, 0.39]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 1.39 (P = 0.16)								
1.2.21 JWH133								
Storr 2009 JWH133 20mg/kg i.p. TNBS (p)	25	39.0323	8	100	94.4695	8	2.2%	-0.98 [-2.04, 0.07]
Subtotal (95% CI)			**8**			**8**	**2.2%**	**-0.98 [-2.04, 0.07]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 1.82 (P = 0.07)								
1.2.22 THC								
Jamontt 2010 THC 10mg/kg i.p. TNBS (p)	1.74	0.3648	11	3.33	0.995	2	1.2%	-3.22 [-5.33, -1.11]
Jamontt 2010 THC 20mg/kg i.p. TNBS (p)	2.21	0.2449	6	3.33	0.995	2	1.1%	-2.10 [-4.26, 0.06]
Jamontt 2010 THC 5mg/kg i.p. TNBS (p)	4.37	0.9798	6	3.33	0.995	2	1.5%	0.92 [-0.80, 2.64]
Subtotal (95% CI)			**23**			**6**	**3.8%**	**-1.40 [-3.97, 1.17]**
Heterogeneity: $Tau^2 = 4.11$; $Chi^2 = 9.99$, df = 2 (P = 0.007); $I^2 = 80\%$								
Test for overall effect: Z = 1.07 (P = 0.28)								
1.2.24 αβ Amyrin								
Matos 2013 αβAmyrin 10mg/kg p.o. DSS (p)	0.16	0.8944	5	0.719	1.5588	3	1.7%	-0.42 [-1.88, 1.04]
Matos 2013 αβAmyrin 10mg/kg p.o. DSS (t)	0.43	0.246	5	0.719	1.5588	2	1.5%	-0.33 [-1.99, 1.33]
Subtotal (95% CI)			**10**			**5**	**3.3%**	**-0.38 [-1.48, 0.71]**
Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 0.01$, df = 1 (P = 0.94); $I^2 = 0\%$								
Test for overall effect: Z = 0.68 (P = 0.50)								
Total (95% CI)			**419**			**338**	**100.0%**	**-1.26 [-1.54, -0.97]**
Heterogeneity: $Tau^2 = 0.66$; $Chi^2 = 139.42$, df = 56 (P < 0.00001); $I^2 = 60\%$								
Test for overall effect: Z = 8.63 (P < 0.00001)								
Test for subgroup differences: $Chi^2 = 38.51$, df = 20 (P = 0.008), $I^2 = 48.1\%$								

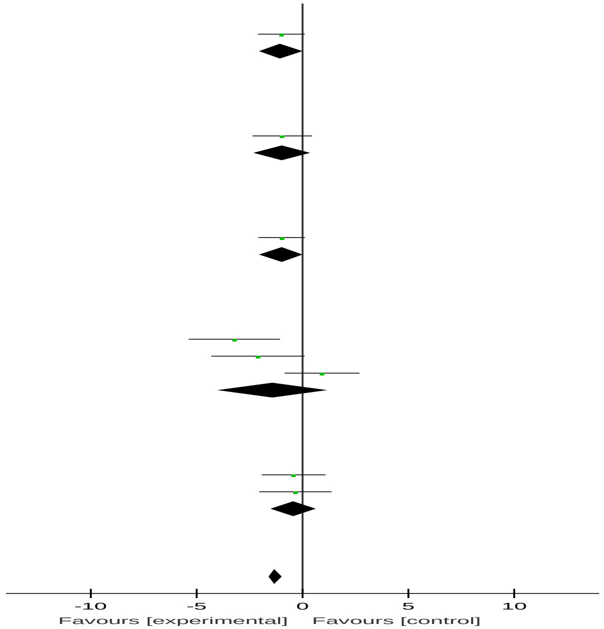


Figure 5. Forest plot of the effects of cannabinoid treatment on MPO activity subdivided by drug type. Time of administration in relation to onset of colitis is given where 'p' represents prophylactic administration, and 't' represents therapeutic administration.

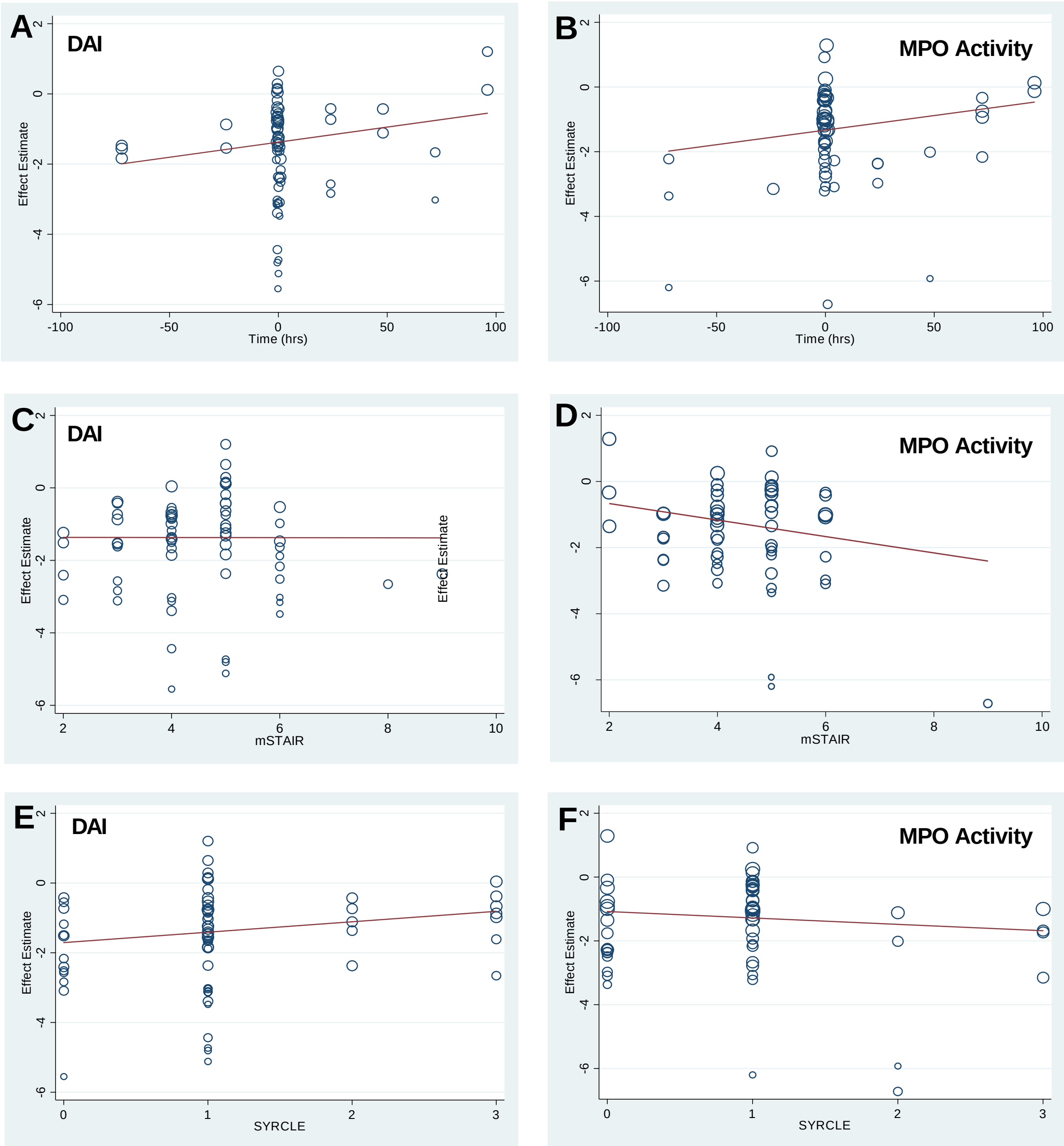


Figure 6. The effect of cannabinoid treatment on experimentally induced colitis determined by DAI (A) and MPO (B) predicted by timing of drug administration in relation to colitis onset. The effect of study quality, determined by mSTAIR score and SYRCLE score, on effect size in DAI (C, E) and MPO (D, F). Study weights are represented by the diameter of the circle, with larger circles representing studies with largest weight in the analysis.

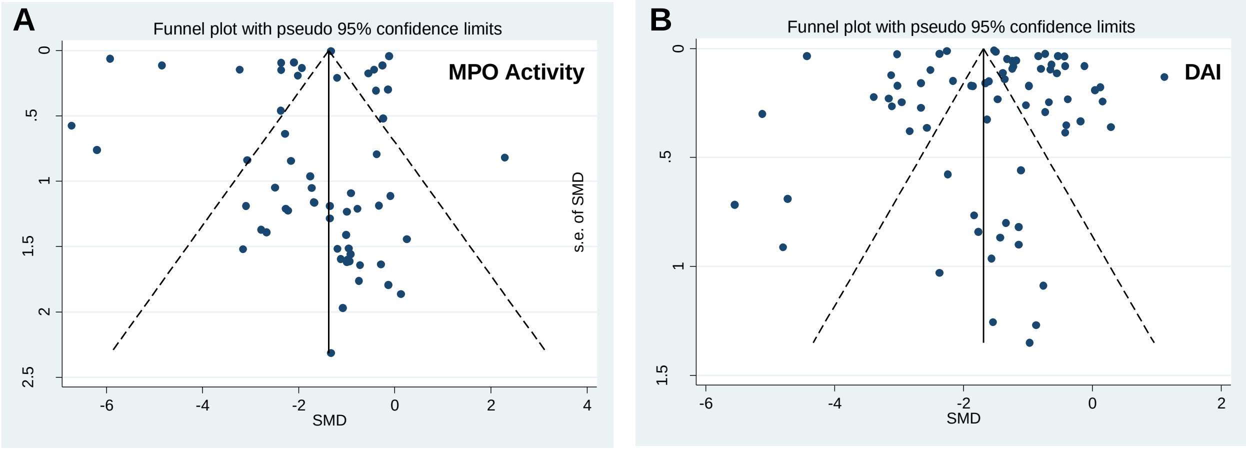


Figure 7. Funnel plots evaluating for publication bias in (A) MPO activity and (B) DAI. Standard error of the standardized mean difference (SE (SMD), y axes) for each study is plotted against its effect size (SMD, x axes).

Cannabinoid class		Drug Description
Endocannabinoids		
	AEA	Anandamide
	PEA	Palmitoylethanolamide
	uPEA	Ultramicronised PEA
Phytocannabinoids		
	Cannabis sativa	Multiple compounds
	CBC	Cannibichromene
	CBD	Cannabidiol
	CBG	Cannabigerol
	CBN	Cannabinol
	THC	Tetrahydrocannabinol
Cannabinomimetics		
	αβ Amyrin	CB_1 and CB_2 agonist
	ACEA	Arachidonyl-2'-chloroethylamide
	Adelmidrol	PEA analogue
	AM1241	CB_2 full agonist, partial CB_1 agonist
	AM841	Peripherally restricted CB_1 agonist
	βCaryophyllene	CB_2 agonist
	CID16020046	GPR55 inverse agonist
	Compound 26	CB_2 agonist
	CP55,940	CB_1 and CB_2 agonist
	HU210	THC analogue
	HU308	CB_2 agonist
	JWH015	CB_2 full agonist, weak CB_1 agonist
	JHW133	CB_2 full agonist, weak CB_1 agonist
	O-1602	GPR18 and GPR55 agonist
	SAB378	Peripherally restricted CB_1 and CB_2 agonist
	WIN55,212-2	CB_1 full agonist
Enzyme Inhibitors		
	AA5HT	FAAH inhibitor
	AM3506	FAAH inhibitor
	AM9053	NAAA inhibitor
	ARN2508	FAAH inhibitor
	compound 39	FAAH inhibitor
	JZL184	MAGL inhibitor
	PF-3845	FAAH inhibitor
	URB597	FAAH inhibitor
Reuptake inhibitors		
	VDM11	AEA reuptake inhibitor

Table 1 – Cannabinoid drugs found by search strategy.

Study	Species	Model	Compound	Route/dosage	Time of administration verses inflammation	Time of assessment post inflammation	Modified STAIR score	SRCYCLE Score
Capasso 2001 (32)	ICR mice	CO	PEA	i.p 2.5-30 mg/kg	20 minutes pre	4 days	4	1
Izzo 2001 (9)	ICR mice	CO	CP 55,940 Cannabinol	i.p. 0.03–10 nmol/m i.p. 10–3000nmol/m	4 days post	20 minutes	3	0
Massa 2004 (25)	C57BL/6N mice	DNBS	SR141716 HU210	i.p. 3mg/kg i.p. 0.05 mg/kg	Pre, 24 and 48 hours post	3 & 7 days	4	2
Mathison 2004 (50)	Spr-Dawley rats	LPS	ACEA JWH133	i.p. 1mg/kg i.p. 1mg/kg	70 minutes post	120 minutes	5	0
D'Argenio 2006 (22)	C57/BJ mice Wistar rats	DNBS TNBS	VDM11 AA-5-HT	s.c. 5mg/kg s.c. 10mg/kg	Post	3 & 7 days	6	0
Kimball 2006 (51)	CD-1 mice	OM	ACEA JWH133	i.p. 10mg/kg i.p. 2.5mg/kg	24 hours pre	3 days	3	1
Capasso 2008 (52)	ICR mice	CO	CBD JWH015	i.p. 5mg/kg i.p. 10mg/kg	20 minutes pre Ach	4 days	5	0
Engel 2008 (53)	AKR mice	TNBS	AEA	i.p. 5mg/kg	30 minutes pre	3 days	3	1
Storr 2008 (54)	C57/BL mice	TNBS	URB597 VDM11	i.p. 5mg/kg i.p.5mg/kg	30 minutes pre or 24 hours post	3 days	4	1
Borelli 2009 (46)	ICR mice	DNBS	CBD	i.p. 1, 2, 5, 10mg/kg	24 hours post	3 days	3	0
Li 2009 (55)	Rats Mice	LPS	HU210 JWH133 AM630 AM251	100 µg.kg 100 µg.kg 3 mg/kg	5 minutes	30 minutes	8	1
Storr 2009 (56)	C57/BL mice	TNBS DSS	JWH133 AM1241 AM630	i.p. 20mg/kg i.p. 10-20 mg/kg i.p. 10mg/kg	30 minutes pre or 24 hours post	1, 3, 5, 7 days	7	1
Cassol Jr 2010 (47)	Wistar rats	CLP	CBD	i.p. 2.5, 4, 10mg/kg	Simultaneous	9 days	8	2
Cluny 2010 (57)	C57/BL mice	DSS TNBS	SAB378 AM251 AM630 WIN55,212-2	i.p 0.1 or 1.0mg/kg i.p 1.0mg/kg i.p 1.0mg/kg i.p 1, 2mg/kg	4 days post	8 days	5	1
Kimball 2010 (58)	CD1 mice	OM	ACEA JWH133	i.p. 1mg/kg i.p. 1mg/kg	30 minutes pre	28 days	4	3
Jamontt 2010 (45)	Wistar rats	TNBS	THC CBD	i.p. 5-20mg/kg i.p. 5-20mg/kg	30 minutes pre	3 days	5	1
Alhouayek 2011 (59)	C57BL/6 mice	TNBS	JZL184	i.p. 16mg/kg	Pre onset	3 days	2	1
Andrejak 2011 (60)	C57/BL mice	TNBS	Compound 39	i.p. 5mg/kg	3 days pre	3 days	6	1
Bento 2011 (61)	CD1 mice	DSS	βCaryophyllene	i.p. 12.5, 25, 50mg/kg	3 -7 days post	7 days	4	1
Defilipis 2011 (49)	OF1 mice	LPS	CBD	i.p. 10mg/kg	6 hours post	120 minutes	6	1
Lin 2011 (43)	C57/BL mice Spr-Dawley rats	LPS	CBD O-1602	i.p. 10mg/kg I.p. 1mg/kg	30 minutes pre	20 minutes	5	1
Schicho 2011 (62)	C57/BL mice	DSS TNBS	O-1602	i.p. 5mg/kg	30 minutes pre	7 days	3	3
Bashashati 2012 (63)	CD1 mice	LPS	AM3506	i.p. 100ug.kg	20 minutes pre	120 minutes	3	0
Izzo 2012 (64)	ICR mice	CO	CBC	i.p. 15mg/kg	20 minutes pre exam	4 days	5	2
Lehmann 2012 (65)	Lewis rats	LPS CASP	HU308	2.5mg/kg	15 minutes post	2 – 16 hours	4	0
Schicho 2012 (42)	C57/BL mice	TNBS	CBD	i.p. 10mg/kg p.o. 20mg/kg p.r. 20mg/kg	30 minutes pre onset	7 days	4	0
Singh 2012 (66)	C57/BL mice	IL-10 -/- DSS	JWH133	i.p. 2.5mg/kg	Simultaneous	7 – 14 days	5	1
Borrelli 2013 (67)	ICR mice	DNBS	CBG	i.p. 30mg/kg	3 days pre	3 days	5	1
Esposito 2014 (33)	CD-1 mice	DSS	PEA	i.p. 10mg/kg	2 days post	7 days	5	2
Li 2013 (68)	C57/BL mice	DSS	WIN55,212-2	i.p. 5mg/kg	Simultaneous	7 days	4	1
Matos 2013 (69)	CD1 mice	DSS	αβ Amyrin	p.o. 1, 3, 10mg/kg	Pre and 3 days post	7 days	6	1
Naftali 2013 (70)	Clinical trial	Crohns	Cannabis sativa extract (THC)	115 mg inhaled	N/A	8 weeks	NA	NA
Romano 2013 (71)	ICR mice	DNBS	CBC	i.p 0.1-1.0mg/kg	24 hours post	3 days	6	0
Wallace 2013 (72)	Wistar rats	DNBS	C. sativa (MMJ) AM630	i.c. 6 mg/kg p.o. 10mg/kg	30 minutes pre and 24 hours post	7 days	4	1
Borelli 2015 (73)	ICR mice	DNBS	PEA	i.p 1mg/kg p.o. 1mg/kg	3 days pre	3 days	5	1

Capasso 2014 (20)	ICR mice	OM	PEA	i.p. 10mg/kg	30 minutes	3 and 7 days	6	2
Fichna 2014 (74)	CD1 mice	DSS DNBS	AM841 CB13	i.p. 0.01, 0.1,1 mg/kg i.p. 0.1 mg/kg	15 minutes pre	3 and 7 days	4	0
Salaga 2014 (75)	C57/BL mice	TNBS DSS	PF3845	i.p. 10mg/kg p.o. 5mg/kg i.c. 5mg/kg	30 minutes	3 and 7 days	2	0
Sardinha 2014 (76)	C57/BL mice	LPS	HU308 AM630 URB597 JZL184	i.v. 2.5mg/kg i.v.2.5mg/kg i.p. 0.6mg/kg i.p. 16mg/kg	15 minutes pre	Simultaneous	6	0
Alhouayek 2015 (77)	CD57/BL mice	TNBS DSS	PEA PF-3845 AM9503	i.p. 10mg/kg i.p. 10mg/kg i.p. 10mg/kg	Simultaneous and 5 days post	7 days	4	1
El bakali 2015 (78)	C57/BL mice	TNBS	Compound 26	p.o. 10mg/kg	2 days pre	7 days	6	0
Impellizzeri 20 15 (79)	CD1 mice	DNBS	uPEA	i.p. 10mg/kg	1 hour post	4 days	9	2
Sasso 2015 (80)	CD1 mice	TNBS DSS	ARN2508	p.o. 5mg/kg	Simultaneous	7 days	8	3
Stančić 2015 (81)	C57/BL mice	DSS TNBS	CID16020046	s.c. 20mg/kg	30 minutes	7 days	6	1
Cordaro 2016 (82)	CD1 mice	DNBS	Adelmidrol	p.o. 10mg/kg	60 minutes post	4 days	4	1
Feng 2016 (83)	C57/BL mice	DSS	WIN55212-2	i.p. 5mg/kg	Simultaneous and 60 hours post	7 days	5	1
Ke 2016 (84)	C57/BL mice	DSS	HU308	i.p. 1mg/kg	Simultaneous and daily	8 days	4	2
Krohn 2016 (40)	CD1 mice	TNBS	Ab-CBD O-1918 AM251 AM630	i.p. 5mg/kg i.p. 5mg/kg i.p. 5mg/kg i.p. 5mg/kg	45 minutes pre	4 days	6	1
Pagano 2016 (39)	ICR mice	DNBS CO	CBD Pure CBD	i.p. 30mg/kg p.o. 60mg/kg	24 hours post	3 days	3	0
Sarnelli 2016 (85)	CD1 mice	DSS	PEA	i.p. 2, 10mg/kg	2 days post	7 days	6	1
Lin 2017 (86)	C57/BL mice	DSS	HU210	i.p. 0.05mg/kg	30 minutes pre	7 days	5	1
Shamran 2017(87)	C57/BL mice	DSS	FAAH-II	i.p. 5 – 40mg/kg	24 hours post	7 days	6	1
Naftali 2017 (35)	Clinical trial	Crohns	CBD	10mg p.o. BD	N/A	8 weeks	NA	NA

CO, croton oil; DNBS, dinitrobenzosulphonic acid; LPS, lipopolysaccharide; TNBS, trinitrobenzosulphonic acid; DSS, dextran sulphate sodium; OM, oil of mustard; CASP, colon ascendens stent peritonitis; IL-10, interleukin 10; PEA, palmitoylethanolamide; AEA, anandamide; CBD, cannabidiol; THC; tetrahydrocannabinol; CBC, cannabichromene; CBG, cannabigerol; MMJ, medicinal cannabis; uPEA, ultramicronised PEA, AB-CBD, abnormal CBD; FAAH-II, fatty acid aminohydrolase II; i.p. intraperitoneal, i.c. intracolonic, p.o. oral administration; s.c. subcutaneous; iv.v intravenous; p.r. per rectum; Ach, acetylcholine.

Table 2. Characteristics of studies included for systematic review.

	No. of Studies	No. of animals	SMD [95% CI]	p value	I^2 (%)	Clinical significance
Endocannabinoids						
PEA	6	118	-1.45 [-1.94, -0.96]	<0.00001	25	High
AEA	1	12	-3.03 [-4.89, -1.17]	0.001	N/A	High
Phytocannabinoids						
CBD	12	181	-0.56 [-0.97, -0.16]	0.006	29	NS
THC	3	44	-0.53 [-1.24, 0.17]	0.14	0	NS
MMJ	1	30	-0.76 [-1.52, -0.00]	0.05	N/A	Moderate
Cannabinomimetics						
αβ Amyrin	4	28	-1.88 [-3.05, -0.72]	0.002	0	High
AM841	4	36	-1.87 [-3.57, -0.17]	0.03	66	High
βCaryophyllene	4	40	-1.52 [-2.32, -0.72]	0.0002	6	High
SAB378	4	56	0.28 [-0.38, 0.94]	0.41	28	NS
WIN55,212-2	4	60	-1.37 [-1.96, -0.78]	<0.00001	0	High
CID16020046	2	16	-2.24 [-3.94, -0.54]	0.01	17	High
HU210	2	24	-2.89 [-6.24, 0.46]	0.09	81	NS
O-1602	2	28	-0.84 [-2.01, 0.33]	0.16	45	NS
ACEA	1	18	-0.87 [-1.85, 0.11]	0.08	N/A	High
Adelmidrol	1	20	-1.85 [-2.94, -0.77]	0.0008	N/A	High
AM1241	1	12	-3.11 [-5.01, -1.22]	0.001	N/A	High
HU308	1	12	-0.73 [-1.92, 0.45]	0.23	N/A	NS
Enzyme inhibitors						
JWH133	4	53	-2.81 [-4.45, -1.17]	0.0008	71	High
PF3845	3	48	-2.21 [-3.11, -1.31]	<0.00001	25	High
AA5HT	1	10	-2.16 [-3.90, -0.43]	0.01	N/A	High
ARN2508	1	12	-2.66 [-4.38, -0.93]	0.002	N/A	High
Compound 39	1	20	-1.47 [-2.48, -0.46]	0.004	N/A	High
JZL184	1	22	-1.24 [-2.16, -0.31]	0.009	N/A	High
URB597	1	18	-4.43 [-6.32, -2.55]	<0.00001	N/A	High
Transport inhibitors						
VDM115	2	30	-3.06 [-4.21, -1.90]	<0.00001	0	High
Total	**68**	**948**	**-1.36 [-1.62, -1.09]**	**<0.00001**	**61**	**High**

Table 3. The effects of cannabinoids on Disease Activity Score caused by experimental colitis grouped by drug

	No. of Studies	No. of animals	SMD [95% CI]	p value	I^2 (%)	Clinical significance
Endocannabinoids						
PEA	7	94	-2.74 [-4.42, -1.06]	0.001	85	High
Phytocannabinoids						
CBD	10	157	-1.03 [-1.40, -0.66]	<0.00001	0	High
THC	3	29	-1.40 [-3.97, 1.17]	0.28	80	NS
CBC	1	10	-2.97 [-5.05, -0.89]	0.005	N/A	High
CBG	1	10	-6.20 [-9.90, -2.50]	0.01	N/A	High
Cannabinomimetics						
βCaryophyllene	4	40	-1.26 [-2.48, -0.05]	0.04	60	High
AM841	4	48	-1.56 [-2.71, -0.41]	0.008	54	High
SAB378	4	42	-0.23 [-0.86, 0.39]	0.46	0	NS
WIN55,212-2	4	52	-1.74 [-2.81, -0.67]	0.001	57	High
αβ Amyrin	2	15	-0.38 [-1.48, 0.71]	0.5	0	NS
CID16020046	2	56	-1.04 [-1.61, -0.48]	0.0003	0	High
HU210	2	24	-0.63 [-1.48, 0.23]	0.15	2	NS
O-1602	2	20	-1.70 [-2.81, -0.60]	0.003	0	High
ACEA	1	16	-3.15 [-4.75, -1.55]	0.0001	N/A	High
AM1241	1	10	-0.96 [-2.31, 0.39]	0.16	N/A	NS
JWH133	1	16	-0.98 [-2.04, 0.07]	0.09	N/A	NS
Ademidrol	1	20	-1.33 [-2.31, -0.34]	0.009	N/A	High
Enzyme inhibitors						
PF3745	3	46	-0.12 [-1.56, 1.32]	0.81	81	NS
AA5HT	1	10	-2.27 [-4.05, -0.49]	0.01	N/A	High
URB597	1	16	-1.00 [-2.06, 0.06]	0.06	N/A	NS
Transport inhibitors						
VDM115	2	26	-1.91 [-3.72, -0.10]	0.04	59	High
Total	**57**	**757**	**-1.26 [-1.54, -0.97]**	**<0.00001**	**48.1**	**High**

Table 4. The effects of cannabinoids on MPO activity caused by experimental colitis grouped by drug

PRISMA Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	6
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	6
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6-7
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6-7

Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	7

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	6
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	8+19
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	8-11+28-29
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	11
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	30-31
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	10-11
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	12
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	11
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	13
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	17
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	13-17
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	1

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

The use of cannabinoids in colitis: a systematic review and meta-analysis

DG Couch, H Maudslay, B Doleman, J N Lund, SE O'Sullivan

School of Medicine, Royal Derby Hospital, University of Nottingham, Nottingham, United Kingdom

Correspondence: Daniel Couch, School of Medicine, Royal Derby Hospital, University of Nottingham, Derby. DE22 3DT UK

E-mail: Couch27@gmail.com

Phone: 01332724701

No disclosures. No funding was recived for this study.

Author contributions:

Conception and design of the study; Couch DG, Lund J, O'Sullivan SE conceived and designed the study

D Couch and H Maudslay collected data.

D Couch, H Maudslay, B Doleman, J Lund and S O'Sullivan analysed data.

D Couch, J Lund and S O'Sullivan were responsible for overall content of the article.

Drafting or revision of the manuscript; all

Approval of the final version of the manuscript: all

Keywords: cannabinoid inflammation gut intestine colitis

Abstract

Background:Clinical trials investigating the use of cannabinoid drugs for the treatment of intestinal inflammation are anticipated secondary to preclinical literature demonstrating efficacy in reducing inflammation.

Methods:We systematically reviewed publications on the benefit of drugs targeting the endo-cannabinoid system in intestinal inflammation. We collated studies examining outcomes for meta-analysis from EMBASE, MEDLINE and Pubmed until March 2017. Quality was assessed according to mSTAIR and SRYCLE score.

Results:From 2008 papers, 51 publications examining the effect of cannabinoid compounds on murine colitis, and two clinical studies were identified. 24 compounds were assessed across 71 endpoints. Cannabidiol, a phytocannabinoid, was the most investigated drug. Macroscopic colitis severity (disease activity index - DAI) and myeloperoxidase activity (MPO) were assessed throughout publications and were meta-analysed using random effects models. Cannabinoids reduced DAI in comparison with vehicle; SMD -1.36, 95% CI -1.62 to-1.09, I^2=61%). FAAH inhibitor URB597 had the largest effect size (SMD-4.43, 95% CI-6.32,-2.55), followed by the synthetic drug AM1241 (SMD –3.11, 95% CI -5.01, -1.22) and the endocannabinoid anandamide (SMD-3.03, 95% CI -4.89,-1.17, I^2 not assessed). Cannabinoids reduced MPO in rodents compared to vehicle; SMD -1.26, 95% CI-1.54 to -0.97, I^2=48.1%. Cannabigerol had the largest effect size (SMD -6.20, 95%CI-9.90, -2.50), followed by the synthetic CB_1 agonist ACEA(SMD -3.15, 95%CI-4.75, -1.55) and synthetic $CB_{1/2}$ agonist WIN55,212-2(SMD-1.74, 95%CI-2.81, -0.67, I^2=57%). We found no evidence of reporting bias. No significant difference was found between the prophylactic and therapeutic use of cannabinoid drugs.

Conclusions:There is abundant pre-clinical literature demonstrating the anti-inflammatory effects of cannabinoid drugs in inflammation of the gut. Larger randomised controlled-trials are warranted.

Table of abbreviations

PPAR - Peroxisome Proliferator Activating Receptor

TRPV1 - Transient receptor potential vanilloid 1

AEA - Anandamide

2-AG - 2-arachidonoyl glycerol

PEA - Palmitoylethanolamide

DNBS - Dinitrobenzene sulphonic acid

OM - Oil of mustard

TNBS - Trinitrobenzene sulphonic acid

DSS - Dextran sulphate sodium

CO - Croton oil

THC - Δ^9-Tetrahydrocannabinol

CBD - Cannabidiol

Ab-CBD - Abnormal cannabidiol

CBG - Cannabigerol

CBN - Cannabinol

MMJ - Medicinal cannabis

MPO - Myeloperoxidase

DAI - Disease activity index

IL-10 - Interleukin-10

SMD - Standard mean difference

CI - Confidence interval

I.c. - Intracolonic

p.o.- Oral

i.v. - Intravenous

p.r. - Per rectum

s.c. - Subcutaneous

Introduction

Inflammatory bowel disease (IBD) affects 200 per 100,000 adults in the United States and 400 per 100,000 in the UK (1,2). Major subtypes consist of Crohns disease and ulcerative colitis. A definitive clinical treatment for these chronic relapsing diseases remains elusive, as currently no therapy exists to reverse the clinical pathology without a risk of significant side effects. 5-ASA agents, corticosteroids, anti-TNFα antibodies and other immunomodulatory drugs have all been shown to induce significant remission in IBD, but are associated with bone marrow suppression, opportunistic infection, infusion reactions and malignancy secondary to immunosuppression (3–5).

The endocannabinoid system (ECS), consisting of multiple receptors and endogenous ligands, controls multiple homeostatic processes including gastrointestinal motility, hunger, perception of pain and immunity (6–10). The receptors of the ECS consist of the classical CB_1 and CB_2 receptors, but also the orphan GPR55 receptor, peroxisome proliferator-activated receptors (PPARs) and transient receptor potential receptor vanilloid (TRPV) receptors. These targets are all found on the cells of gut mucosa, submucosa, enteric nervous and immune systems. Endocannabinoids, such as anandamide (AEA) and 2-arachiodoylglycerol (2-AG), are intercellular lipid signalling molecules derived on demand from membrane precursors (11). They are metabolised by fatty acid amide hydrolase (FAAH) as well as N-acyl ethanolamine-hydrolysing acid amidase (NAAA) in the case of AEA, and monoacylglycerol lipase (MAGL) in the case of 2-AG (12–14). Palmitoylethanolamide (PEA), also metabolised by NAAA, has been shown to activate PPARα and may increase local concentrations of AEA or the affinity of AEA to the CB_1 receptor and is therefore included as an atypical cannabinoid (15,16). Phytocannabinoids include Δ-^{9} tetrahydrocannabinol (THC), cannabidiol (CBD), cannabigerol (CBG), cannibichromene (CBC) and up to 60 others and are isolated from C*annabis Sativa* (11). THC and CBD have found place in clinical practice in the treatment of childhood epilepsy and muscular spasticity in multiple sclerosis (17,18). A growing collection of synthetic cannabinoid agonists have been derived possessing selective high affinity for the CB_1, CB_2, GPR55 and TRPV1 receptors, and have been investigated pre-clinically for roles in gut motility, satiety and immunity (8).

Under inflammatory conditions CB_1, CB_2 and both PPARα and PPARγ expression increases on the submucosa and on adjacent immune cells, whereas GPR55 and TRPV1 expression decreases on the mucosa, but increases on enteric nervous tissue (19–21). Levels of AEA, 2-AG and PEA are upregulated *in vitro*, and also in animal *in vivo* and human *ex-vivo* models of intestinal inflammation (22–24). Early experimentation in murine models demonstrated cannabinoids prevent the onset of experimental murine colitis or reduced its severity (25). Since these initial findings, many reports, including clinical trials, have now investigated the effect of cannabinoid ligands, or the effect of blockade of their metabolising enzymes, on inflammation of the gut.

There is a significant amount of promising preclinical evidence for the use of cannabinoid agents in the treatment of colitis. Within this study we aimed to gather all preclinical and clinical evidence for the use of these drugs in colitis, and where possible, perform meta-analyses across studies in order to assess the efficacy of cannabinoids for further clinical trials. Where possible clinically relevant experimental endpoints were assessed.

Methods

Search Strategy

All studies evaluating the effect of cannabinoid drugs on inflammation of the colon were searched from March 1980 until March 2017 by two independent researchers in Medline, EMBASE and Pubmed. Keywords included cannabidiol, tetrahydrocannabinol, anandamide, 2-AG, cannibichromene, cannabigerol, cannabinoid, cannabis sativa, colon, intestine, gut, inflammation, Crohns, ulcerative and colitis. Names of synthetic cannabinoid agents were also included. References from included studies were searched by hand. Pre-specified inclusion and exclusion criteria were used to prevent bias. Experiments must have been be performed in the context of administration of cannabinoid drugs to inflammatory states of the colon in humans or animals, either experimental or due to endogenous disease (Crohns disease or ulcerative colitis). *In vitro* studies or studies not examining the effect of cannabinoids in intestinal inflammation specifically, or studies using cannabinoid antagonists as a primary agent were excluded. A PRISMA checklist is included in the appendix.

Data Acquisition

The mode of colitis induction in preclinical studies was recorded in addition to the timing of cannabinoid application. For the purposes of meta-analysis, data on the macroscopic or histological disease scores (disease activity index – DAI) and myeloperoxidase (MPO) activity were collected. If the exact number of animals was not available, the lowest number of animals within the range given were used for the experimental group, and the highest number used for the control/vehicle group. Where studies reported the effects of more than one cannabinoid sharing a single control group for comparison, control group numbers were equally distributed between comparisons to avoid unit of analysis issues. WebPlotDigitiser (version 3.11) was used to extract values from figures in published articles where no data values were given in the text.

Quality

Quality of included studies were assessed by two independent researchers to quantify risk of bias according to the six-point criteria developed by the Cochrane Collaboration risk of bias tool (26). In order to assess the quality of preclinical studies, the STAIR and Arrive preclinical assessment tools were adapted (27,28). Each of the below were awarded one point: randomisation, assessor blinding, results replicated in a second species, dose-response experiments, results replicated in a second model of colitis, n=5 or greater in each group, the use of clinically relevant endpoint to assess response of colitis, definitive statement of animal numbers in each group, a statement regarding the housing of animals and a statement describing the location and timing of animal experimentation (i.e. in animal housing or a separate cage, time of day etc), giving a highest possible score of 10.

Data analysis

Where possible, data were grouped into DAI and MPO activity, and subdivided by species and compound. Data from each group were analysed as forest plots using Cochrane Review Manager Software (Review Manager 5.3, Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014), and as funnel plots using Stat (Stat Corp. 2009 Stat Statistical Software: Release 11. College Station, TX, USA). Funnel plot asymmetry was tested using Egger's linear regression test. A P value of <0.05 was considered statistically significant. As differing studies measured MPO activity and DAI using various scales, we present effect estimates as standardized mean differences (SMD) with 95% confidence intervals (CI). We used the following SMD values to assess results for clinical significance: < -0.5 small clinical significance, -0.5 to -0.8 moderate clinical significance and >-0.8 high clinical significance. Due to clinical heterogeneity between the various studies, a random-effects model was used. We assessed statistical heterogeneity using the I^2 statistic, with >50% regarded as evidence of statistical heterogeneity. We assessed the quality of evidence using the previously validated SYRCLE criteria, with studies graded out of 10 (29). Studies were weighted by sample size and statistical significance was set at a minimum of p<0.05.

Results

Search results and study characteristics

The search strategy returned 2008 results from which 199 relevant publications were identified. From these, 53 publications comprising 106 experiments examining 35 compounds met the inclusion criteria (figure 1, table 1 and 2). Thirty four studies were included in the meta-analysis.

Forty-three publications studied the effects of cannabinoids on experimental murine colitis, 5 in rats, and 3 in both mice and rats. Two clinical trials examined the effect of a cannabinoid (THC and CBD) in Crohns disease. Within animal publications, 43 used caustic agents (Di-nitrobenzine sulphonic acid (DNBS), trinitrobenzene sulphonic acid (TNBS), oil of mustard (OM), dextran sulphate sodium (DSS) and croton oil (CO)) to induce colitis, 6 used intravenous or topical lipopolysaccharide, 2 induced colonic inflammation using surgical arterial ligation or puncture of the colon and 1 induced colitis with interleukin-10 (IL-10) knock-down and DSS (figure 2A). Across all publications, including clinical trials, 71 endpoints were examined to evaluate the effect of cannabinoid drugs on colitis. Forty-nine publications (89 experiments) examined more than one endpoint. Of these endpoints MPO and DAI were the most consistently used (34 and 26 studies respectively), and were therefore selected for meta-analysis. Incidence of endpoints is given in figure 2B.

The effect of 7 phytocannabinoids were studied across 18 publications; cannabinol (CBN), CBD, THC, CBC, CBG, medicinal cannabis (MMJ) and abnormal CBD (Ab-CBD). 4 endocannabinoids were studied across 11 publications (PEA, ultramicronized PEA (uPEA), Arachidonyl-2'-chloroethylamide (ACEA) and AEA), 15 synthetic cannabinoid agonists were studied across 22 publications (AM841, Adelmidrol , HU210, CP55,940, WIN55,212-2, AM1241, JHW015, JWH133, βCaryophyllene, O-1602, HU308, αβ amyrin CID 16020046 compound 26 and SAB378), and 9 compounds targeting the catabolism or transport of endogenous cannabinoids were studied across 13 publications (ARN2508, PF-3845, compound 39, JZL184, AA5HT, VDM11, URB597, AM9053, AM3506). These compounds are delineated by class in table 1. The degree of positivity or negativity of the outcomes of these studies are displayed in figure 2C. Twenty-three studies investigated underlying receptor mechanisms using knock-out (KO) animals or receptor antagonists.

Of the 105 experiments comparing cannabinoids with vehicle or placebo, 67 (63.8%) favoured cannabinoids, 34 (32.3%) reported no difference, and 4 (3.8%) favoured vehicle. Mice were used in 89 experiments (68.5% of which favoured cannabinoids), rats in 14 (71.4% favoured cannabinoids), in 4 experiments both mice and rats were used showing no difference between cannabinoids and vehicle. In the two clinical trials, no difference in primary outcome was found between the use of THC cigarettes or oral CBD and placebo. 11 of 14 publications (78.6%) using synthetic CB_2 receptor agonists favoured cannabinoid use over vehicle, and a further 11 of 13 (84.6%) favoured using FAAH inhibitors over vehicle. The outcome of all cannabinoids across publications is given in figure 2C.

Two clinical trials examining the effect of CBD and THC in Crohns disease were found. Naftali et al. (2013) conducted a placebo controlled study in Crohns disease patients, comparing THC 115mg inhaled alone with placebo. Disease activity was compared between groups by means of validated questionnaire (Crohns disease activity index – CDAI) after 8 weeks of treatment. A non-significant reduction in clinical disease remission as defined by the authors was found at the end of the study period, however a secondary endpoint of reduction in overall activity scores was found between groups (p=0.028). In a second study, Naftali et al. (2017) compared oral CBD 10 mg p.o. twice daily with placebo in Crohns disease, using CDAI in an identical fashion. No reduction in disease activity was detected between groups. In both studies the authors measured changes in serum C-reactive protein (CRP), within both experimental and placebo groups CRP levels were below 5 units per ml at the end of the study periods. Clinically, CRP levels greater than 5 units per ml are considered indicative of inflammatory disease. Within both studies the combination of CBD and THC within a single study were not assessed.

Of the 104 experiments where timing of drug administration of drug was stated, 37 administered cannabinoids therapeutically, of which 62.2% favoured cannabinoid treatment. 19 experiments administered cannabinoids prophylactically, of which 52.6% favoured cannabinoid treatment. 48 experiments administered cannabinoids both prophylactically and therapeutically, of which 75% favoured cannabinoid treatment versus vehicle.

Meta-Analysis

34 studies reported the same endpoints of disease activity index or myeloperoxidase activity allowing for meta-analysis. Of the remaining studies heterogeneity of endpoints prevented further meta-analysis.

Crohns Disease Activity Index (CDAI).

The use of two phytocannabinoids, THC or CBD, in two human studies were meta-analysed. Phytocannabinoid use decreased severity scores in comparison with placebo (mean difference (MD) -74.97, 95% CI –229, 0.79, I^2=75%. Figure 3). THC alone had a significant effect on reducing CDAI (MD-154.00, 95% CI -2.68.57, -44.43), whereas CBD alone did not (MD +4.00 95% CI -1.5.39, +113.39).

Disease Activity Index (DAI)

Thirty-four publications examined the effects of 25 cannabinoid drugs across 68 experiments, within mouse and rat models (total n = 948, n = 519 experimental vs 429 in control groups). Cannabinoid drugs reduced DAI in comparison with vehicle; SMD -1.36, 95% CI -1.62 to-1.09, I^2=61% (figure 4, table 3). On subgroup analysis there was significant difference between drug subtypes (P<0.001). DAI was significantly reduced in mice (SMD -1.49, 95% CI -1.77 to -1.22; I^2=61%). Seven experiments within one publication examined the effects of cannabinoids on rat colitis (THC and CBD, both conducted in a dose response manner), but did not reach significance at any concentration; SMD -0.29, 95% CI -0.77 to 0.20, I^2=0%. SMD and confidence intervals for individual drugs on DAI are given in table 3.

The largest effect size in DAI reduction was caused by an enzyme inhibitor: the FAAH inhibitor URB597 (SMD-4.43, 95% CI-6.32,-2.55). The largest effect size of DAI reduction by an endocannabinoid was AEA (SMD-3.03, 95% CI -4.89,-1.17), the largest effect size of DAI reduction by a phytocannabinoid was CBD (SMD -0.56, 95% CI-0.97, -0.16, I^2= 29%), and the largest synthetic cannabinoid effect size on DAI was AM1241 (SMD –3.11, 95% CI -5.01, -1.22). SMD and confidence intervals of individual drugs on DAI are given in table 4. Eighteen of twenty-five drugs had a large effect size, one had a moderate effect size, and six had no significant effect on DAI.

Myeloperoxidase Activity (MPO)

Twenty-six publications investigated the effects of 21 cannabinoid drugs on MPO activity throughout 57 individual experiments (total n = 757, n = 419 in experimental vs 338 in control groups). Cannabinoid drugs reduced MPO in comparison with vehicle; SMD -1.26, 95% CI-1.54 to -0.97, I^2=48.1% (figure 5, table 4). Overall, there was significant heterogeneity between studies and there was significant subgroup difference (I^2=48.1%, P<0.008). MPO was significantly reduced in mice and rats (SMD -1.28, 95% CI -1.59 to -0.98 I^2=61% and -1.06, 95% CI -1.99 to -0.13, I^2=56% respectively).

The largest effect size in MPO reduction was caused by the phytocannabinoid CBG (SMD -6.20, 95% CI -9.90 to -2.50, I^2 not assessed). The largest effect size by an endocannabinoid was PEA (SMD -2.74, 95% CI -4.42, -1.06, I^2=85%), the largest synthetic cannabinoid effect size on MPO was caused by ACEA (SMD -3.15, 95% CI -4.75, -1.55, I^2 not assessed), and the largest effect size of any enzyme or transport inhibitor was AA5HT (SMD -2.27, 95% CI -4.05, -0.49, I^2 not assessed). SMD and confidence intervals of individual drugs on MPO activity are given in table 4. Thirteen of 21 cannabinoid drugs had a large clinical effect, the remaining of which had no significant effect on MPO.

Time of administration

From the 50 publications examining the effect of cannabinoids on murine colitis, 28 studies administered cannabinoid agents either simultaneously with colitis onset, or prophylactically. 17 administered drugs between 15 minutes and 7 days after the onset of colitis. Additionally 7 studies compared the benefit of prophylactic cannabinoid use to therapeutic, but did not find any difference in efficacy. To investigate if timing of drug treatment affected DAI or MPO we compared study size-weighted effect sizes (dependent variable) with time of administration (covariate) using meta-regression. We found that timing of drug administration weakly predicted effect size in reducing DAI and MPO, although this was of borderline statistical significance (P=0.09 R^2=11% and P=0.055 R^2=41% respectively, figure 6 A and B).

Quality and risk of bias

Of the 53 papers, 21 used randomisation in their design, 7 reported blinding of assessment, 5 replicated their results in a second species, and 14 replicated their findings in a second model of colitis. 50 reported n≥5 in control and experimental groups. 15 publications reported specific numbers within groups. All papers reported a clinically relevant endpoint. Median study quality modified STAIR score was 5 out of 10 (mean 4.9, SD 2.29). Using meta-regression, higher quality scores predicted greater reductions in MPO activity (P=0.043 R^2=65%, figure 6 D), but not in DAI (P=0.98 R^2= 35%, Figure 6 C).

The SYRCLE risk of bias score for each endpoint showed a trend to larger reduction in DAI in studies with a larger risk of bias (P=0.084 R^2=69%, figure 6 E), but not MPO (P=0.345 R^2=8%, figure 6F).

Publication Bias

Funnel plots comparing MPO activity and DAI were constructed and analysed statistically for bias. The presence of publication bias was not found in either group (MPO; Egger's statistic P=0.570, figure 7A; DAI; Egger's statistic P=0.274, figure 7B).

Discussion

The aim of this study was to determine the efficacy of cannabinoid drugs in reducing gut inflammation to aid the design of further clinical studies. We found 53 studies that examined this effect using endocannabinoids, phytocannabinoids, synthetic cannabinoids, and enzyme and reuptake inhibitors across multiple models of murine and human colitis. In both qualitative assessment and meta-analysis, these controlled studies demonstrate that the use of cannabinoid drugs are beneficial in reducing colonic inflammation in rats and mice, with unclear effects in human subjects.

In animal studies, cannabinoids were shown to reduce inflammation both qualitatively, and at meta-analysis. Across experiments included in this review CB_2 agonists, FAAH inhibitors and CBD were the most widely studied and showed the greatest therapeutic benefit across all endpoints. Subgroup analyses suggested that CBG caused the greatest reduction in MPO activity scores followed by synthetic CB_1 agonist ACEA. However both agents were only studied within a single publication. In the MPO analysis the most studied drug was CBD, with 157 animals across 7 publications, demonstrating a significant effect on MPO activity reduction. Similarly, within DAI analysis CBD was again the most studied single drug including 181 animal across 6 publications. Although CBD demonstrated a significant effect on DAI reduction, the largest reduction in DAI was caused by the FAAH antagonist URB597, studied in one publication. There was statistical heterogeneity in both MPO and DAI analyses, which was partially accounted for by subgroup differences. At meta-regression, factors leading to subgroup differences were quality, timing and risk of bias.

Receptor targets were explored in 23 publications using receptor-specific agonists or antagonists, and receptor knock-down. In murine colitis, agonism of the CB_1 or CB_2 receptor brought about reduction in inflammation, and at subgroup analysis use of the synthetic CB_1/CB_2 agonists acting demonstrated the greatest reduction in disease scores and MPO activity. In addition, agonism of the PPARα, GPR55 and GPR18 receptors also reduced inflammation of the colon. The wide variation in the measured inflammatory endpoints across these studies prevented further meta-analysis. Interestingly the use of the peripherally restricted synthetic agonist SAB378, which agonises both CB_1 and CB_2 receptors, had no significant effect on either MPO activity or DAI. This is in contrast to *ex vivo* explant human colonic data, which demonstrated that cannabinoid agonism with AEA or CBD was beneficial in colonic mucosal inflammation, which were peripherally restricted by definition of the explant model (30,31). Izzo et al. (9) found through receptor antagonism that the effect of CBN in preventing hypermobility caused by croton oil was mediated by CB_1, but not CB_2. PEA was investigated by Capasso et al. (20,32) using two models of inflammation-induced hypermotility. Using receptor antagonists in both experiments Capasso et al. found that PEA, in an OM model, acted through CB_1 but not CB_2 or PPARα, but in a CO model PEA was still effective, but did not act through CB_1 or CB_2. This suggests that the mechanism by which PEA acts as an anti-inflammatory agent was not mediated by a single receptor, but by receptor co-dependence. ACEA was investigated for receptor mechanism in two publications, both of which found ACEA dependent on CB_1. None of the reviewed studies investigated a mechanism of action for AEA in gut inflammation, however one *ex vivo* human study from Harvey et al. found that AEA prevented increased cytokine production in experimentally inflamed human mucosa was dependent on CB_2, although the authors did not report antagonism of any other receptor (31).

The specific mechanism by which manipulation of the cannabinoid system affects inflammation is not clear. Esposito et al. (33) demonstrated that PEA brought about anti-inflammatory effects on enteric glial cells acting at toll-like receptor 4, suggesting that rather than acting at an epithelial mucosal level, acts at either at innate immune colonies or the enteric nervous system. This hypothesis as recently been evidence by a study demonstrating that both CBD and PEA do not act on the immune response of epithelial cells, but are likely to require the presence of these other cells types, acting through down regulation of NF-κβ (34), but is challenged by Cluny et al, demonstrating that peripherally restricted cannabinoids have a diminished effect on inflammation. Nevertheless it is clear that the mechanism of action of cannabinoids does not simply lie at the epithelial level, but is likely to reside within the gut-brain axis.

From the clinical literature we found two randomised placebo-controlled studies examining the effect of phytocannabinoids in humans. Our analysis found no overall effect of THC or CBD on disease scores, however there was large statistical and clinical heterogeneity between these studies. We found from meta-analysis that inhaled THC did have a beneficial effect on CDAI at 8 weeks, whereas CBD did not. There may be several reasons for this heterogeneity, firstly in all groups, small cohort sizes were used which may have overestimated positive or negative effects in both studies, making meaningful conclusions regarding the use of CBD or THC in inflammatory bowel disease difficult. Secondly, within the Naftali et al. (2017) study, very low doses of CBD were utilized compared to the use of CBD in other clinical trials, which commonly used 600mg twice daily (35). A recent trial in drug-resistant epilepsy used $20 mg.kg^{-1}$ daily for 4 weeks, with a small number of participants experiencing side effects such as vomiting and diarrhoea (36). It is likely that in adult males such 10mg doses had no clinical effect on Crohns disease as insufficient plasma concentrations may have been reached due to the poor bioavailability of oral CBD. A major flaw within the Naftali et al. 2013 trial is that sham cigarettes contained cannabis sativa flowers in which active cannabinoids had been removed. However, it is unlikely that other compounds present in cannabis (such as terpenes) which are known to have an anti-inflammatory effect had also been removed, which may have introduced positive bias into the study (37). However, despite these drawbacks, the Naftali et al. 2013 trial demonstrated a significant reduction in pain and the use of steroid therapy, with increased sleep and satisfaction levels with THC use compared to placebo. Although not included in this analysis, a study from Storr et al. (38) demonstrated that although cannabis use provided symptomatic relief from Crohns disease, the risk of salvage surgery was increased within 6 months of use (odds ratio = 5.03, 95% confidence interval = 1.45-17.46). However these findings have not yet been supported from randomised, blinding controlled trials. We may suggest, therefore, that phytocannabinoid use may be a future therapy in intestinal inflammation, although before firm conclusions are drawn, further clinical studies examining their effects be conducted at higher, therapeutic dosages with adequately powered cohort sizes. As MMJ use in inflammatory bowel disease has been justified because of its effects on appetite and diarrhoea, studies may be designed to examine these quality of life-affecting endpoints directly.

We found that most of the existing cannabinoid-gut research focusses on the therapeutic potential of CBD. This is unsurprising as CBD is currently used clinically, is well tolerated, and has shown consistently positive results. Nine studies found a positive, dose dependent effect on local inflammatory cytokine expression, COX2 activation, MPO activity, enteric glial cell activation and caspase-3 production, with associated improvements in macroscopic and histologic grades of inflammation (39–46). One study also showed that intraperitoneal CBD administration decreased oxidative-stress scores of peripheral lung and brain tissue following intestinal inflammation (47), adding to the existing evidence that CBD maintains the gut barrier during inflammation (48). Despite being the most-studied drug, the mechanism by which CBD acts was not made clear by this review. One study by De Fillipis et al (44), found that hyper-motility caused by LPS administration in mice was reduced by CBD through a CB_1 dependent mechanism. Similarly, Capasso et al. in 2008 found that CBD prevented croton oil-induced hypermotility via CB_1. *In vitro*, de Fillipis et al. in 2011 demonstrated that in human explant tissue S100B levels, as a marker of glial cell activation was decreased by CBD in a PPARγ dependent mechanism (although other antagonists were not investigated) (49).

The timing of cannabinoid administration correlated with reduction in effect on colitis activity, although did not reach statistical significance. There was a correlation between time of drug administration and effect size in both DAI and MPO reduction, with earlier administration of cannabinoids drugs producing a greater effect size, suggesting that in clinical trials cannabinoids may be used prophylactically and therapeutically. There is promise therefore that compounds targeting the endocannabinoid system may be able to not only prevent colonic inflammation, but treat established intestinal inflammatory conditions. As it is not clear if cannabinoids are more effective when treating new-onset or established intestinal inflammation, further study designs should investigate this endpoint specifically.

One important potential area for research is the combination of cannabinoid drugs with existing treatments for inflammatory bowel disease. In clinical practice it is common to treat patients with acute severe Crohns and ulcerative colitis with combination of agents, such as antibiotic, anti-TNFα, and corticosteroid therapy. One study compared the efficacy of CBD and THC with that of sulphasalazine, a 5-ASA, a drug commonly used in clinical practice (45). Although in this study CBD and THC efficacy were comparable to that of sulphasalazine, the authors did not examine for the potential additive or subtractive effect of these agents in the context of colitis.

The findings of this study are limited by several factors typically seen in meta-analyses and systematic reviews. We found significant heterogeneity between sub-groups in both DAI and MPO analyses, and suggested that 11% and 41% of this was due to the difference in time of administration in terms of changes in DAI and MPO respectively. Additionally we found a high risk of bias study design, and median study quality to be relatively low. Meta-regression demonstrated these factors significantly correlated with study outcomes. Although we did not analyse for differences between scoring systems and mode of colitis, these factors may have also contributed to heterogeneity and influenced outcome. We sought to overcome this variability between scoring systems with random effects analysis. Additionally within this review we have examined the effect of cannabinoid drugs *en mass*, which may have affected the overall outcome of meta-analyses. It is possible that some articles may have not been identified in initial searches, or conference abstracts missed from the search period. Lastly, where control groups were compared to multiple experimental groups within the same set of experiments variance and SMD may be exaggerated, leading to further bias.

In conclusion, we have shown in this systematic review and meta-analysis that cannabinoid drugs are beneficial in treating experimentally-induced murine models of colitis. These positive findings support the development of further human clinical trials. Current literature converges on CBD, and in order to avoid research bias the effect of all cannabinoid drugs, including the large number of currently un-investigated phytocannabinoid drugs, should also be investigated.

References

1. Kappelman MD, Rifas–Shiman SL, Kleinman K, Ollendorf D, Bousvaros A, Grand RJ, et al. The Prevalence and Geographic Distribution of Crohn's Disease and Ulcerative Colitis in the United States. Clin Gastroenterol Hepatol [Internet]. 2007 Dec;5(12):1424–9.

2. Rubin GP, Hungin AP, Kelly PJ, Ling J. Inflammatory bowel disease: epidemiology and management in an English general practice population. Aliment Pharmacol Ther [Internet]. 2000 Dec;14(12):1553–9.

3. Sandborn WJ, Rutgeerts P, Feagan BG, Reinisch W, Olson A, Johanns J, et al. Colectomy Rate Comparison After Treatment of Ulcerative Colitis With Placebo or Infliximab. Gastroenterology [Internet]. 137:1250–60.

4. Sutherland LR, Martin F, Bailey RJ, Fedorak RN, Poleski M, Dallaire C, et al. A Randomized, Placebo-Controlled, Double-Blind Trial of Mesalamine in the Maintenance of Remission of Crohn's Disease. Gastroenterology [Internet]. 1997;112:1069–77.

5. Herrinton LJ, Liu L, Weng X, Lewis JD, Hutfless S, Allison JE. Role of Thiopurine and Anti-TNF Therapy in Lymphoma in Inflammatory Bowel Disease. Am J Gastroenterol [Internet]. 2011 Dec 25;106(12):2146–53.

6. Alhamoruni A, Wright KL, Larvin M, O'Sullivan SE. Cannabinoids mediate opposing effects on inflammation-induced intestinal permeability. Br J Pharmacol. 2012;165:2598–610.

7. Storr M a., Sharkey K a. The endocannabinoid system and gut-brain signalling. Curr Opin Pharmacol. 2007;7:575–82.

8. Lee Y, Jo J, Chung HY, Pothoulakis C, Im E. Endocannabinoids in the gut. Am J Physiol Gastrointest Liver Physiol [Internet]. 2016 Aug 18;ajpgi.00294.2015.

9. Izzo a a, Fezza F, Capasso R, Bisogno T, Pinto L, Iuvone T, et al. Cannabinoid CB1-receptor mediated regulation of gastrointestinal motility in mice in a model of intestinal inflammation. Br J Pharmacol. 2001;134:563–70.

10. Di Marzo V, Izzo a a. Endocannabinoid overactivity and intestinal inflammation. Gut. 2006;55:1373–6.

11. Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, et al. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science [Internet]. 1992 Dec 18;258(5090):1946–9.

12. Freund TF, Katona I, Piomelli D. Role of endogenous cannabinoids in synaptic signaling. Physiol Rev [Internet]. 2003 Jul;83(3):1017–66.

13. Muccioli GG. Endocannabinoid biosynthesis and inactivation, from simple to complex. Vol. 15, Drug Discovery Today. 2010. p. 474–83.

14. Blankman JL, Simon GM, Cravatt BF. A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. Chem Biol [Internet]. 2007 Dec;14(12):1347–56.

15. O'Sullivan SE. Cannabinoids go nuclear: evidence for activation of peroxisome proliferator-activated receptors. Br J Pharmacol [Internet]. 2007 Nov;152(5):576–82.

16. Smart D, Jonsson K-O, Vandevoorde S, Lambert DM, Fowler CJ. "Entourage" effects of N-acyl ethanolamines at human vanilloid receptors. Comparison of effects upon anandamide-induced vanilloid receptor activation and upon anandamide metabolism. Br J Pharmacol [Internet]. 2002 Jun;136(3):452–8.

17. Leocani L, Nuara A, Houdayer E, Schiavetti I, Del Carro U, Amadio S, et al. Sativex(®) and clinical-neurophysiological measures of spasticity in progressive multiple sclerosis. J Neurol [Internet]. 2015 Nov;262(11):2520–7.

18. Reddy DS, Golub VM. The Pharmacological Basis of Cannabis Therapy for Epilepsy. J Pharmacol Exp Ther [Internet]. 2016 Mar 1;357(1):45–55.

19. Suárez J, Romero-Zerbo Y, Márquez L, Rivera P, Iglesias M, Bermúdez-Silva FJ, et al. Ulcerative colitis impairs the acylethanolamide-based anti-inflammatory system reversal by 5-aminosalicylic acid and glucocorticoids. PLoS One [Internet]. 2012 Jan;7(5):e37729.

20. Capasso R, Orlando P, Pagano E, Aveta T, Buono L, Borrelli F, et al. Palmitoylethanolamide normalizes intestinal motility in a model of post-inflammatory accelerated transit: involvement of CB_1 receptors and TRPV1 channels. Br J Pharmacol [Internet]. 2014 Sep;171(17):4026–37.

21. Akbar A, Yiangou Y, Facer P, Brydon WG, Walters JRF, Anand P, et al. Expression of the TRPV1 receptor differs in quiescent inflammatory bowel disease with or without abdominal pain. Gut [Internet]. 2010 Jun 1;59(6):767–74.

22. D'Argenio G, Valenti M, Scaglione G, Cosenza V, Sorrentini I, Di Marzo V. Up-regulation of anandamide levels as an endogenous mechanism and a pharmacological strategy to limit colon inflammation. FASEB J. 2006;20:568–70.

23. D'Argenio G, Petrosino S, Gianfrani C, Valenti M, Scaglione G, Grandone I, et al. Overactivity of the intestinal endocannabinoid system in celiac disease and in methotrexate-treated rats. J Mol Med. 2007;85:523–30.

24. Karwad MA, Macpherson T, Wang B, Theophilidou E, Sarmad S, Barrett DA, et al. Oleoylethanolamine and palmitoylethanolamine modulate intestinal permeability in vitro via TRPV1 and PPARα. FASEB J [Internet]. 2016 Sep 13;fj.201500132.

25. Massa F, Marsicano G, Hermann H, Cannich A, Monory K, Cravatt BF, et al. The endogenous cannabinoid system protects against colonic inflammation. J Clin Invest [Internet]. 2004 Apr 15;113(8):1202–9.

26. Higgins JPT, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ [Internet]. 2011;343.

27. Fisher M, Feuerstein G, Howells DW, Hurn PD, Kent TA, Savitz SI, et al. Update of the stroke therapy academic industry roundtable preclinical recommendations. Stroke [Internet]. 2009 Jun;40(6):2244–50.

28. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. PLOS Biol [Internet]. 2010;8(6).

29. Hooijmans CR, Rovers MM, de Vries RBM, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE's risk of bias tool for animal studies. BMC Med Res Methodol [Internet]. 2014 Mar 26;14:43.

30. Harvey BS, Sia TC, Wattchow D a., Smid SD. Interleukin 17A evoked mucosal damage is attenuated by cannabidiol and anandamide in a human colonic explant model. Cytokine [Internet]. 2014;65(2):236–44.

31. Nicotra LL, Vu M, Harvey BS, Smid SD. Prostaglandin ethanolamides attenuate damage in a human explant colitis model. Prostaglandins Other Lipid Mediat [Internet]. 2013;100–101:22–9.

32. Capasso R, Izzo AA, Fezza F, Pinto A, Capasso F, Mascolo N, et al. Inhibitory effect of palmitoylethanolamide on gastrointestinal motility in mice. Br J Pharmacol [Internet]. 2001 Nov;134(5):945–50.

33. Esposito G, Capoccia E, Turco F, Palumbo I, Lu J, Steardo A, et al. Palmitoylethanolamide improves colon inflammation through an enteric glia/toll like receptor 4-dependent PPAR-α activation. Gut [Internet]. 2014;1300–12.

34. Couch DG, Tasker C, Theophilidou E, Lund JN, O'Sullivan SE. Cannabidiol and palmitoylethanolamide are anti-inflammatory in the acutely inflamed human colon. Clin Sci [Internet]. 2017 Nov 1;131(21):2611–26.

35. Naftali T, Mechulam R, Marii A, Gabay G, Stein A, Bronshtain M, et al. Low-Dose Cannabidiol Is Safe but Not Effective in the Treatment for Crohn's Disease, a Randomized Controlled Trial. Dig Dis Sci [Internet]. 2017 Mar 27;

36. Devinsky O, Cross JH, Laux L, Marsh E, Miller I, Nabbout R, et al. Trial of Cannabidiol for Drug-Resistant Seizures in the Dravet Syndrome. N Engl J Med [Internet]. 2017 May 25;376(21):2011–20.

37. de las Heras B, Hortelano S. Molecular basis of the anti-inflammatory effects of terpenoids. Inflamm Allergy Drug Targets [Internet]. 2009 Mar;8(1):28–39.

38. Storr M, Devlin S, Kaplan GG, Panaccione R, Andrews CN. Cannabis Use Provides Symptom Relief in Patients with Inflammatory Bowel Disease but Is Associated with Worse Disease Prognosis in Patients with Crohn's Disease. Inflamm Bowel Dis [Internet]. 2014 Mar;20(3):472–80.

39. Pagano E, Capasso R, Piscitelli F, Romano B, Parisi OA, Finizio S, et al. An Orally Active Cannabis Extract with High Content in Cannabidiol attenuates Chemically-induced Intestinal Inflammation and Hypermotility in the Mouse. Front Pharmacol [Internet]. 2016 Oct 4;7:341.

40. Krohn RM, Parsons SA, Fichna J, Patel KD, Yates RM, Sharkey KA, et al. Abnormal cannabidiol attenuates experimental colitis in mice, promotes wound healing and inhibits neutrophil recruitment. J Inflamm (Lond) [Internet]. 2016 Dec 14;13(1):21.

41. Harvey BS, Nicotra LL, Vu M, Smid SD. Cannabinoid CB2 receptor activation attenuates cytokine-evoked mucosal damage in a human colonic explant model without changing epithelial permeability. Cytokine. 2013;63:209–17.

42. Schicho R, Storr M. Topical and systemic cannabidiol improves trinitrobenzene sulfonic acid colitis in mice. Pharmacology [Internet]. 2012 Jan;89(3–4):149–55.

43. Lin X-H, Yuece B, Li Y-Y, Feng Y-J, Feng J-Y, Yu L-Y, et al. A novel CB receptor GPR55 and its ligands are involved in regulation of gut movement in rodents. Neurogastroenterol Motil [Internet]. 2011 Sep;23(9):862-e342.

44. de Filippis D, Iuvone T, D'amico A, Esposito G, Steardo L, Herman AG, et al. Effect of cannabidiol on sepsis-induced motility disturbances in mice: involvement of CB receptors and fatty acid amide hydrolase. Neurogastroenterol Motil [Internet]. 2008 Aug;20(8):919–27.

45. Jamontt JM, Molleman A, Pertwee RG, Parsons ME. The effects of delta 9-tetrahydrocannabinol and cannabidiol alone and in combination on damage, inflammation and in vitro motility disturbances in rat colitis. Br J Pharmacol. 2010;160(November 2009):712–23.

46. Borrelli F, Aviello G, Romano B, Orlando P, Capasso R, Maiello F, et al. Cannabidiol, a safe and non-psychotropic ingredient of the marijuana plant Cannabis sativa, is protective in a murine model of colitis. J Mol Med. 2009;87:1111–21.

47. Cassol-Jr OJ, Comim CM, Silva BR, Hermani F V., Constantino LS, Felisberto F, et al. Treatment with cannabidiol reverses oxidative stress parameters, cognitive impairment and mortality in rats submitted to sepsis by cecal ligation and puncture. Brain Res [Internet]. 2010 Aug 12;1348:128–38.

48. Alhamoruni A, Lee C, Wright KL, Larvin M, O'Sullivan SE. Pharmacological effects of cannabinoids on the Caco-2 cell culture model of intestinal permeability. J Pharmacol Exp Ther. 2010;335(1):92–102.

49. de Filippis D, Esposito G, Cirillo C, Cipriano M, de Winter BY, Scuderi C, et al. Cannabidiol reduces intestinal inflammation through the control of neuroimmune axis. PLoS One [Internet]. 2011;6(12):1–8.

50. Mathison R, Ho W, Pittman QJ, Davison JS, Sharkey K a. Effects of cannabinoid receptor-2 activation on accelerated gastrointestinal transit in lipopolysaccharide-treated rats. Br J Pharmacol. 2004;142:1247–54.

51. Kimball ES, Schneider CR, Wallace NH, Hornby PJ. Agonists of cannabinoid receptor 1 and 2 inhibit experimental colitis induced by oil of mustard and by dextran sulfate sodium. Am J Physiol Gastrointest Liver Physiol. 2006;291:G364–71.

52. Capasso R, Borrelli F, Aviello G, Romano B, Scalisi C, Capasso F, et al. Cannabidiol, extracted from Cannabis sativa, selectively inhibits inflammatory hypermotility in mice. Br J Pharmacol. 2008;154:1001–8.

53. Engel M a., Kellermann C a., Rau T, Burnat G, Hahn EG, Konturek PC. Ulcerative colitis in AKR mice is attenuated by intraperitoneally administered anandamide. J Physiol Pharmacol. 2008;59(Cd):673–89.

54. Storr MA, Keenan CM, Emmerdinger D, Zhang H, Yüce B, Sibaev A, et al. Targeting endocannabinoid degradation protects against experimental colitis in mice: involvement of CB1 and CB2 receptors. J Mol Med (Berl) [Internet]. 2008 Aug;86(8):925–36.

55. li y. -y., li y. -n., ni j. -b., chen c. -j., lv s., chai s. -y., et al. Involvement of cannabinoid-1 and cannabinoid-2 receptors in septic ileus. Neurogastroenterol Motil [Internet]. 2010 Mar 1;22(3):350-e88.

56. Storr M a., Keenan CM, Zhang H, Patel KD, Makriyannis A, Sharkey K a. Activation of the cannabinoid 2 receptor (CB2) protects against experimental colitis. Inflamm Bowel Dis. 2009;15(11):1678–85.

57. Cluny NL, Keenan CM, Duncan M, Fox A, Lutz B, Sharkey K a. (SAB378), a Peripherally Restricted Cannabinoid CB 1 / CB 2 Receptor Agonist , Inhibits Gastrointestinal Motility but Has No Effect on Experimental Colitis in Mice. 2010;334(3):973–80.

58. Kimball ES, Wallace NH, Schneider CR, D ’andrea MR, Hornby PJ, Storr M. Small intestinal cannabinoid receptor changes following a single colonic insult with oil of mustard in mice. Front Pharmacol [Internet]. 2010;1(132):1–8.

59. Alhouayek M, Lambert DM, Delzenne NM, Cani PD, Muccioli GG. Increasing endogenous 2-arachidonoylglycerol levels counteracts colitis and related systemic inflammation. FASEB J. 2011;25:2711–21.

60. Andrzejak V, Muccioli GG, Body-Malapel M, El Bakali J, Djouina M, Renault N, et al. New FAAH inhibitors based on 3-carboxamido-5-aryl-isoxazole scaffold that protect against experimental colitis. Bioorg Med Chem [Internet]. 2011 Jun 15;19(12):3777–86.

61. Bento AF, Marcon R, Dutra RC, Claudino RF, Cola M, Leite DFP, et al. β-Caryophyllene inhibits dextran sulfate sodium-induced colitis in mice through CB2 receptor activation and PPARγ pathway. Am J Pathol [Internet]. 2011 Mar;178(3):1153–66.

62. Schicho R, Bashashati M, Bawa M, McHugh D, Saur D, Hu H-M, et al. The atypical cannabinoid O-1602 protects against experimental colitis and inhibits neutrophil recruitment. Inflamm Bowel Dis [Internet]. 2011 Aug;17(8):1651–64.

63. Bashashati M, Storr M a., Nikas SP, Wood JT, Godlewski G, Liu J, et al. Inhibiting fatty acid amide hydrolase normalizes endotoxin-induced enhanced gastrointestinal motility in mice. Br J Pharmacol. 2012;165:1556–71.

64. Izzo AA, Capasso R, Aviello G, Borrelli F, Romano B, Piscitelli F, et al. Inhibitory effect of cannabichromene, a major non-psychotropic cannabinoid extracted from Cannabis sativa, on inflammation-induced hypermotility in mice. Br J Pharmacol [Internet]. 2012 Jun;166(4):1444–60.

65. Lehmann C, Kianian M, Zhou J, Küster I, Kuschnereit R, Whynot S, et al. Cannabinoid receptor 2 activation reduces intestinal leukocyte recruitment and systemic inflammatory mediator release in acute experimental sepsis. Crit Care [Internet]. 2012 Dec 12;16(2):R47.

66. Singh UP, Singh NP, Singh B, Price RL, Nagarkatti M, Nagarkatti PS. Cannabinoid receptor-2 (CB2) agonist ameliorates colitis in IL-10(-/-) mice by attenuating the activation of T cells and promoting their apoptosis. Toxicol Appl Pharmacol [Internet]. 2012 Jan 15;258(2):256–67.

67. Borrelli F, Fasolino I, Romano B, Capasso R, Maiello F, Coppola D, et al. Beneficial effect of the non-psychotropic plant cannabinoid cannabigerol on experimental inflammatory bowel disease. Biochem Pharmacol [Internet]. 2013;85(9):1306–16.

68. Li YY, Yuece B, Cao HM, Lin HX, Lv S, Chen JC, et al. Inhibition of p38/Mk2 signaling pathway improves the anti-inflammatory effect of WIN55 on mouse experimental colitis. Lab Investig [Internet]. 2013 Mar 4;93(3):322–33.

69. Matos I, Bento AF, Marcon R, Claudino RF, Calixto JB. Preventive and therapeutic oral administration of the pentacyclic triterpene α,β-amyrin ameliorates dextran sulfate sodium-induced colitis in mice: the relevance of cannabinoid system. Mol Immunol [Internet]. 2013 Jul;54(3–4):482–92.

70. Naftali T, Bar-Lev Schleider L, Dotan I, Lansky EP, Sklerovsky Benjaminov F, Konikoff FM. Cannabis induces a clinical response in patients with Crohn’s disease: a prospective placebo-controlled study. Clin Gastroenterol Hepatol [Internet]. 2013 Oct;11(10):1276–1280.e1.

71. Romano B, Borrelli F, Fasolino I, Capasso R, Piscitelli F, Cascio M, et al. The cannabinoid TRPA1 agonist cannabichromene inhibits nitric oxide production in macrophages and ameliorates murine colitis. Br J Pharmacol [Internet]. 2013 May;169(1):213–29.

72. Wallace JL, Flannigan KL, McKnight W, Wang L, Ferraz JGP, Tuitt D. Pro-resolution, protective and anti-nociceptive effects of a cannabis extract in the rat gastrointestinal tract. J Physiol Pharmacol [Internet]. 2013 Apr;64(2):167–75.

73. Borrelli F, Romano B, Petrosino S, Pagano E, Capasso R, Coppola D, et al. Palmitoylethanolamide, a naturally occurring lipid, is an orally effective intestinal anti-inflammatory agent. Br J Pharmacol [Internet]. 2015;172:142–58.

74. Fichna J, Sałaga M, Stuart J, Saur D, Sobczak M, Zatorski H, et al. Selective inhibition of FAAH produces antidiarrheal and antinociceptive effect mediated by endocannabinoids and cannabinoid-like fatty acid amides. Neurogastroenterol Motil [Internet]. 2014 Apr;26(4):470–81.

75. Sałaga M, Mokrowiecka A, Zakrzewski PK, Cygankiewicz A, Leishman E, Sobczak M, et al. Experimental colitis in mice is attenuated by changes in the levels of endocannabinoid metabolites induced by selective inhibition of fatty acid amide hydrolase (FAAH). J Crohns Colitis [Internet]. 2014 Sep 1;8(9):998–1009.

76. Sardinha J, Kelly MEM, Zhou J, Lehmann C. Experimental cannabinoid 2 receptor-mediated immune modulation in sepsis. Mediators Inflamm [Internet]. 2014;2014:978678.

77. Alhouayek M, Bottemanne P, Subramanian K V, Lambert DM, Makriyannis A, Cani PD, et al. N-Acylethanolamine-hydrolyzing acid amidase inhibition increases colon N-palmitoylethanolamine levels and counteracts murine colitis. FASEB J [Internet]. 2015 Feb;29(2):650–61.

78. El Bakali J, Muccioli GG, Body-Malapel M, Djouina M, Klupsch F, Ghinet A, et al. Conformational Restriction Leading to a Selective CB2 Cannabinoid Receptor Agonist Orally Active Against Colitis. ACS Med Chem Lett [Internet]. 2015 Feb 12;6(2):198–203.

79. Impellizzeri D, Di Paola R, Cordaro M, Gugliandolo E, Casili G, Morittu VM, et al. Adelmidrol, a palmitoylethanolamide analogue, as a new pharmacological treatment for the management of acute and chronic inflammation. Biochem Pharmacol [Internet]. 2016 Nov 1;119:27–41.

80. Sasso O, Migliore M, Habrant D, Armirotti A, Albani C, Summa M, et al. Multitarget fatty acid amide hydrolase/cyclooxygenase blockade suppresses intestinal inflammation and protects against nonsteroidal anti-inflammatory drug-dependent gastrointestinal damage. FASEB J [Internet]. 2015 Jun;29(6):2616–27.

81. Stančić A, Jandl K, Hasenöhrl C, Reichmann F, Marsche G, Schuligoi R, et al. The GPR55 antagonist CID16020046 protects against intestinal inflammation. Neurogastroenterol Motil. 2015;27(10).

82. Cordaro M, Impellizzeri D, Gugliandolo E, Siracusa R, Crupi R, Esposito E, et al. Adelmidrol, a Palmitoylethanolamide Analogue, as a New Pharmacological Treatment for the Management of Inflammatory Bowel Disease. Mol Pharmacol [Internet]. 2016 Oct 3;90(5):549–61.

83. Feng Y-J, Li Y-Y, Lin X-H, Li K, Cao M-H. Anti-inflammatory effect of cannabinoid agonist WIN55, 212 on mouse experimental colitis is related to inhibition of p38MAPK. World J Gastroenterol [Internet]. 2016 Nov 21;22(43):9515–24.

84. Ke P, Shao B-Z, Xu Z-Q, Wei W, Han B-Z, Chen X-W, et al. Activation of Cannabinoid Receptor 2 Ameliorates DSS-Induced Colitis through Inhibiting NLRP3 Inflammasome in Macrophages. Allen IC, editor. PLoS One [Internet]. 2016 Sep 9;11(9):e0155076.

85. Sarnelli G, D'Alessandro A, Iuvone T, Capoccia E, Gigli S, Pesce M, et al. Palmitoylethanolamide Modulates Inflammation-Associated Vascular Endothelial Growth Factor (VEGF) Signaling via the Akt/mTOR Pathway in a Selective Peroxisome Proliferator-Activated Receptor Alpha (PPAR-α)-Dependent Manner. PLoS One [Internet]. 2016;11(5):e0156198.

86. Lin S, Li Y, Shen L, Zhang R, Yang L, Li M, et al. The Anti-Inflammatory Effect and Intestinal Barrier Protection of HU210 Differentially Depend on TLR4 Signaling in Dextran Sulfate Sodium-Induced Murine Colitis. Dig Dis Sci [Internet]. 2017 Feb 19;62(2):372–86.

87. Shamran H, Singh NP, Zumbrun EE, Murphy A, Taub DD, Mishra MK, et al. Fatty acid amide hydrolase (FAAH) blockade ameliorates experimental colitis by altering microRNA expression and suppressing inflammation. Brain Behav Immun [Internet]. 2017 Jan;59:10–20.

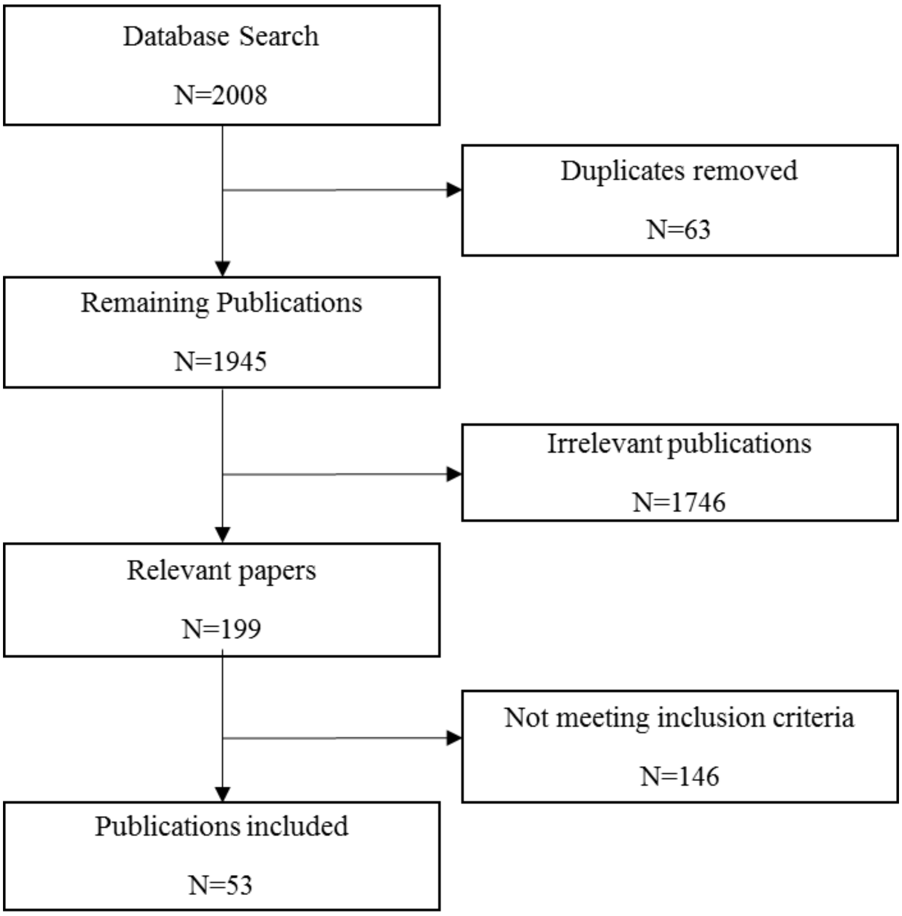


Figure 1. Record identification process

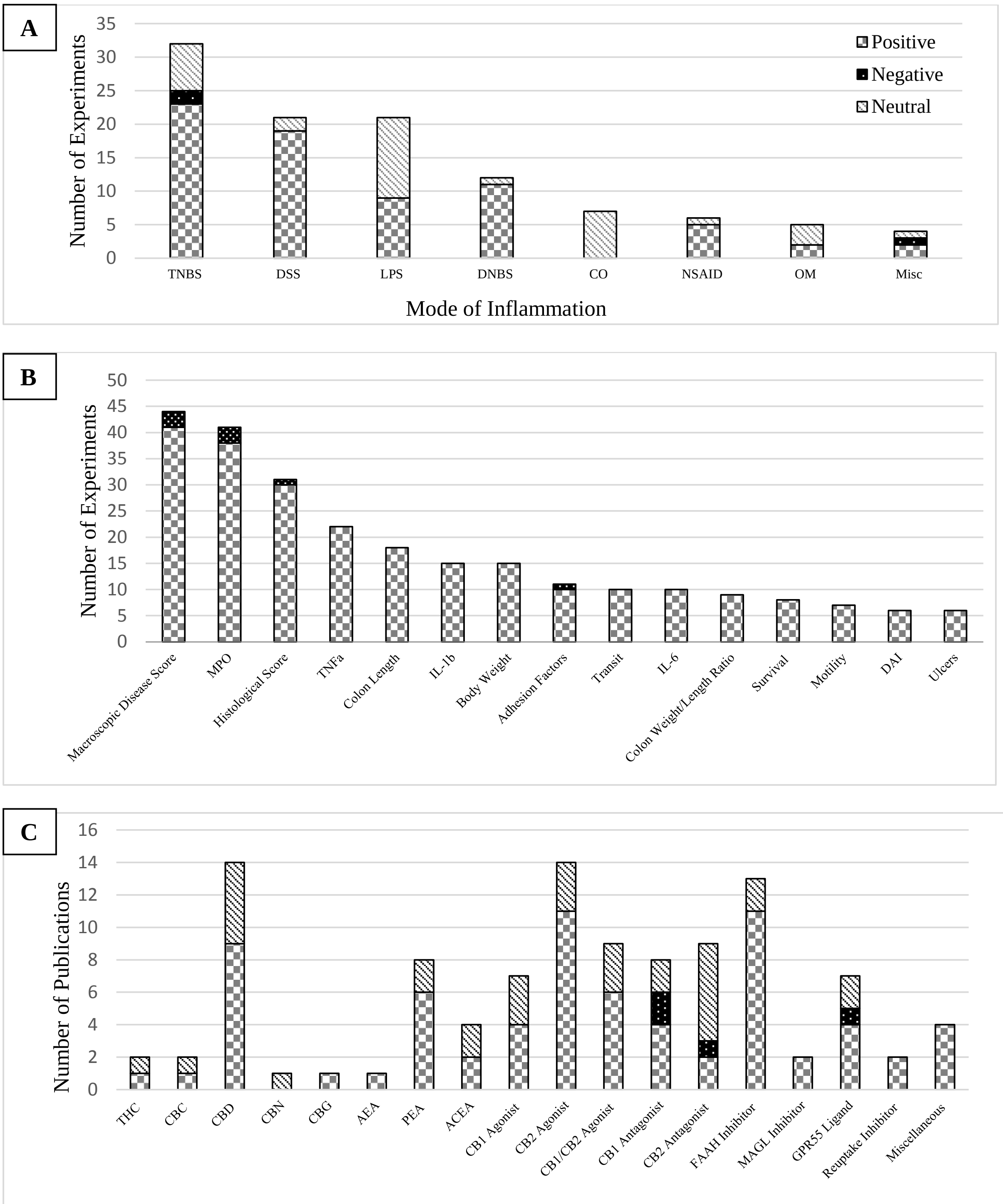


Figure 2. Positive, negative and neutral outcomes of cannabinoid treatment across modes of inflammation (**A**). Incidence of endpoints across all experiments comparing cannabinoid treatment with control (**B**). The effect of cannabinoid drugs compared to control across all endpoints expressed as primary drug investigated (**C**).

	Cannabinoids			Placebo				Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI
Naftali 2003 THC 115mg INH, 8 wks	152	109	11	306	143	10	50.0%	-154.00 [-263.57, -44.43]
Naftali 2017 CBD 10mg p.o. BD 8 weeks	220	122	10	216	121	9	50.0%	4.00 [-105.39, 113.39]
Total (95% CI)			**21**			**19**	**100.0%**	**-74.97 [-229.81, 79.87]**

Heterogeneity: Tau² = 9361.92; Chi² = 4.00, df = 1 (P = 0.05); I² = 75%

Test for overall effect: Z = 0.95 (P = 0.34)

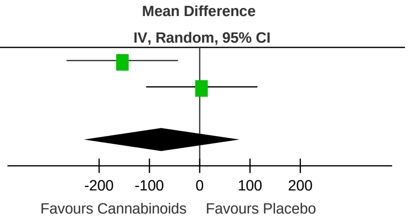
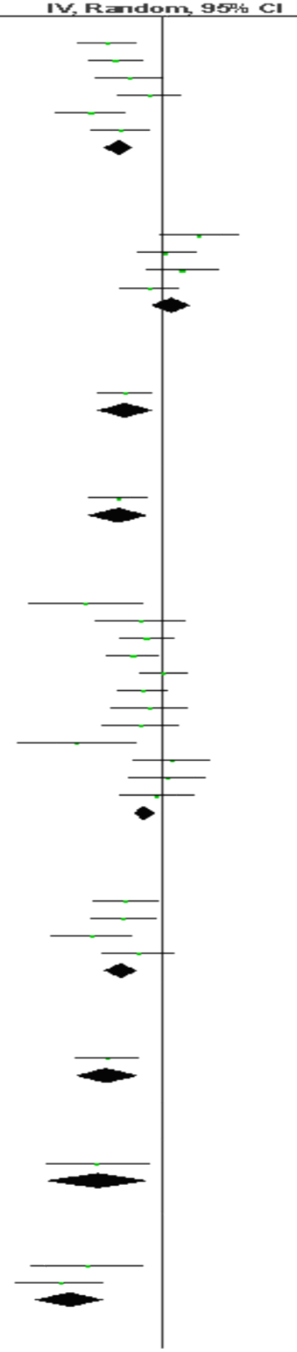


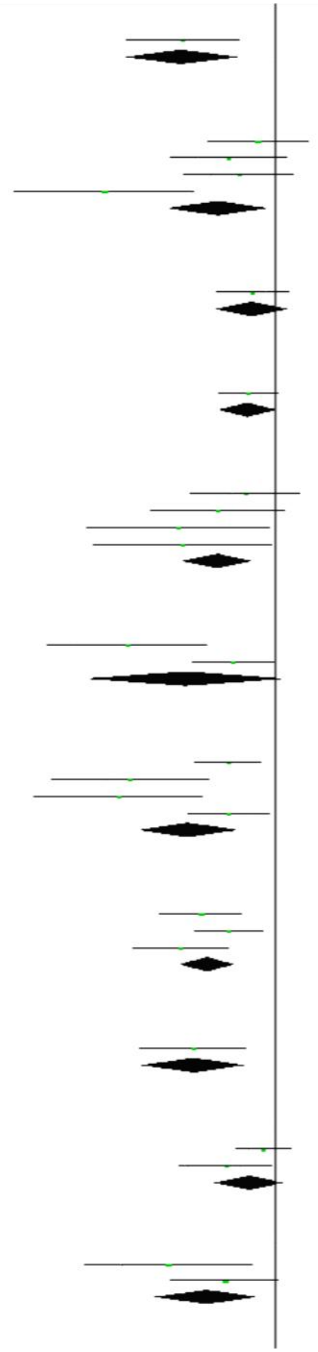
Figure 3. Forest plot of the effects of cannabinoid treatment on Crohns Disease, assessed by reduction in CDAI in human studies.

Study or Subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight	Std. Mean Difference IV, Random, 95% CI
1.1.1 PEA								
Borrelli 2014 PEA 1mg/kg p.o. DNBS (t)	50.1	4.5033	12	77.3	19.7454	12	1.9%	-1.83 [-2.81, -0.85]
Borrelli 2014 PEA 1mg/kg i.p. DNBS (t)	48.5	24.2487	12	84.94	20.7846	12	2.0%	-1.56 [-2.49, -0.62]
Esposito 2014 PEA 2mg/kg i.p. DSS (t)	1.12	0.6325	10	1.8	0.4249	5	1.7%	-1.11 [-2.28, 0.06]
Esposito 2014 PEA 10mg/kg i.p. DSS (t)	0.72	2.846	10	1.8	0.4249	5	1.8%	-0.43 [-1.52, 0.66]
Impellizzeri 2015 PEA 10mg/kg i.p. DNBS (t)	40.66	31.3065	10	100	13.0286	10	1.7%	-2.37 [-3.57, -1.17]
Alhouayek 2015 PEA 10mg/kg i.p. DSS (p)	78.3	15.8114	10	100	13.5978	10	1.9%	-1.41 [-2.41, -0.41]
Subtotal (95% CI)			**64**			**54**	**11.1%**	**-1.45 [-1.94, -0.96]**
Heterogeneity: Tau² = 0.09; Chi² = 6.62, df = 5 (P = 0.25); I² = 25%								
Test for overall effect: Z = 5.74 (P < 0.00001)								
1.1.2 SAB378								
Cluny 2010 SAB378 0.1mg/kg i.p. DSS (t)	9.53	1.3294	8	7.2	2.5456	4	1.6%	1.21 [-0.13, 2.54]
Cluny 2010 SAB378 1mg/kg i.p. DSS (t)	7.5	2.2627	8	7.2	2.5456	8	1.9%	0.12 [-0.86, 1.10]
Cluny 2010 SAB378 0.1mg/kg i.p. TNBS (p)	7.45	1.5274	8	6.2	2.2627	4	1.7%	0.65 [-0.59, 1.89]
Cluny 2010 SAB378 1mg/kg i.p.TNBS (p)	5.12	2.5456	8	6.2	2.2627	8	1.9%	-0.42 [-1.42, 0.57]
Subtotal (95% CI)			**32**			**24**	**7.1%**	**0.28 [-0.38, 0.94]**
Heterogeneity: Tau² = 0.13; Chi² = 4.19, df = 3 (P = 0.24); I² = 28%								
Test for overall effect: Z = 0.83 (P = 0.41)								
1.1.3 JZL184								
Alhouayek 2011 JZL184 16mg/kg i.p. TNBS (p)	2.7	2.3216	11	5.13	1.3266	11	2.0%	-1.24 [-2.16, -0.31]
Subtotal (95% CI)			**11**			**11**	**2.0%**	**-1.24 [-2.16, -0.31]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 2.61 (P = 0.009)								
1.1.4 Compound 39								
Andrzejak 2011 Compound 39 5mg/kg i.p. TNBS (p)	2.2	0.9487	10	4.2	1.5811	10	1.9%	-1.47 [-2.48, -0.46]
Subtotal (95% CI)			**10**			**10**	**1.9%**	**-1.47 [-2.48, -0.46]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 2.84 (P = 0.004)								
1.1.5 CBD								
Borrelli 2009 CBD 10mg/kg i.p. DNBS (p)	2.26	0.7155	5	4.2	0.6485	5	1.1%	-2.57 [-4.47, -0.66]
Jamontt 2010 CBD 10mg/kg i.p. TNBS (p)	2.9	3.3166	11	5.51	2.8523	2	1.4%	-0.74 [-2.29, 0.80]
Krohn 2016 abCBD 5mg/kg i.p. TNBS (t)	4.05	2.3085	10	5.38	2.5298	10	2.0%	-0.53 [-1.42, 0.37]
Schicho 2012 CBD 10mg.kg i.p. TNBS (p)	4.3	0.995	11	5.9	1.99	11	2.0%	-0.98 [-1.87, -0.08]
Schicho 2012 CBD 20mg/kg p.o. TNBS (p)	5.57	0.995	11	5.5	1.8905	11	2.1%	0.04 [-0.79, 0.88]
Schicho 2012 CBD 20mg/kg p.r. TNBS (p)	6.15	1.3266	11	7.07	1.3266	11	2.1%	-0.67 [-1.53, 0.20]
Borrelli 2009 CBD 1mg/kg i.p. DNBS (p)	3.9	0.6485	5	4.2	0.6485	5	1.6%	-0.42 [-1.68, 0.84]
Borrelli 2009 CBD 2.5mg/kg i.p. DNBS (p)	3.55	0.9391	5	4.2	0.6485	5	1.6%	-0.73 [-2.03, 0.58]
Borrelli 2009 CBD 5mg/kg i.p. DNBS (p)	2.13	0.6708	5	4.2	0.6485	5	1.0%	-2.83 [-4.85, -0.81]
Jamontt 2010 CBD 5mg/kg i.p. TNBS (p)	6.7	5.3066	11	5.15	2.8523	3	1.6%	0.29 [-0.99, 1.57]
Jamontt 2010 CBD 15mg/kg i.p. TNBS (p)	5.52	1.99	11	5.15	2.8523	3	1.6%	0.16 [-1.12, 1.44]
Jamontt 2010 CBD 20mg/kg i.p. TNBS (p)	4.65	4.6433	11	5.51	2.8523	3	1.6%	-0.18 [-1.46, 1.10]
Subtotal (95% CI)			**107**			**74**	**19.9%**	**-0.56 [-0.97, -0.16]**
Heterogeneity: Tau² = 0.14; Chi² = 15.43, df = 11 (P = 0.16); I² = 29%								
Test for overall effect: Z = 2.75 (P = 0.006)								
1.1.6 WIN 522,212								
Cluny 2010 WIN 52212-2 2mg/kg i.p. DSS (t)	4.2	1.9799	8	7.2	2.5456	8	1.8%	-1.24 [-2.34, -0.15]
Cluny 2010 WIN 52212-2 2mg/kg i.p. TNBS (t)	3.7	1.1314	8	6.2	2.2627	8	1.8%	-1.32 [-2.43, -0.21]
Feng 2016 WIN55,212 5mg/kg i.p. DSS (t)	5.05	2.2627	8	9.77	1.4142	8	1.6%	-2.37 [-3.73, -1.00]
Li 2013 WIN55212-2 5mg/kg i.p. DSS (t)	2.29	0.9798	6	3.5	1.7146	6	1.7%	-0.80 [-2.00, 0.40]
Subtotal (95% CI)			**30**			**30**	**6.9%**	**-1.37 [-1.96, -0.78]**
Heterogeneity: Tau² = 0.00; Chi² = 2.98, df = 3 (P = 0.39); I² = 0%								
Test for overall effect: Z = 4.55 (P < 0.00001)								
1.1.7 Adelmidrol								
Cordaro 2016 Ademidrol 10mg/kg p.o DNBS (t)	4.3	1.8974	10	7.2	0.9487	10	1.8%	-1.85 [-2.94, -0.77]
Subtotal (95% CI)			**10**			**10**	**1.8%**	**-1.85 [-2.94, -0.77]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 3.34 (P = 0.0008)								
1.1.8 AA5HT								
D'Argenio 2006 AA5HT10mg/kg s.c DNBS (t)	1.51	0.8497	5	4.2	1.3416	5	1.2%	-2.16 [-3.90, -0.43]
Subtotal (95% CI)			**5**			**5**	**1.2%**	**-2.16 [-3.90, -0.43]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 2.44 (P = 0.01)								
1.1.9 VDM115								
D'Argenio 2006 VDM11 5mg/kg s.c. DNBS (t)	0.7054	1.1628	5	4.2	1.3416	5	1.1%	-2.51 [-4.40, -0.63]
Storr 2008 VDM11 5mg/kg i.p. TNBS (p)	3.37	1.0752	10	8.09	1.5495	10	1.5%	-3.39 [-4.85, -1.93]
Subtotal (95% CI)			**15**			**15**	**2.6%**	**-3.06 [-4.21, -1.90]**
Heterogeneity: Tau² = 0.00; Chi² = 0.52, df = 1 (P = 0.47); I² = 0%								
Test for overall effect: Z = 5.19 (P < 0.00001)								

Std. Mean Difference
IV, Random, 95% CI



1.1.10 AEA								
Engel 2008 AEA 5mg/kg i.p. TNBS (p)	2.6	1.7146	6	8.64	1.9596	6	1.1%	-3.03 [-4.89, -1.17]
Subtotal (95% CI)			**6**			**6**	**1.1%**	**-3.03 [-4.89, -1.17]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 3.19 (P = 0.001)								
1.1.11 AM841								
Fichna 2014 AM841 0.01mg/kg i.p. DSS (p)	7.55	0.8083	6	8.08	0.9798	2	1.3%	-0.55 [-2.19, 1.10]
Fichna 2014 AM841 0.1mg/kg i.p. DSS (p)	5.03	1.8861	6	8.08	0.9798	2	1.1%	-1.50 [-3.40, 0.40]
Fichna 2014 AM841 1mg/kg i.p. DSS (p)	5.3	2.2045	6	8.08	0.9798	2	1.2%	-1.18 [-2.97, 0.62]
Fichna 2014 AM841 1mg/kg i.p. TNBS (p)	4.04	0.2939	6	6.04	0.3674	6	0.6%	-5.55 [-8.49, -2.61]
Subtotal (95% CI)			**24**			**12**	**4.2%**	**-1.87 [-3.57, -0.17]**
Heterogeneity: Tau² = 1.93; Chi² = 8.72, df = 3 (P = 0.03); I² = 66%								
Test for overall effect: Z = 2.16 (P = 0.03)								
1.1.12 HU308								
Ke 2016 HU308 1mg/kg i.p. DSS (t)	2.3	1.9596	6	3.6	1.2247	6	1.7%	-0.73 [-1.92, 0.45]
Subtotal (95% CI)			**6**			**6**	**1.7%**	**-0.73 [-1.92, 0.45]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 1.21 (P = 0.23)								
1.1.13 ACEA								
Kimball 2006 ACEA 10 mg/kg i.p.OM (p)	64	33	9	100	45	9	1.9%	-0.87 [-1.85, 0.11]
Subtotal (95% CI)			**9**			**9**	**1.9%**	**-0.87 [-1.85, 0.11]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 1.74 (P = 0.08)								
1.1.14 αβ Amyrin								
Matos 2013 αβAmyrin 1mg/kg p.o. DSS (p)	3.7	1.3416	5	5.11	0.44	2	1.2%	-0.98 [-2.79, 0.84]
Matos 2013 αβAmyrin 3mg/kg p.o. DSS (p)	2.4	1.3416	5	5.11	0.44	2	0.9%	-1.88 [-4.09, 0.34]
Matos 2013 αβAmyrin 10mg/kg p.o. DSS (p)	1.29	1.118	5	5.11	0.44	2	0.6%	-3.16 [-6.15, -0.17]
Matos 2013 αβAmyrin 10mg/kg p.o. DSS (t)	0.75	1.3416	5	5.11	0.44	2	0.6%	-3.02 [-5.92, -0.12]
Subtotal (95% CI)			**20**			**8**	**3.3%**	**-1.88 [-3.05, -0.72]**
Heterogeneity: Tau² = 0.00; Chi² = 2.25, df = 3 (P = 0.52); I² = 0%								
Test for overall effect: Z = 3.17 (P = 0.002)								
1.1.15 HU210								
Lin 2017 HU210 0.05mg/kg i.p. DSS (p)	2.5	0.2449	6	3.6	0.1715	6	0.7%	-4.80 [-7.41, -2.20]
Massa 2004 HU210 0.05mg/kg i.p. DNBS (p)	1.77	0.8944	5	3.77	1.5875	7	1.6%	-1.36 [-2.69, -0.04]
Subtotal (95% CI)			**11**			**13**	**2.3%**	**-2.89 [-6.24, 0.46]**
Heterogeneity: Tau² = 4.80; Chi² = 5.32, df = 1 (P = 0.02); I² = 81%								
Test for overall effect: Z = 1.69 (P = 0.09)								
1.1.16 JWH133								
Kimball 2006 JWH133 2.5mg/kg i.p. OM (p)	41	30	9	100	42.4264	8	1.8%	-1.54 [-2.66, -0.42]
Singh 2012 JWH133 2.5mg/kg i.p. DSS (t)	0.99	0.098	6	2.8	0.4899	6	0.7%	-4.73 [-7.30, -2.16]
Singh 2012 JWH133 2.5mg/kg i.p. IL10-/- (t)	2.8	0.7348	6	7.6	0.9798	6	0.7%	-5.12 [-7.86, -2.37]
Storr 2009 JWH133 20mg/kg i.p. TNBS (p)	4.7	2.2045	6	7.6	1.1758	6	1.6%	-1.52 [-2.87, -0.16]
Subtotal (95% CI)			**27**			**26**	**4.8%**	**-2.81 [-4.45, -1.17]**
Heterogeneity: Tau² = 1.86; Chi² = 10.34, df = 3 (P = 0.02); I² = 71%								
Test for overall effect: Z = 3.36 (P = 0.0008)								
1.1.17 PF3845								
Salaga 2014 PF3845 5mg/kg i.p. TNBS (p)	4.79	0.8485	8	7.5	1.2445	8	1.5%	-2.41 [-3.78, -1.03]
Salaga 2014 PF3845 5mg/kg p.o. TNBS (p)	3.44	0.9899	8	5.66	1.6971	8	1.8%	-1.51 [-2.66, -0.36]
Salaga 2014 PF3845 5mg.kg i.c. TNBS (p)	3.55	0.8485	8	7.36	1.4142	8	1.4%	-3.09 [-4.66, -1.51]
Subtotal (95% CI)			**24**			**24**	**4.7%**	**-2.21 [-3.11, -1.31]**
Heterogeneity: Tau² = 0.16; Chi² = 2.68, df = 2 (P = 0.26); I² = 25%								
Test for overall effect: Z = 4.81 (P < 0.00001)								
1.1.18 ARN2508								
Sasso 2015 ARN2508 5mg/kg p.o. TNBS (p)	5.7	0.9798	6	8.05	0.6124	6	1.2%	-2.66 [-4.38, -0.93]
Subtotal (95% CI)			**6**			**6**	**1.2%**	**-2.66 [-4.38, -0.93]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 3.02 (P = 0.002)								
1.1.19 O-1602								
Schicho 2011 O-1602 5mg/kg i.p. DSS (p)	3	11.1	9	6.2	2.4	9	2.0%	-0.38 [-1.31, 0.56]
Schicho 2011 O-1602 5mg/kg i.p. TNBS (p)	2.3	1.5652	5	4.9	1.3416	5	1.4%	-1.61 [-3.15, -0.08]
Subtotal (95% CI)			**14**			**14**	**3.4%**	**-0.84 [-2.01, 0.33]**
Heterogeneity: Tau² = 0.34; Chi² = 1.80, df = 1 (P = 0.18); I² = 45%								
Test for overall effect: Z = 1.41 (P = 0.16)								
1.1.20 CID 16020046								
Stancic 2015 CID16020046 20mg/kg i.p. DSS (p)	6.6	1	4	10.6	1	4	0.7%	-3.48 [-6.24, -0.71]
Stancic 2015 CID16020046 20mg/kg i.p. TNBS (p)	2.1	0.8	4	3.6	0.8	4	1.2%	-1.63 [-3.41, 0.15]
Subtotal (95% CI)			**8**			**8**	**1.9%**	**-2.24 [-3.94, -0.54]**
Heterogeneity: Tau² = 0.30; Chi² = 1.21, df = 1 (P = 0.27); I² = 17%								
Test for overall effect: Z = 2.58 (P = 0.010)								



1.1.21 URB597								
Storr 2008 URB597 5mg/kg i.p. TNBS (p)	2.73	1.062	9	8.08	1.23	9	1.1%	-4.43 [-6.32, -2.55]
Subtotal (95% CI)			**9**			**9**	**1.1%**	**-4.43 [-6.32, -2.55]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 4.62 (P < 0.00001)								
1.1.22 AM1241								
Storr 2009 AM1241 20mg/kg i.p. TNBS (p)	3.26	0.9798	6	7.14	1.2982	6	1.1%	-3.11 [-5.01, -1.22]
Subtotal (95% CI)			**6**			**6**	**1.1%**	**-3.11 [-5.01, -1.22]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 3.22 (P = 0.001)								
1.1.23 MMF								
Wallace 2013 MMF 10mg/kg i.c. DNBS (t)	3.45	5.5426	12	7	3.7335	18	2.2%	-0.76 [-1.52, -0.00]
Subtotal (95% CI)			**12**			**18**	**2.2%**	**-0.76 [-1.52, -0.00]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 1.97 (P = 0.05)								
1.1.24 THC								
Jamontt 2010 THC 5mg/kg i.p. TNBS (p)	6.5	2.3216	11	6.2	1.6583	3	1.6%	0.13 [-1.15, 1.40]
Jamontt 2010 THC 10mg/kg i.p. TNBS (p)	2.9	3.3166	11	6.2	1.6583	4	1.7%	-1.03 [-2.25, 0.19]
Jamontt 2010 THC 20mg/kg i.p. TNBS (p)	4.73	2.3216	11	6.2	1.6583	4	1.7%	-0.63 [-1.81, 0.54]
Subtotal (95% CI)			**33**			**11**	**5.1%**	**-0.53 [-1.24, 0.17]**
Heterogeneity: Tau² = 0.00; Chi² = 1.68, df = 2 (P = 0.43); I² = 0%								
Test for overall effect: Z = 1.48 (P = 0.14)								
1.1.26 βCaryophyllene								
Bento 2011 βCaryophyllene 12.5mg/kg i.p. DSS (p)	7.44	2.3255	5	9.4	1.8559	5	1.6%	-0.84 [-2.17, 0.49]
Bento 2011 βCaryophyllene 25mg/kg i.p. DSS (p)	5.78	2.6386	5	9.4	1.8559	5	1.4%	-1.43 [-2.91, 0.05]
Bento 2011 βCaryophyllene 50mg/kg i.p. DSS (p)	3.77	1.364	5	9.4	1.8559	5	1.0%	-3.12 [-5.27, -0.97]
Bento 2011 βCaryophyllene 50mg/kg i.p. DSS (t)	5.88	1.9677	5	9.4	1.8559	5	1.4%	-1.66 [-3.22, -0.11]
Subtotal (95% CI)			**20**			**20**	**5.4%**	**-1.52 [-2.32, -0.72]**
Heterogeneity: Tau² = 0.04; Chi² = 3.18, df = 3 (P = 0.36); I² = 6%								
Test for overall effect: Z = 3.71 (P = 0.0002)								
Total (95% CI)			**519**			**429**	**100.0%**	**-1.36 [-1.62, -1.09]**
Heterogeneity: Tau² = 0.67; Chi² = 169.13, df = 66 (P < 0.00001); I² = 61%								
Test for overall effect: Z = 10.16 (P < 0.00001)								
Test for subgroup differences: Chi² = 84.23, df = 24 (P < 0.00001), I² = 71.5%								

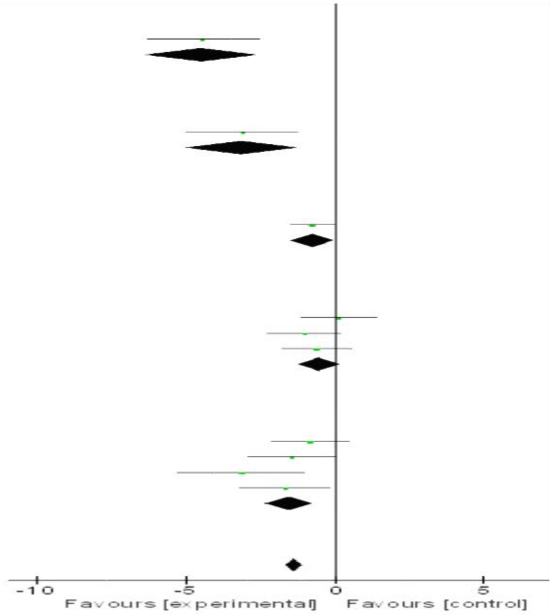
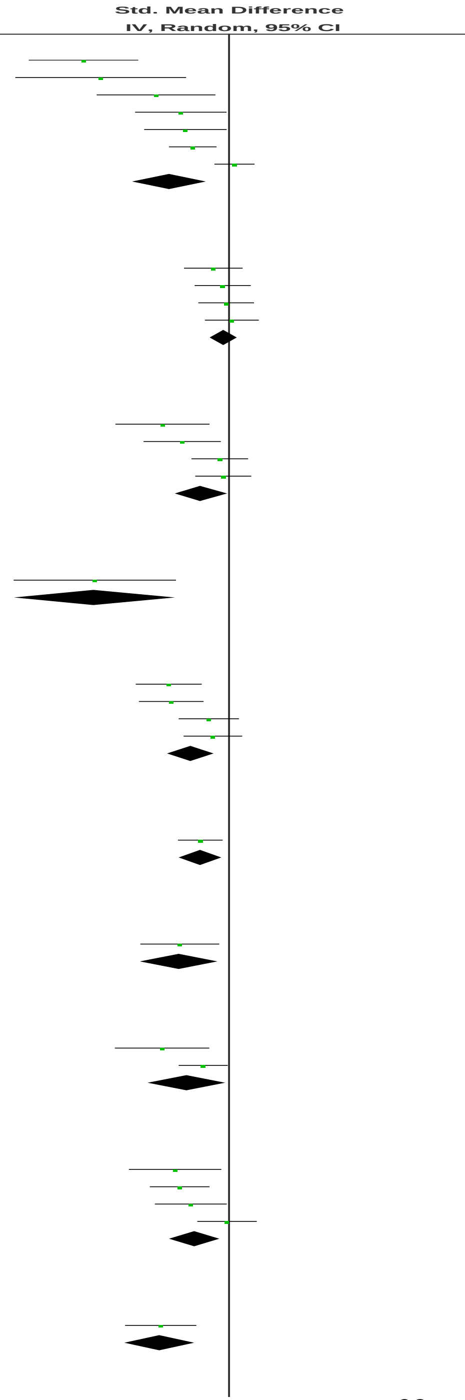
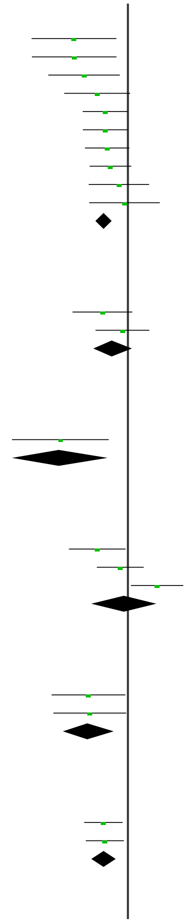


Figure 4. Forest plot of the effects of cannabinoid treatment on Disease Activity Score subdivided by drug type. Time of administration in relation to onset of colitis is given where 'p' represents prophylactic administration, and ’t’ represents therapeutic administration.

	Experimental			Control				Std. Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI
1.2.1 PEA								
Impellizzeri 2015 PEA 10mg/kg i.p. DNBS (t)	40.91	6.7357	10	100	9.8284	10	0.9%	-6.72 [-9.20, -4.23]
Esposito 2014 PEA 10mg/kg i.p. DSS (t)	7.8	1.4207	6	17.9	1.7321	3	0.5%	-5.92 [-9.82, -2.02]
Borrelli 2014 PEA 1mg/kg p.o. DNBS (t)	34.4	5.6	4	68.69	11.2	4	0.8%	-3.37 [-6.07, -0.67]
Borrelli 2014 PEA 1mg/kg i.p. DNBS (t)	39.19	28.14	4	100	18.4	4	1.2%	-2.22 [-4.29, -0.16]
Esposito 2014 PEA 2mg/kg i.p. DSS (t)	11.9	2.9394	6	17.9	1.7321	3	1.4%	-2.01 [-3.87, -0.15]
Alhouayek 2015 PEA 10mg/kg i.p. DSS (p)	43.14	33.2039	10	100	31.939	10	2.2%	-1.67 [-2.72, -0.62]
Alhouayek 2015 PEA 10mg/kg i.p. TNBS (p)	116.4	84.3379	10	100	26.2469	10	2.5%	0.25 [-0.63, 1.13]
Subtotal (95% CI)			**50**			**44**	**9.5%**	**-2.74 [-4.42, -1.06]**
Heterogeneity: $Tau^2 = 3.94$; $Chi^2 = 39.60$, df = 6 (P < 0.00001); $I^2 = 85\%$								
Test for overall effect: Z = 3.20 (P = 0.001)								
1.2.2 SAB378								
Cluny 2010 SAB378 1mg/kg i.p.TNBS (p)	44.5	39.802	5	100	89.6663	5	1.9%	-0.72 [-2.03, 0.58]
Cluny 2010 SAB378 0.1mg/kg i.p. TNBS (p)	77.85	37.7895	5	100	89.66	5	2.0%	-0.29 [-1.54, 0.96]
Cluny 2010 SAB378 1mg/kg i.p. DSS (t)	85.7	56.1253	5	100	127.523	5	2.0%	-0.13 [-1.37, 1.11]
Cluny 2010 SAB378 0.1mg/kg i.p. DSS (t)	116.04	102.1062	8	100	127.52	4	2.0%	0.13 [-1.07, 1.34]
Subtotal (95% CI)			**23**			**19**	**7.9%**	**-0.23 [-0.86, 0.39]**
Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 0.93$, df = 3 (P = 0.82); $I^2 = 0\%$								
Test for overall effect: Z = 0.74 (P = 0.46)								
1.2.3 βCaryophyllene								
Bento 2011 βCaryophyllene 50mg/kg i.p. DSS (p)	0.4438	0.208	5	1.218	0.246	5	1.2%	-3.07 [-5.20, -0.94]
Bento 2011 βCaryophyllene 50mg/kg i.p. DSS (t)	0.68	0.2012	5	1.218	0.246	5	1.5%	-2.16 [-3.90, -0.42]
Bento 2011 βCaryophyllene 12.5mg/kg i.p. DSS (p)	0.69	1.5652	5	1.218	0.246	5	2.0%	-0.43 [-1.69, 0.84]
Bento 2011 βCaryophyllene 25mg/kg i.p. DSS (p)	0.615	2.9069	5	1.218	0.246	5	2.0%	-0.26 [-1.51, 0.98]
Subtotal (95% CI)			**20**			**20**	**6.6%**	**-1.26 [-2.48, -0.05]**
Heterogeneity: $Tau^2 = 0.90$; $Chi^2 = 7.49$, df = 3 (P = 0.06); $I^2 = 60\%$								
Test for overall effect: Z = 2.04 (P = 0.04)								
1.2.4 CBG								
Borrelli 2013 CBG 30mg/kg i.p. DNBS (t)	6.279	3.1752	5	68.966	12.522	5	0.5%	-6.20 [-9.90, -2.50]
Subtotal (95% CI)			**5**			**5**	**0.5%**	**-6.20 [-9.90, -2.50]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 3.28 (P = 0.001)								
1.2.5 WIN 55212-2								
Feng 2016 WIN55,212 5mg/kg i.p. DSS (t)	47.2	23.1931	8	185.25	62.2254	8	1.7%	-2.78 [-4.26, -1.30]
Li 2013 WIN55212-2 5mg/kg i.p. DSS (t)	45.3	20.9304	8	184.3	66.468	8	1.8%	-2.67 [-4.11, -1.22]
Cluny 2010 WIN 52212-2 2mg/kg i.p. TNBS (t)	32.8	19.6774	5	100	89.6663	5	1.9%	-0.94 [-2.28, 0.41]
Cluny 2010 WIN 52212-2 2mg/kg i.p. DSS (t)	24.4	20.3482	5	100	128.1267	5	1.9%	-0.74 [-2.05, 0.56]
Subtotal (95% CI)			**26**			**26**	**7.3%**	**-1.74 [-2.81, -0.67]**
Heterogeneity: $Tau^2 = 0.68$; $Chi^2 = 7.05$, df = 3 (P = 0.07); $I^2 = 57\%$								
Test for overall effect: Z = 3.20 (P = 0.001)								
1.2.6 Ademidrol								
Cordaro 2016 Ademidrol 10mg/kg p.o DNBS (t)	786	388.9602	10	1,428	528.1004	10	2.3%	-1.33 [-2.31, -0.34]
Subtotal (95% CI)			**10**			**10**	**2.3%**	**-1.33 [-2.31, -0.34]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 2.63 (P = 0.009)								
1.2.9 AA5HT								
D'Argenio 2006 AA5HT10mg/kg s.c DNBS (t)	35.2	19.0066	5	100	31.0813	5	1.4%	-2.27 [-4.05, -0.49]
Subtotal (95% CI)			**5**			**5**	**1.4%**	**-2.27 [-4.05, -0.49]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 2.50 (P = 0.01)								
1.2.10 VDM115								
D'Argenio 2006 VDM11 5mg/kg s.c. DNBS (t)	16	15.4289	5	100	31.0813	5	1.1%	-3.09 [-5.23, -0.96]
Storr 2008 VDM11 5mg/kg i.p. TNBS (p)	17.2	9.051	8	100.1	92.7724	8	2.2%	-1.19 [-2.28, -0.10]
Subtotal (95% CI)			**13**			**13**	**3.3%**	**-1.91 [-3.72, -0.10]**
Heterogeneity: $Tau^2 = 1.06$; $Chi^2 = 2.42$, df = 1 (P = 0.12); $I^2 = 59\%$								
Test for overall effect: Z = 2.07 (P = 0.04)								
1.2.11 AM841								
Fichna 2014 AM841 1mg/kg i.p. DSS (p)	16.15	10.748	8	47.45	14.99	2	1.2%	-2.49 [-4.58, -0.40]
Fichna 2014 AM841 1mg/kg i.p. TNBS (p)	18.43	7.3539	8	39.43	9.8995	8	1.9%	-2.28 [-3.61, -0.94]
Fichna 2014 AM841 0.1mg/kg i.p. DSS (p)	27.14	8.8813	8	47.45	14.99	3	1.6%	-1.76 [-3.37, -0.15]
Fichna 2014 AM841 0.01mg/kg i.p. DSS (p)	44.9	27.4357	8	47.45	14.99	3	1.9%	-0.09 [-1.42, 1.24]
Subtotal (95% CI)			**32**			**16**	**6.5%**	**-1.56 [-2.71, -0.41]**
Heterogeneity: $Tau^2 = 0.74$; $Chi^2 = 6.57$, df = 3 (P = 0.09); $I^2 = 54\%$								
Test for overall effect: Z = 2.66 (P = 0.008)								
1.2.12 ACEA								
Kimball 2006 ACEA 10 mg/kg i.p.OM (p)	5.7	13.2	9	199	87.3098	7	1.6%	-3.15 [-4.75, -1.55]
Subtotal (95% CI)			**9**			**7**	**1.6%**	**-3.15 [-4.75, -1.55]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 3.86 (P = 0.0001)								



1.2.13 CBD								
Pagano 2016 CBD 30mg/kg i.p. DNBS (t)	4.62	0.5814	5	6.06	0.5143	5	1.4%	-2.37 [-4.19, -0.55]
Pagano 2016 CBD 60mg/kg p.o. DNBS (t)	5.7	1.7889	5	9.03	0.2236	5	1.4%	-2.36 [-4.18, -0.54]
Jamontt 2010 CBD 20mg/kg i.p. TNBS (p)	2.87	1.3266	11	6.05	2.3548	3	1.7%	-1.93 [-3.45, -0.40]
Jamontt 2010 CBD 10mg/kg i.p. TNBS (p)	4.6	0.3317	11	6.05	2.3548	3	1.8%	-1.35 [-2.75, 0.06]
Schicho 2012 CBD 20mg/kg p.r. TNBS (p)	69.4	21.8197	10	105.55	43.9557	10	2.4%	-1.00 [-1.94, -0.06]
Krohn 2016 abCBD 5mg/kg i.p. TNBS (t)	60.03	32.5715	10	100	43.6394	10	2.4%	-0.99 [-1.94, -0.05]
Schicho 2012 CBD 10mg.kg i.p. TNBS (p)	80.5	17.7088	10	106.9	35.1013	10	2.4%	-0.91 [-1.84, 0.02]
Schicho 2012 CBD 20mg/kg p.o. TNBS (p)	70.83	50.7444	11	101.38	18.5731	11	2.5%	-0.77 [-1.64, 0.10]
Jamontt 2010 CBD 15mg/kg i.p. TNBS (p)	4.1	4.9749	11	6.05	2.3548	3	1.9%	-0.39 [-1.68, 0.89]
Jamontt 2010 CBD 5mg/kg i.p. TNBS (p)	5.5	3.6483	11	6.05	2.3548	2	1.7%	-0.14 [-1.65, 1.36]
Subtotal (95% CI)			**95**			**62**	**19.5%**	**-1.03 [-1.40, -0.66]**
Heterogeneity: Tau² = 0.00; Chi² = 8.33, df = 9 (P = 0.50); I² = 0%								
Test for overall effect: Z = 5.47 (P < 0.00001)								
1.2.14 HU210								
Massa 2004 HU210 0.05mg/kg i.p. DNBS (p)	2.9	9.1679	5	100	103.4489	7	2.0%	-1.12 [-2.39, 0.15]
Lin 2017 HU210 0.05mg/kg i.p. DSS (p)	1.74	0.4164	6	3.21	8.0833	6	2.1%	-0.24 [-1.37, 0.90]
Subtotal (95% CI)			**11**			**13**	**4.1%**	**-0.63 [-1.48, 0.23]**
Heterogeneity: Tau² = 0.01; Chi² = 1.02, df = 1 (P = 0.31); I² = 2%								
Test for overall effect: Z = 1.44 (P = 0.15)								
1.2.15 CBC								
Romano 2013 CBC 1mg.kg i.p. DNBS (t)	33.14	7.2001	5	67.64	12.9692	5	1.2%	-2.97 [-5.05, -0.89]
Subtotal (95% CI)			**5**			**5**	**1.2%**	**-2.97 [-5.05, -0.89]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 2.80 (P = 0.005)								
1.2.16 PF3745								
Salaga 2014 PF3845 5mg/kg p.o. TNBS (p)	61.01	29.1297	7	100	24.8701	7	2.0%	-1.35 [-2.55, -0.15]
Salaga 2014 PF3845 5mg.kg i.c. TNBS (p)	86.36	48.1964	8	100	25.7104	8	2.3%	-0.33 [-1.32, 0.66]
Salaga 2014 PF3845 5mg/kg i.p. TNBS (p)	142	35.3553	8	100	25.4558	8	2.2%	1.29 [0.18, 2.40]
Subtotal (95% CI)			**23**			**23**	**6.5%**	**-0.12 [-1.56, 1.32]**
Heterogeneity: Tau² = 1.31; Chi² = 10.40, df = 2 (P = 0.006); I² = 81%								
Test for overall effect: Z = 0.16 (P = 0.87)								
1.2.17 O-1602								
Schicho 2011 O-1602 5mg/kg i.p. TNBS (p)	21.98	33.541	5	100	46.7338	5	1.6%	-1.73 [-3.31, -0.16]
Schicho 2011 O-1602 5mg/kg i.p. DSS (p)	67.06	16.7705	5	100.31	19.0066	5	1.6%	-1.68 [-3.23, -0.12]
Subtotal (95% CI)			**10**			**10**	**3.3%**	**-1.70 [-2.81, -0.60]**
Heterogeneity: Tau² = 0.00; Chi² = 0.00, df = 1 (P = 0.96); I² = 0%								
Test for overall effect: Z = 3.01 (P = 0.003)								
1.2.18 CID16020046								
Stancic 2015 CID16020046 20mg/kg i.p. TNBS (p)	313.5	69.2207	14	435	138.4413	14	2.6%	-1.08 [-1.88, -0.28]
Stancic 2015 CID16020046 20mg/kg i.p. DSS (p)	172	53.1315	14	428.37	344.2325	14	2.6%	-1.01 [-1.80, -0.22]
Subtotal (95% CI)			**28**			**28**	**5.1%**	**-1.04 [-1.61, -0.48]**
Heterogeneity: Tau² = 0.00; Chi² = 0.01, df = 1 (P = 0.91); I² = 0%								
Test for overall effect: Z = 3.63 (P = 0.0003)								



1.2.19 URB597								
Storr 2008 URB597 5mg/kg i.p. TNBS (p)	30.5	6.5054	8	100	92.7724	8	2.2%	-1.00 [-2.06, 0.06]
Subtotal (95% CI)			**8**			**8**	**2.2%**	**-1.00 [-2.06, 0.06]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 1.85 (P = 0.06)								
1.2.20 AM1241								
Storr 2009 AM1241 20mg/kg i.p. TNBS (p)	32.9	49.6407	5	100	74.4611	5	1.9%	-0.96 [-2.31, 0.39]
Subtotal (95% CI)			**5**			**5**	**1.9%**	**-0.96 [-2.31, 0.39]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 1.39 (P = 0.16)								
1.2.21 JWH133								
Storr 2009 JWH133 20mg/kg i.p. TNBS (p)	25	39.0323	8	100	94.4695	8	2.2%	-0.98 [-2.04, 0.07]
Subtotal (95% CI)			**8**			**8**	**2.2%**	**-0.98 [-2.04, 0.07]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 1.82 (P = 0.07)								
1.2.22 THC								
Jamontt 2010 THC 10mg/kg i.p. TNBS (p)	1.74	0.3648	11	3.33	0.995	2	1.2%	-3.22 [-5.33, -1.11]
Jamontt 2010 THC 20mg/kg i.p. TNBS (p)	2.21	0.2449	6	3.33	0.995	2	1.1%	-2.10 [-4.26, 0.06]
Jamontt 2010 THC 5mg/kg i.p. TNBS (p)	4.37	0.9798	6	3.33	0.995	2	1.5%	0.92 [-0.80, 2.64]
Subtotal (95% CI)			**23**			**6**	**3.8%**	**-1.40 [-3.97, 1.17]**
Heterogeneity: Tau² = 4.11; Chi² = 9.99, df = 2 (P = 0.007); I² = 80%								
Test for overall effect: Z = 1.07 (P = 0.28)								
1.2.24 αβ Amyrin								
Matos 2013 αβAmyrin 10mg/kg p.o. DSS (p)	0.16	0.8944	5	0.719	1.5588	3	1.7%	-0.42 [-1.88, 1.04]
Matos 2013 αβAmyrin 10mg/kg p.o. DSS (t)	0.43	0.246	5	0.719	1.5588	2	1.5%	-0.33 [-1.99, 1.33]
Subtotal (95% CI)			**10**			**5**	**3.3%**	**-0.38 [-1.48, 0.71]**
Heterogeneity: Tau² = 0.00; Chi² = 0.01, df = 1 (P = 0.94); I² = 0%								
Test for overall effect: Z = 0.68 (P = 0.50)								
Total (95% CI)			**419**			**338**	**100.0%**	**-1.26 [-1.54, -0.97]**
Heterogeneity: Tau² = 0.66; Chi² = 139.42, df = 56 (P < 0.00001); I² = 60%								
Test for overall effect: Z = 8.63 (P < 0.00001)								
Test for subgroup differences: Chi² = 38.51, df = 20 (P = 0.008), I² = 48.1%								

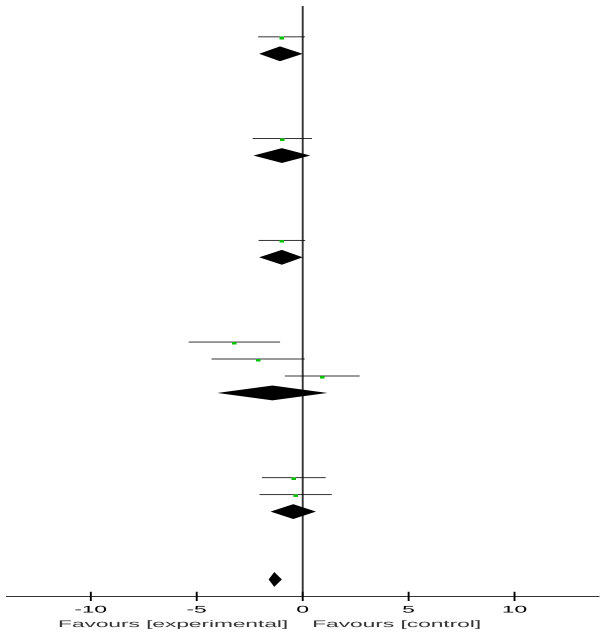


Figure 5. Forest plot of the effects of cannabinoid treatment on MPO activity subdivided by drug type. Time of administration in relation to onset of colitis is given where 'p' represents prophylactic administration, and 't' represents therapeutic administration.

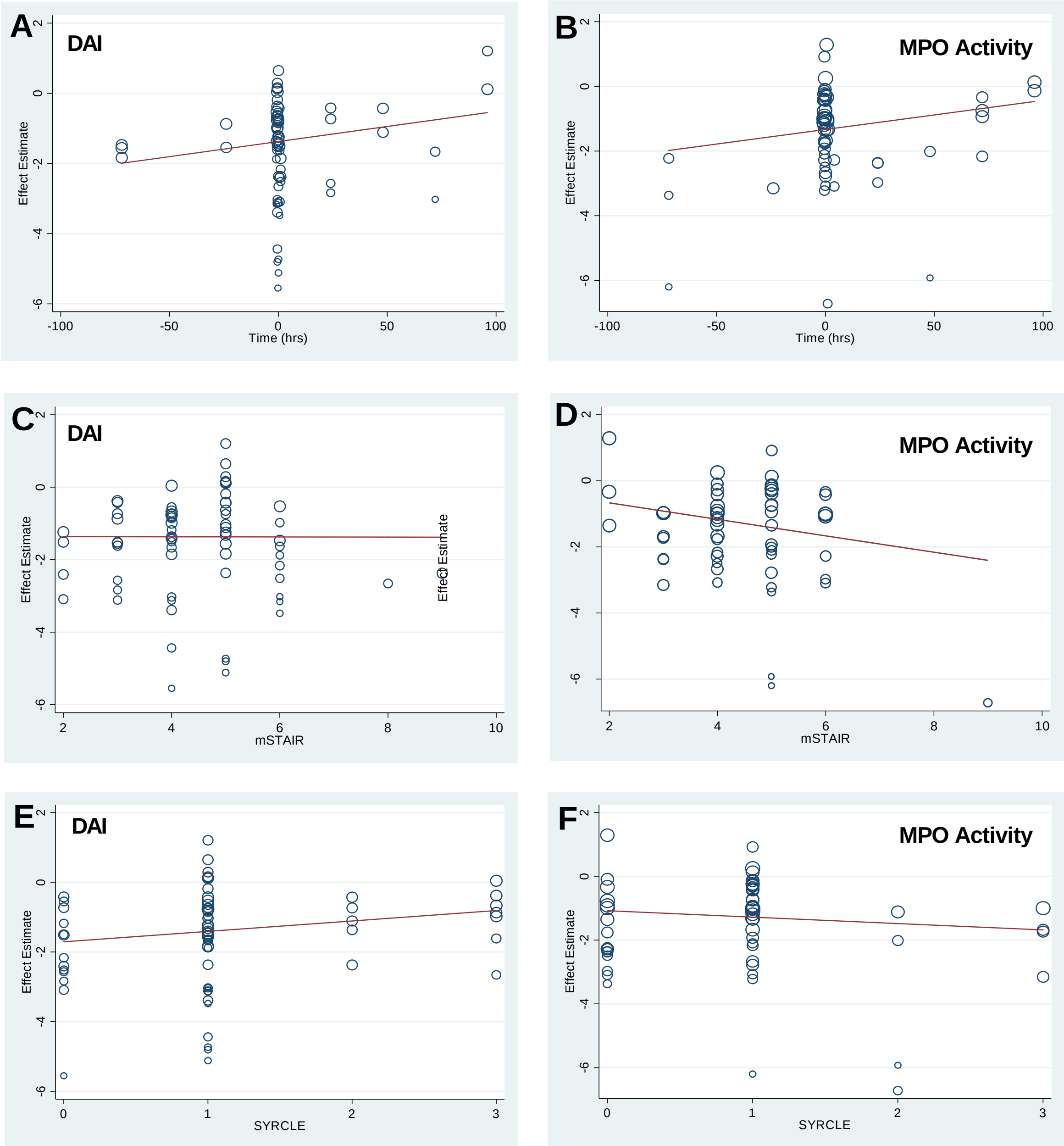


Figure 6. The effect of cannabinoid treatment on experimentally induced colitis determined by DAI (A) and MPO (B) predicted by timing of drug administration in relation to colitis onset. The effect of study quality, determined by mSTAIR score and SYRCLE score, on effect size in DAI (C, E) and MPO (D, F). Study weights are represented by the diameter of the circle, with larger circles representing studies with largest weight in the analysis.

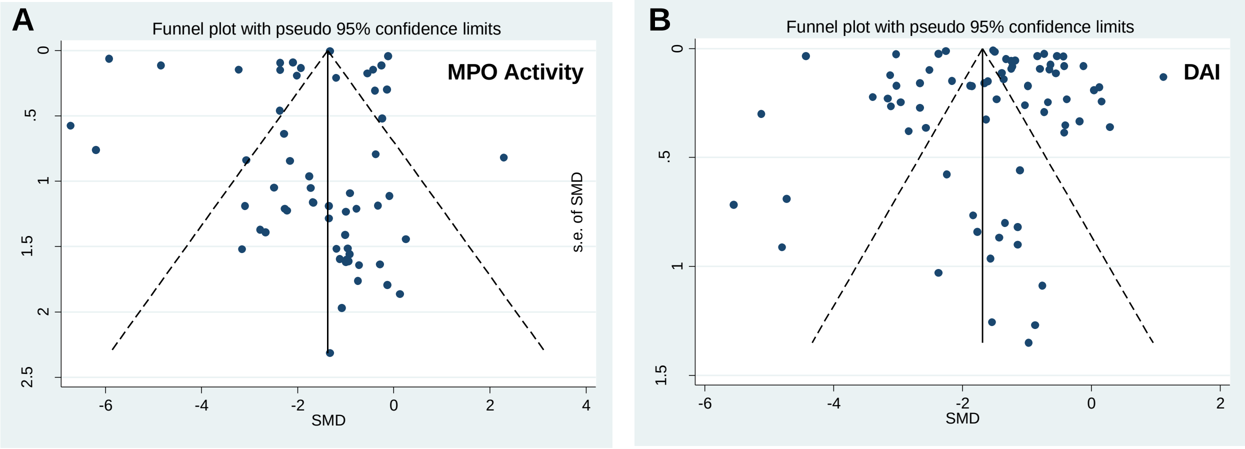


Figure 7. Funnel plots evaluating for publication bias in (A) MPO activity and (B) DAI. Standard error of the standardized mean difference (SE (SMD), y axes) for each study is plotted against its effect size (SMD, x axes).

Endocannabinoids		
	AEA	Anandamide
	PEA	Palmitoylethanolamide
	uPEA	Ultramicronised PEA
Phytocannabinoids		
	Cannabis sativa	Multiple compounds
	CBC	Cannibichromene
	CBD	Cannabidiol
	CBG	Cannabigerol
	CBN	Cannabinol
	THC	Tetrahydrocannabinol
Cannabinomimetics		
	αβ Amyrin	CB_1 and CB_2 agonist
	ACEA	Arachidonyl-2'-chloroethylamide
	Adelmidrol	PEA analogue
	AM1241	CB_2 full agonist, partial CB_1 agonist
	AM841	Peripherally restricted CB_1 agonist
	βCaryophyllene	CB_2 agonist
	CID16020046	GPR55 inverse agonist
	Compound 26	CB_2 agonist
	CP55,940	CB_1 and CB_2 agonist
	HU210	THC analogue
	HU308	CB_2 agonist
	JWH015	CB_2 full agonist, weak CB_1 agonist
	JHW133	CB_2 full agonist, weak CB_1 agonist
	O-1602	GPR18 and GPR55 agonist
	SAB378	Peripherally restricted CB_1 and CB_2 agonist
	WIN55,212-2	CB_1 full agonist
Enzyme Inhibitors		
	AA5HT	FAAH inhibitor
	AM3506	FAAH inhibitor
	AM9053	NAAA inhibitor
	ARN2508	FAAH inhibitor
	compound 39	FAAH inhibitor
	JZL184	MAGL inhibitor
	PF-3845	FAAH inhibitor
	URB597	FAAH inhibitor
Reuptake inhibitors		
	VDM11	AEA reuptake inhibitor

Table 1 – Cannabinoid drugs found by search strategy.

Study	Species	Model	Compound	Route/dosage	Time of administration verses inflammation	Time of assessment post inflammation	Modified STAIR score	SRCYCLE Score
Capasso 2001 (32)	ICR mice	CO	PEA	i.p 2.5-30 mg/kg	20 minutes pre	4 days	4	1
Izzo 2001 (9)	ICR mice	CO	CP 55,940 Cannabinol	i.p. 0.03–10 nmol/m i.p. 10–3000nmol/m	4 days post	20 minutes	3	0
Massa 2004 (25)	C57BL/6N mice	DNBS	SR141716 HU210	i.p. 3mg/kg i.p. 0.05 mg/kg	Pre, 24 and 48 hours post	3 & 7 days	4	2
Mathison 2004 (50)	Spr-Dawley rats	LPS	ACEA JWH133	i.p. 1mg/kg i.p. 1mg/kg	70 minutes post	120 minutes	5	0
D'Argenio 2006 (22)	C57/BJ mice Wistar rats	DNBS TNBS	VDM11 AA-5-HT	s.c. 5mg/kg s.c. 10mg/kg	Post	3 & 7 days	6	0
Kimball 2006 (51)	CD-1 mice	OM	ACEA JWH133	i.p. 10mg/kg i.p. 2.5mg/kg	24 hours pre	3 days	3	1
Capasso 2008 (52)	ICR mice	CO	CBD JWH015	i.p. 5mg/kg i.p. 10mg/kg	20 minutes pre Ach	4 days	5	0
Engel 2008 (53)	AKR mice	TNBS	AEA	i.p. 5mg/kg	30 minutes pre	3 days	3	1
Storr 2008 (54)	C57/BL mice	TNBS	URB597 VDM11	i.p. 5mg/kg i.p.5mg/kg	30 minutes pre or 24 hours post	3 days	4	1
Borelli 2009 (46)	ICR mice	DNBS	CBD	i.p. 1, 2, 5, 10mg/kg	24 hours post	3 days	3	0
Li 2009 (55)	Rats Mice	LPS	HU210 JWH133 AM630 AM251	100 µg.kg 100 µg.kg 3 mg/kg	5 minutes	30 minutes	8	1
Storr 2009 (56)	C57/BL mice	TNBS DSS	JWH133 AM1241 AM630	i.p. 20mg/kg i.p. 10-20 mg/kg i.p. 10mg/kg	30 minutes pre or 24 hours post	1, 3, 5, 7 days	7	1
Cassol Jr 2010 (47)	Wistar rats	CLP	CBD	i.p. 2.5, 4, 10mg/kg	Simultaneous	9 days	8	2
Cluny 2010 (57)	C57/BL mice	DSS TNBS	SAB378 AM251 AM630 WIN55,212-2	i.p 0.1 or 1.0mg/kg i.p 1.0mg/kg i.p 1.0mg/kg i.p 1, 2mg/kg	4 days post	8 days	5	1
Kimball 2010 (58)	CD1 mice	OM	ACEA JWH133	i.p. 1mg/kg i.p. 1mg/kg	30 minutes pre	28 days	4	3
Jamontt 2010 (45)	Wistar rats	TNBS	THC CBD	i.p. 5-20mg/kg i.p. 5-20mg/kg	30 minutes pre	3 days	5	1
Alhouayek 2011 (59)	C57BL/6 mice	TNBS	JZL184	i.p. 16mg/kg	Pre onset	3 days	2	1
Andrejak 2011 (60)	C57/BL mice	TNBS	Compound 39	i.p. 5mg/kg	3 days pre	3 days	6	1
Bento 2011 (61)	CD1 mice	DSS	βCaryophyllene	i.p. 12.5, 25, 50mg/kg	3 -7 days post	7 days	4	1
Defilipis 2011 (49)	OF1 mice	LPS	CBD	i.p. 10mg/kg	6 hours post	120 minutes	6	1
Lin 2011 (43)	C57/BL mice Spr-Dawley rats	LPS	CBD O-1602	i.p. 10mg/kg I.p. 1mg/kg	30 minutes pre	20 minutes	5	1
Schicho 2011 (62)	C57/BL mice	DSS TNBS	O-1602	i.p. 5mg/kg	30 minutes pre	7 days	3	3
Bashashati 2012 (63)	CD1 mice	LPS	AM3506	i.p. 100ug.kg	20 minutes pre	120 minutes	3	0
Izzo 2012 (64)	ICR mice	CO	CBC	i.p. 15mg/kg	20 minutes pre exam	4 days	5	2
Lehmann 2012 (65)	Lewis rats	LPS CASP	HU308	2.5mg/kg	15 minutes post	2 – 16 hours	4	0
Schicho 2012 (42)	C57/BL mice	TNBS	CBD	i.p. 10mg/kg p.o. 20mg/kg p.r. 20mg/kg	30 minutes pre onset	7 days	4	0
Singh 2012 (66)	C57/BL mice	IL-10 -/- DSS	JWH133	i.p. 2.5mg/kg	Simultaneous	7 – 14 days	5	1
Borrelli 2013 (67)	ICR mice	DNBS	CBG	i.p. 30mg/kg	3 days pre	3 days	5	1
Esposito 2014 (33)	CD-1 mice	DSS	PEA	i.p. 10mg/kg	2 days post	7 days	5	2
Li 2013 (68)	C57/BL mice	DSS	WIN55,212-2	i.p. 5mg/kg	Simultaneous	7 days	4	1
Matos 2013 (69)	CD1 mice	DSS	αβ Amyrin	p.o. 1, 3, 10mg/kg	Pre and 3 days post	7 days	6	1
Naftali 2013 (70)	Clinical trial	Crohns	Cannabis sativa extract (THC)	115 mg inhaled	N/A	8 weeks	NA	NA
Romano 2013 (71)	ICR mice	DNBS	CBC	i.p 0.1-1.0mg/kg	24 hours post	3 days	6	0
Wallace 2013 (72)	Wistar rats	DNBS	C. sativa (MMJ) AM630	i.c. 6 mg/kg p.o. 10mg/kg	30 minutes pre and 24 hours post	7 days	4	1
Borelli 2015 (73)	ICR mice	DNBS	PEA	i.p 1mg/kg p.o. 1mg/kg	3 days pre	3 days	5	1

Capasso 2014 (20)	ICR mice	OM	PEA	i.p. 10mg/kg	30 minutes	3 and 7 days	6	2
Fichna 2014 (74)	CD1 mice	DSS DNBS	AM841 CB13	i.p. 0.01, 0.1,1 mg/kg i.p. 0.1 mg/kg	15 minutes pre	3 and 7 days	4	0
Salaga 2014 (75)	C57/BL mice	TNBS DSS	PF3845	i.p. 10mg/kg p.o. 5mg/kg i.c. 5mg/kg	30 minutes	3 and 7 days	2	0
Sardinha 2014 (76)	C57/BL mice	LPS	HU308 AM630 URB597 JZL184	i.v. 2.5mg/kg i.v.2.5mg/kg i.p. 0.6mg/kg i.p. 16mg/kg	15 minutes pre	Simultaneous	6	0
Alhouayek 2015 (77)	CD57/BL mice	TNBS DSS	PEA PF-3845 AM9503	i.p. 10mg/kg i.p. 10mg/kg i.p. 10mg/kg	Simultaneous and 5 days post	7 days	4	1
El bakali 2015 (78)	C57/BL mice	TNBS	Compound 26	p.o. 10mg/kg	2 days pre	7 days	6	0
Impellizzeri 2015 (79)	CD1 mice	DNBS	uPEA	i.p. 10mg/kg	1 hour post	4 days	9	2
Sasso 2015 (80)	CD1 mice	TNBS DSS	ARN2508	p.o. 5mg/kg	Simultaneous	7 days	8	3
Stančić 2015 (81)	C57/BL mice	DSS TNBS	CID16020046	s.c. 20mg/kg	30 minutes	7 days	6	1
Cordaro 2016 (82)	CD1 mice	DNBS	Adelmidrol	p.o. 10mg/kg	60 minutes post	4 days	4	1
Feng 2016 (83)	C57/BL mice	DSS	WIN55212-2	i.p. 5mg/kg	Simultaneous and 60 hours post	7 days	5	1
Ke 2016 (84)	C57/BL mice	DSS	HU308	i.p. 1mg/kg	Simultaneous and daily	8 days	4	2
Krohn 2016 (40)	CD1 mice	TNBS	Ab-CBD O-1918 AM251 AM630	i.p. 5mg/kg i.p. 5mg/kg i.p. 5mg/kg i.p. 5mg/kg	45 minutes pre	4 days	6	1
Pagano 2016 (39)	ICR mice	DNBS CO	CBD Pure CBD	i.p. 30mg/kg p.o. 60mg/kg	24 hours post	3 days	3	0
Sarnelli 2016 (85)	CD1 mice	DSS	PEA	i.p. 2, 10mg/kg	2 days post	7 days	6	1
Lin 2017 (86)	C57/BL mice	DSS	HU210	i.p. 0.05mg/kg	30 minutes pre	7 days	5	1
Shamran 2017(87)	C57/BL mice	DSS	FAAH-II	i.p. 5 – 40mg/kg	24 hours post	7 days	6	1
Naftali 2017 (35)	Clinical trial	Crohns	CBD	10mg p.o. BD	N/A	8 weeks	NA	NA

CO, croton oil; DNBS, dinitrobenzosulphonic acid; LPS, lipopolysaccharide; TNBS, trinitrobenzosulphonic acid; DSS, dextran sulphate sodium; OM, oil of mustard; CASP, colon ascendens stent peritonitis; IL-10, interleukin 10; PEA, palmitoylethanolamide; AEA, anandamide; CBD, cannabidiol; THC; tetrahydrocannabinol; CBC, cannabichromene; CBG, cannabigerol; MMJ, medicinal cannabis; uPEA, ultramicronised PEA, AB-CBD, abnormal CBD; FAAH-II, fatty acid aminohydrolase II; i.p. intraperitoneal, i.c. intracolonic, p.o. oral administration; s.c. subcutaneous; iv.v intravenous; p.r. per rectum; Ach, acetylcholine.

Table 2. Characteristics of studies included for systematic review.

	No. of Studies	No. of animals	SMD [95% CI]	p value	I^2 (%)	Clinical significance
Endocannabinoids						
PEA	6	118	-1.45 [-1.94, -0.96]	<0.00001	25	High
AEA	1	12	-3.03 [-4.89, -1.17]	0.001	N/A	High
Phytocannabinoids						
CBD	12	181	-0.56 [-0.97, -0.16]	0.006	29	NS
THC	3	44	-0.53 [-1.24, 0.17]	0.14	0	NS
MMJ	1	30	-0.76 [-1.52, -0.00]	0.05	N/A	Moderate
Cannabinomimetics						
αβ Amyrin	4	28	-1.88 [-3.05, -0.72]	0.002	0	High
AM841	4	36	-1.87 [-3.57, -0.17]	0.03	66	High
βCaryophyllene	4	40	-1.52 [-2.32, -0.72]	0.0002	6	High
SAB378	4	56	0.28 [-0.38, 0.94]	0.41	28	NS
WIN55,212-2	4	60	-1.37 [-1.96, -0.78]	<0.00001	0	High
CID16020046	2	16	-2.24 [-3.94, -0.54]	0.01	17	High
HU210	2	24	-2.89 [-6.24, 0.46]	0.09	81	NS
O-1602	2	28	-0.84 [-2.01, 0.33]	0.16	45	NS
ACEA	1	18	-0.87 [-1.85, 0.11]	0.08	N/A	High
Adelmidrol	1	20	-1.85 [-2.94, -0.77]	0.0008	N/A	High
AM1241	1	12	-3.11 [-5.01, -1.22]	0.001	N/A	High
HU308	1	12	-0.73 [-1.92, 0.45]	0.23	N/A	NS
Enzyme inhibitors						
JWH133	4	53	-2.81 [-4.45, -1.17]	0.0008	71	High
PF3845	3	48	-2.21 [-3.11, -1.31]	<0.00001	25	High
AA5HT	1	10	-2.16 [-3.90, -0.43]	0.01	N/A	High
ARN2508	1	12	-2.66 [-4.38, -0.93]	0.002	N/A	High
Compound 39	1	20	-1.47 [-2.48, -0.46]	0.004	N/A	High
JZL184	1	22	-1.24 [-2.16, -0.31]	0.009	N/A	High
URB597	1	18	-4.43 [-6.32, -2.55]	<0.00001	N/A	High
Transport inhibitors						
VDM115	2	30	-3.06 [-4.21, -1.90]	<0.00001	0	High
Total	**68**	**948**	**-1.36 [-1.62, -1.09]**	**<0.00001**	**61**	**High**

Table 3. The effects of cannabinoids on Disease Activity Score caused by experimental colitis grouped by drug

	No. of Studies	No. of animals	SMD [95% CI]	p value	I^2 (%)	Clinical significance
Endocannabinoids						
PEA	7	94	-2.74 [-4.42, -1.06]	0.001	85	High
Phytocannabinoids						
CBD	10	157	-1.03 [-1.40, -0.66]	<0.00001	0	High
THC	3	29	-1.40 [-3.97, 1.17]	0.28	80	NS
CBC	1	10	-2.97 [-5.05, -0.89]	0.005	N/A	High
CBG	1	10	-6.20 [-9.90, -2.50]	0.01	N/A	High
Cannabinomimetics						
βCaryophyllene	4	40	-1.26 [-2.48, -0.05]	0.04	60	High
AM841	4	48	-1.56 [-2.71, -0.41]	0.008	54	High
SAB378	4	42	-0.23 [-0.86, 0.39]	0.46	0	NS
WIN55,212-2	4	52	-1.74 [-2.81, -0.67]	0.001	57	High
αβ Amyrin	2	15	-0.38 [-1.48, 0.71]	0.5	0	NS
CID16020046	2	56	-1.04 [-1.61, -0.48]	0.0003	0	High
HU210	2	24	-0.63 [-1.48, 0.23]	0.15	2	NS
O-1602	2	20	-1.70 [-2.81, -0.60]	0.003	0	High
ACEA	1	16	-3.15 [-4.75, -1.55]	0.0001	N/A	High
AM1241	1	10	-0.96 [-2.31, 0.39]	0.16	N/A	NS
JWH133	1	16	-0.98 [-2.04, 0.07]	0.09	N/A	NS
Ademidrol	1	20	-1.33 [-2.31, -0.34]	0.009	N/A	High
Enzyme inhibitors						
PF3745	3	46	-0.12 [-1.56, 1.32]	0.81	81	NS
AA5HT	1	10	-2.27 [-4.05, -0.49]	0.01	N/A	High
URB597	1	16	-1.00 [-2.06, 0.06]	0.06	N/A	NS
Transport inhibitors						
VDM115	2	26	-1.91 [-3.72, -0.10]	0.04	59	High
Total	**57**	**757**	**-1.26 [-1.54, -0.97]**	**<0.00001**	**48.1**	**High**

Table 4. The effects of cannabinoids on MPO activity caused by experimental colitis grouped by drug

PRISMA Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	6
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	6
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6-7
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6-7
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	7

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	6
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	8+19
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	8-11+28-29
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	11
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	30-31
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	10-11
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	12
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	11
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	13
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	17
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	13-17
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	1

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097