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PRO-RESOLUTION, PROTECTIVE AND ANTI-NOCICEPTIVE EFFECTS OF A CANNABIS EXTRACT IN THE RAT GASTROINTESTINAL TRACT

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Cannabis is widely used for treating a number of gastrointestinal ailments, but its use is associated with several adverse effects, particularly when the route of administration is *via* smoking. In the present study, we tested the effects (in rats) of a simple extract of medicinal cannabis (called “MFF”) for its ability to promote resolution of colitis, to prevent gastric damage induced by naproxen, and to reduce gastric distention-induced visceral pain. Intracolonic, but not oral administration of MFF dose-dependently reduced the severity of hapten-induced colitis, an effect not reduced by pre-treatment with antagonists of CB1 or CB2 receptors. Significant improvement of symptoms (diarrhea, weight loss) and healing of ulcerated tissue was evident with MFF treatment at doses that did not produce detectable urinary levels of 9- Δ -tetrahydrocannabinol (THC). MFF increased colonic hydrogen sulfide synthesis in healthy rats, but not in rats with colitis, and had no effect on colonic prostaglandin E₂ synthesis. Orally, but not systemically administered MFF dose-dependently reduced the severity of naproxen-induced gastric damage, and a CB1 antagonist reversed this effect. MFF prevented gastric distention-induced visceral pain *via* a CB2-dependent mechanism. These results demonstrate that a simple extract of medicinal cannabis can significantly enhance resolution of inflammation and injury, as well as prevent injury, in the gastrointestinal tract. Interestingly, different cannabinoid receptors were involved in some of the effects. MFF may serve as the basis for a simple preparation of cannabis that would produce beneficial effects in the GI tract with reduced systemic toxicity.

Key words: *cannabis, gastrointestinal tract, gastric damage,*

Abbreviations: DNBS, dinitrobenzene sulfonic acid; GI, gastrointestinal tract; IBD, inflammatory bowel disease; MPO, myeloperoxidase; NSAID, nonsteroidal anti-inflammatory drug; P5P, pyroxidal-5'-phosphate; THC, 9- Δ -tetrahydrocannabinol

INTRODUCTION

While best known for its psychotropic effects, cannabis has long been known to have analgesic, immunomodulatory and anti-inflammatory effects (1, 2). In the GI tract, positive effects on motility and pain sensitivity have been documented (3). The receptors mediating these effects have been characterized, at least in animal models. Thus, the psychotropic effects of cannabis and the inhibitory effects on gastric motility, mainly attributable to THC, are generally mediated *via* the CB1 receptor, while actions *via* the CB2 receptor have been reported to account for the effects of cannabis to promote resolution of inflammation (4, 5). THC inhibits gastric motility through CB1 receptors.

Use of cannabis by patients suffering from inflammatory bowel disease (IBD) is common (6, 7). In one study of 100 ulcerative colitis and 191 Crohn's disease patients (6), approximately 50% in both subsets had tried cannabis for symptom relief. Current use of cannabis was reported by 12% of ulcerative colitis and 16% of Crohn's disease patients in that

study. The most common reasons given for its use were to reduce diarrhea and pain, and to boost appetite. Cannabis use was highest in patients with a history of abdominal surgery, abdominal pain and low quality of life (6).

Targeting the cannabinoid system as a strategy to treat IBD is supported by a number of findings. Expression of the CB1 and CB2 receptors is increased on the gut epithelium in human IBD, and cannabinoids have been shown to promote healing of the epithelium (8). A number of studies of laboratory animals have demonstrated that intraperitoneal administration of cannabinoid receptor agonists reduce the severity of colitis (9, 10), while colitis was more severe in mice lacking CB1 receptors (9, 11) or CB2 receptors (11) and in mice treated with a CB1 antagonist (12).

Because of concerns primarily with the psychoactive properties of cannabinoids (4), efforts have been made to identify specific components of cannabis that might be used as therapies, as well as on the development of selective agonists of cannabinoid receptors. But selective agonists of CB receptors and some components of cannabis may not provide the full range of beneficial activities as seen with cannabis itself. Adverse effects directly related to the smoking of cannabis (1, 13) have further driven research into components of this substance that may be effective and cause less adverse effects when taken by other routes. Many patients seek simple, cannabis-based therapies that can be used *via* other routes. For treatment of

gastrointestinal disorders, for example, topical exposure of the mucosa to the therapeutic agent may be more effective and allow for lower doses to be used.

In the present study, we examined the effects of intraluminal delivery of a simple extract of cannabis (subsequently referred to as "MFF") to modulate the severity of colitis in rats, and to reduce visceral pain. We also tested the effects of the extract on susceptibility of the stomach to damage induced by a nonsteroidal anti-inflammatory drug (NSAID; naproxen), and evaluated the possible contribution of CB1 and CB2 receptors to the observed effects of the cannabis extract.

MATERIAL AND METHODS

Animals

The Animal Care Committee of McMaster University approved all experiments, and all procedures were conducted in accordance with the guidelines of the Canadian Council on Animal Care.

Male Wistar rats (Charles River Breeding Laboratories, Saint Constant, QC, Canada) were used for the experiments. Rats were housed in micro-isolator cages in the Central Animal Facilities. The cages were equipped with filter hoods and kept under controlled temperature (20°C) with a 12:12 h light-dark cycle and free access to food and water. Animals were fed with a standard rodent diet.

Materials

The CB1 antagonist (AM251; *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide) and the CB2 antagonist (AM630; 6-iodopravadoline) were obtained from Tocris Bioscience, (Ellisville, Missouri, USA). Solutions of these compounds in 50% DMSO were freshly prepared each day. The drugs were administered intraperitoneally (i.p.) at 1 ml/kg body weight, and control rats received the same volume of vehicle. DNBS and naproxen sodium were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA).

Test drugs

An extract of medicinal cannabis (MFF) was prepared. The cannabis (*C. sativa*) and was homogenized in absolute ethanol (10 mg/ml), then centrifuged (1000 g) for 10 min. The supernatant was dried under a stream of nitrogen and the residue was reconstituted in canola oil such that each 1 ml contained the ethanolic extract of 100 mg of cannabis. The MFF was stored at 4°C and was prepared freshly each week.

Colitis

As described in detail previously (14), modified slightly from the original description of hapten-induced colitis (15). Using a pediatric catheter, 30 mg of dinitrobenzene sulfonic acid (DNBS) in 0.5 mL of 50% ethanol was instilled into the distal colon of the rats, approximately 8 cm proximal to the rectum.

Each day the rats were examined and weighed, and a "disease activity index" was determined, which consisted of the sums of scores for diarrhea (score of 1 for loose stool, 2 for watery diarrhea), blood in the stool (score of 1 if present) and weight loss (score of 1 for loss of 1–10% of original body weight, score of 2 for loss of 11–15%, score of 3 for loss of 16–20%). Rats were euthanized if weight loss exceeded 20% of the starting body weight. The individual performing this scoring was blind as to the treatments the rats received.

Beginning 24 hours after administration of DNBS, groups of at least 6 rats each began to receive twice-daily treatments with MFF or vehicle, intracolonicallly (i.c.) or orally. The treatments were continued for 7 days. MFF was tested intracolonicallly at 1, 6 and 10 mg/kg, and orally at 10 mg/kg. Four hours after the morning administration of MFF or vehicle on day 7, the rats were euthanized in a randomized order and the severity of colitis was blindly evaluated using the criteria outlined in *Table 1* (16). Samples of the distal colon were frozen for subsequent measurement of myeloperoxidase (MPO) activity, as an index of granulocyte infiltration (17). Samples of the distal colon were also used for measurement of prostaglandin E₂ or hydrogen sulfide synthesis. PGE₂ was measured as described previously (18) using ELISA kits obtained from Cedarlane Laboratories (Burlington, ON, Canada). Hydrogen sulfide (H₂S) synthesis was measured using a zinc-trapping assay, modified slightly (19) from a previously described assay (20). In some experiments, a urine sample was drawn from the bladder of the rats (at time of sacrifice) for measurement of Δ9-tetrahydrocannabinol (THC) concentrations (21), using an ELISA kit obtained from BioQuant (San Diego, CA).

To investigate the potential role of cannabinoid receptors in the effects of MFF in the colon, experiments similar to those described above were performed using groups of at least 5 rats. Rats with colitis, treated intracolonicallly with MFF (10 mg/kg) or vehicle, were also treated twice-daily (30 min before MFF, i.p.) with a CB1 antagonist (AM251; 3.3 mg/kg) (22), a CB2 antagonist (AM630; 3 mg/kg) (23) or vehicle. The selected doses of these antagonists have been shown to effectively block the target receptors *in vivo* (24, 25). The severity of colitis was assessed on day 7, as described above.

Naproxen-induced gastric damage

Rats were deprived of food, but not water, for 18–20 hours. Groups of at least 5 rats each were given vehicle or MFF (1, 3 and 10 mg/kg) orally or intraperitoneally (10 mg/kg) thirty minutes prior to oral administration of naproxen (30 mg/kg). Three hours later the rats were euthanized and the gastric mucosa was blindly evaluated for hemorrhagic damage. Erosions were measured (in mm) and a "gastric damage score" was calculated by summing the lengths of all erosions in the stomach (26). Tissue samples were taken from the corpus region of the stomach and processed for measurement of PGE₂ synthesis, as described above. Urine was collected, as described above, for measurement of THC concentration.

Table 1. Criteria for scoring colonic damage and inflammation.

Score	Appearance
0	Normal
1	Localized hyperemia, no ulcers
2	Ulceration without hyperemia or bowel wall thickening
3	Ulceration with inflammation at one site
4	Two or more sites of ulceration and inflammation
5	Ulceration at multiple sites or extending >1 cm along the length of the colon
6–10	When an area of damage extended >2 cm along the length of colon, the score was increased by 1 for each additional cm of involvement

In addition, the score was increased by 1 or 2 if there were mild or severe adhesions, respectively, by 1 if diarrhea was evident, and by 1 if rectal bleeding was evident. The maximum colon thickness (mm) was also added to the score.

To investigate the potential role of cannabinoid receptors in the effects of MFF on the stomach, experiments similar to those described above were performed using groups of at least 5 rats. In these experiments, the dose of naproxen was increased to 60 mg/kg so that a greater level of injury was induced, in order that we might detect any increase or decrease in the protective effects of MFF when rats were pre-treated (i.p.) with a CB1 antagonist (AM251; 3.3 mg/kg), or a CB2 antagonist (AM630; 3 mg/kg) as compared to vehicle. The antagonists or vehicle were administered 30 min prior to oral administration of MFF (10 mg/kg) or vehicle. Naproxen was administered orally 30 min later. Gastric damage was blindly scored, as described above, 3 hours after naproxen administration.

Visceral pain

Visceral pain in response to graded gastric distention was measured as described in detail previously (27, 28). In brief, the rats were fasted for 18 hours then anesthetized with a mixture of ketamine hydrochloride (75 mg/kg) and xylazine (10 mg/kg). A mid-line laparotomy was performed and a ball-shaped balloon (2 cm diameter) catheter was inserted into the stomach through a small incision in the proximal duodenum. Care was taken to keep the manipulation of the stomach to a minimum. Supplemental anesthesia was given as necessary. The balloon catheter was connected to a computerized barostat system (Distender, G&J Electronic Inc., Toronto, ON, Canada). During the experiment continuous recordings of heart rate were performed through a surface electrocardiogram, obtained through three needle electrodes applied to the left and right shoulders, and the right hind leg. The signal was amplified and recorded onto a personal computer using a commercial data acquisition program (Experimenter's Workbench, DataWave Technologies, Berthoud, CO, USA). Heart rate was measured for 60 sec before, during and after each distension. Following each distension, 10 min was allowed for recovery. Changes in heart rate were recorded in response to distention of the intragastric balloon catheter to pressures of 20, 40 and 60 mm Hg. To control for the effect of gastric distention over time, the data

were presented as average change from resting heart rate using the mean heart rate recorded during distension. Groups were compared using the average change in heart rate during each distension. The rats were treated intraperitoneally with MFF (3 mg/kg) or vehicle, 15 min before the first distention.

To examine if MFF would affect visceral pain in response to gastric distention, a group of rats received an intraperitoneal injection of a single dose (3 mg/kg) of MFF 15 min prior to distention. To determine if CB1 and/or CB2 receptors were involved in the effects of MFF, rats were pretreated i.p. with AM251 (CB1 antagonist; 3.3 mg/kg), AM630 (CB2 antagonist; 3 mg/kg) or vehicle 10 min prior to administration of MFF.

Statistical analyses

All data are presented as the mean \pm S.E.M., with samples sizes of at least 5 per group. Comparisons among groups of data were performed by a one-way analysis of variance followed by the Dunnett's Multiple Comparison test. For non-parametric data (e.g., colonic damage scores), the Mann-Whitney U test was used. A p-value less than 5% was considered significant.

RESULTS

MFF dose-dependently reduced the severity of colitis

Intracolonic administration of DNBS resulted in extensive damage and inflammation of the distal colon, which was accompanied by loss of body weight, diarrhea and bleeding. MFF significantly reduced the severity of colitis when given intracolonic. *Fig. 1* shows the significant reduction ($p < 0.01$) of the colonic damage score by MFF at a dose of 10 mg/kg, intracolonic. The effects of MFF were dose-dependent. Thus, the median score for the vehicle-treated group was 7.5, while those for MFF at 1, 6 and 10 mg/kg were, respectively, 7.0, 4.5 ($p < 0.05$) and 3.5 ($p < 0.01$), with 6 to 9 rats per group. MFF did not significantly affect the severity of colitis when administered orally at 10 mg/kg (median score of 7.9).

Fig. 2 shows the body weight and disease activity index data for MFF (10 mg/kg, i.c.) versus vehicle. These data show that the impact of MFF was rapid, with positive changes in body weight being observed early in the course of treatment (significantly increased above the vehicle group by the 2nd day of treatment). Similarly, there was rapid improvement of the disease activity index soon after initiating treatment with MFF, with a marked reduction in diarrhea and bleeding in the rats treated with MFF.

The significant recovery of body weight in the group treated with MFF may have been to some extent related to increased food intake. To determine this, we carried out a study in which rats with or without colitis were treated with vehicle or MFF (10 mg/kg, i.c.) and food intake and body weight were monitored daily. As shown in *Table 2*, food intake was significantly decreased in all rats in which colitis was induced, and it remained significantly lower than the healthy rats throughout the 3-day period of treatment. Rats treated with MFF exhibited numerically greater food intake than the vehicle-treated rats, but it was highly variable within that group and the marked differences were not statistically significant. There was no effect of MFF on food intake or weight gain in healthy rats. Despite the lack of significant effect of MFF in food intake in rats with colitis, there was a significant increase in body weight in those rats by the 2nd and 3rd day of treatment.

Despite the significant beneficial effect of intracolonic MFF on the severity of colitis (macroscopic damage and disease activity score), this treatment had no effect on tissue MPO

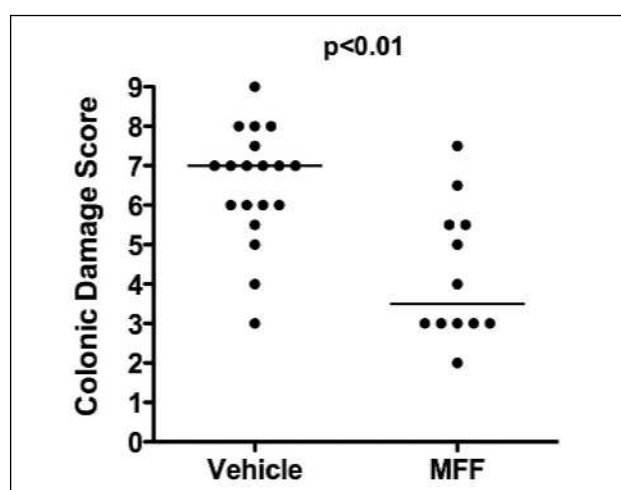


Fig. 1. The cannabis extract, MFF, significantly reduced the severity of colonic damage. Rats with colitis induced by dinitrobenzene sulfonic acid were given MFF (10 mg/kg) twice-daily, (i.c.), for 7 days. The criteria for scoring damage are outlined in *Table 1*. Horizontal lines represent the median score for each group. (ANOVA and Mann-Whitney test).

activity (Fig. 3). It should be noted that in the rats with colitis, tissue samples were always taken from regions of overt mucosal damage. While MPO activity was markedly elevated in rats with colitis as compared to healthy controls, MFF treatment of

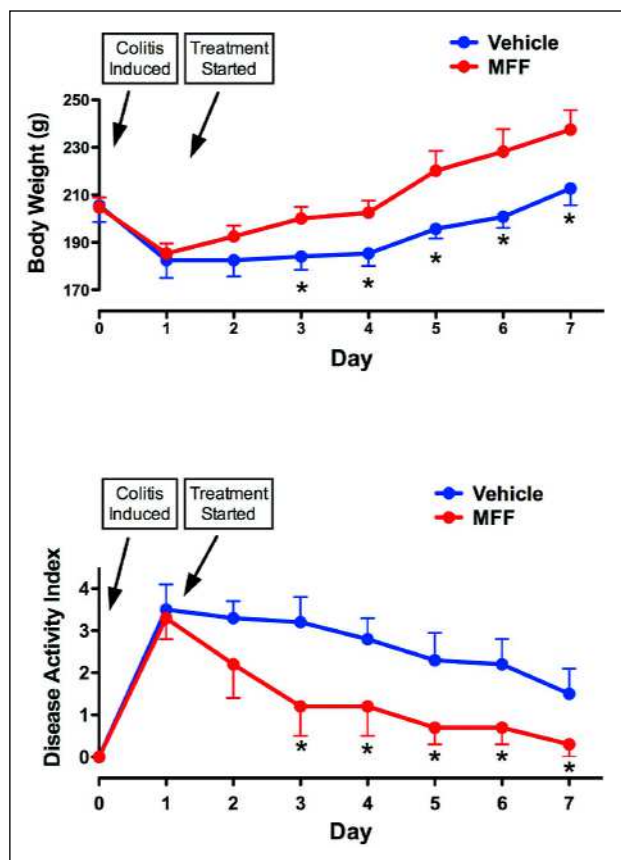


Fig. 2. Beneficial effects of intracolonic treatment with MFF (10 mg/kg), a cannabis extract, in rats with colitis induced by dinitrobenzene sulfonic acid. Twice-daily treatment with MFF beginning one day after induction of colitis resulted in significantly greater body weight gain and reduction of the disease activity index (a composite score for bleeding, stool consistency and body weight loss). Data are shown as the mean \pm S.E.M. ($n \geq 5$ per group; * $p < 0.05$ versus the vehicle-treated group; ANOVA and Dunnett's test (body weight) or Mann-Whitney test (disease activity)).

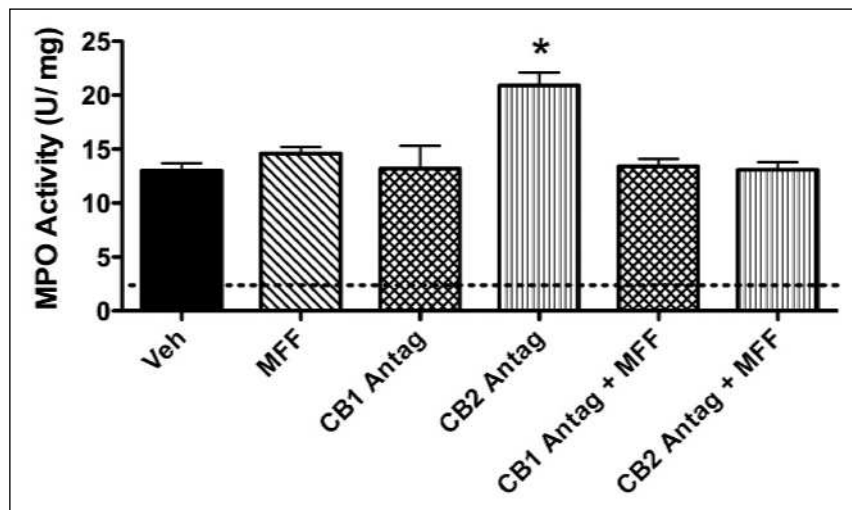


Fig. 3. Colonic myeloperoxidase (MPO) activity in rats with colitis treated with MFF (i.e., 10 mg/kg), with a CB1 or CB2 antagonist (i.p.) or a combination of one of the antagonists and MFF. The only significant difference observed was in the group treated with the CB2 antagonist alone (* $p < 0.05$ versus vehicle-treated; ANOVA and Dunnett's test). The dotted line represents the mean colonic MPO activity in healthy controls. The CB1 and CB2 antagonists used were AM251 (3.3 mg/kg) and AM630 (3 mg/kg), respectively. Each group consisted of at least 6 rats.

healthy rats or rats with colitis did not significantly affect tissue MPO activity. Treatment of rats with colitis with a CB2 antagonist resulted in a significant (~50%) increase in MPO activity. However, when rats were co-treated with MFF and the CB2 antagonist, no increase in MPO activity was observed. No effect on MPO was observed in rats treated with a CB1 antagonist (with or without MFF).

Effects of MFF on urinary THC levels in rats with colitis

THC levels were below levels of detection (< 2 ng/ml) in rats treated intracolonicly twice-daily for 7 days with MFF at doses of 1–10 mg/kg. However, with oral dosing of MFF at the highest dose (10 mg/kg), THC levels in urine were detectable in 5 of 6 rats (the mean for the 5 rats was 5.5 ± 1.3 ng/ml).

Potential mechanisms of pro-resolution effects of MFF

The roles of CB1 and CB2 receptors, and of mucosal synthesis of prostaglandin E_2 and hydrogen sulfide (H_2S), in the pro-resolution effects of MFF were investigated. Rats with colitis treated twice-daily with the CB1 antagonist for 7 days did not exhibit any significant worsening of colonic damage scores (7.8 ± 1.2 versus 7.7 ± 0.9 in vehicle-treated rats) or any change in tissue MPO activity (Fig. 3). However, in the rats with colitis that were treated with the CB2 antagonist, there was a significant increase in MPO (Fig. 3; $p < 0.05$) and a significant worsening of colitis (colonic damage score of 11.8 ± 0.8 versus 7.7 ± 0.9 in vehicle-treated rats; $p < 0.05$). When MFF was also administered to the rats with colitis, the CB1 and CB2 antagonists had no effect on colonic MPO activity as compared to the vehicle-treated group (Fig. 3), and did not attenuate the beneficial effects of MFF on the colonic damage score (colonic damage scores of 3.6 ± 0.7 and 4.0 ± 0.9 for CB1 and CB2 antagonists, respectively, versus 3.7 ± 0.6 for vehicle + MFF).

In healthy rats, colonic PGE_2 synthesis was not affected by intracolonic treatment with MFF (32 ± 4 ng/g versus 30 ± 5 ng/g in vehicle-treated). In rats with colitis, colonic PGE_2 synthesis was markedly increased (~4-fold; $p < 0.01$) over that in healthy rats (Fig. 4). Treatment with the CB2 antagonist resulted in a significant reduction of colonic PGE_2 synthesis (by ~50%; $p < 0.05$), while treatment with the CB1 antagonist had no effect. In rats with colitis treated with MFF, the CB2 antagonist-induced decrease in colonic PGE_2 synthesis was no longer evident (Fig. 4).

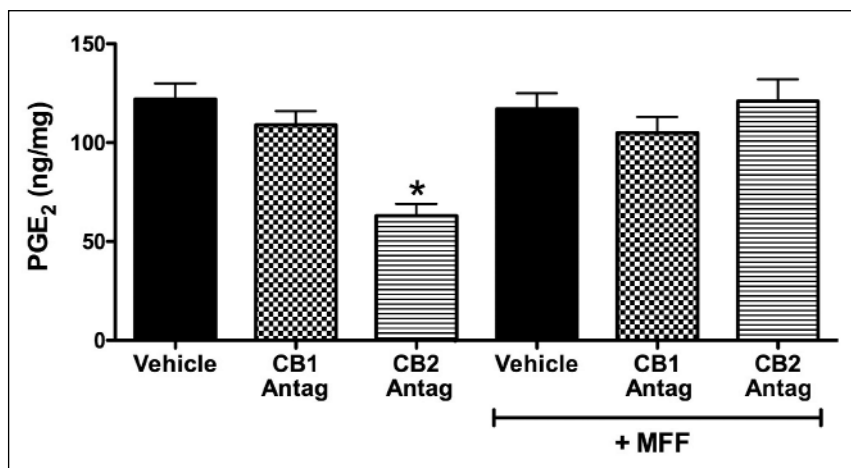


Fig. 4. Effects of cannabinoid antagonists and MFF (10 mg/kg) on colonic prostaglandin E₂ (PGE₂) synthesis in rats with colitis. The CB2 antagonist (AM630; 3 mg/kg) significantly reduced colonic PGE₂ synthesis as compared to vehicle treatment (**p*<0.05; ANOVA and Dunnett's test), while the CB1 antagonist (AM251; 3.3 mg/kg) had no effect. However, in rats also treated with MFF, there was no effect of the CB1 or CB2 antagonist on colonic PGE₂ synthesis. Data shown are the mean \pm S.E.M. (*n*≥5 per group).

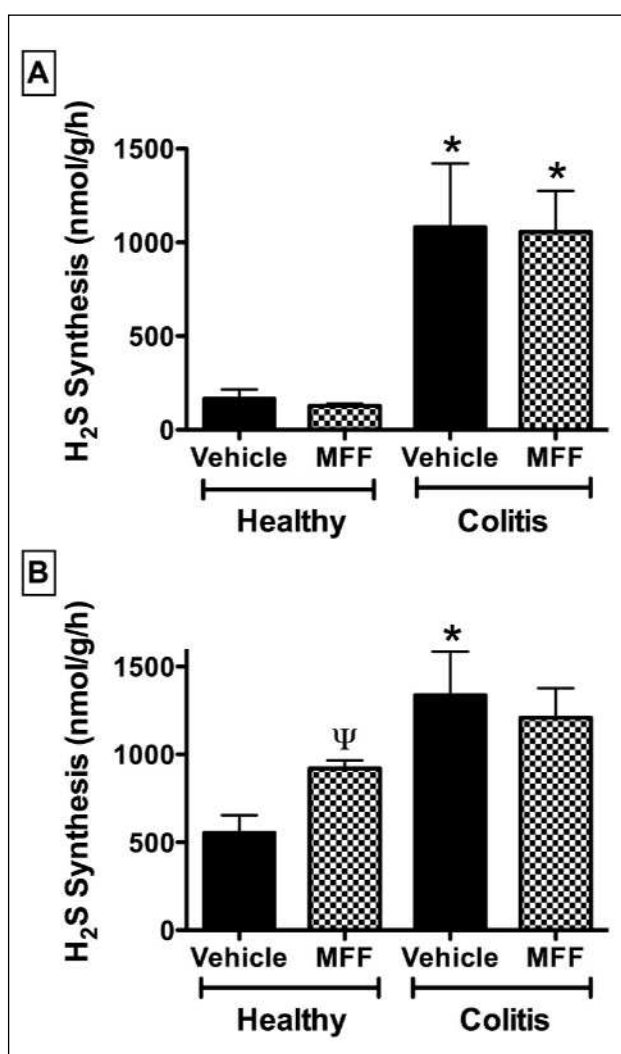


Fig. 5. Effects of intracolonic treatment with MFF on colonic hydrogen sulfide (H₂S) synthesis in healthy rats and rats with colitis.

Panels A and B show H₂S production *via* pyroxidal-5'-phosphate (P5P)-dependent and -independent pathways, respectively (**p*<0.05 versus the corresponding healthy controls). In healthy rats, MFF significantly increased H₂S synthesis *via* the P5P-independent pathway (^ψ*p*<0.05 versus the corresponding vehicle-treated group; ANOVA and Dunnett's test). *n*≥5 per group.

H₂S synthesis is markedly elevated in experimental colitis and contributes significantly to resolution of inflammation and to healing of ulcers (19, 29-31). H₂S is produced *via* both pyroxidal-5'-phosphate dependent (P5P) and -independent pathways. H₂S production by colonic tissue from healthy rats averaged 165 \pm 51 nmol/g/h and 554 \pm 100 nmol/g/h *via* the P5P-dependent and P5P-independent pathways, respectively (Fig. 5). Treatment of healthy rats with MFF intracolonic at 10 mg/kg twice-daily for 7 days had no effect on colonic H₂S synthesis *via* the P5P-dependent pathways. However, H₂S synthesis *via* the P5P-independent pathways was significantly increased in rats treated with MFF (by 72%; *p*<0.01; Fig. 5B).

In colitis, production of H₂S *via* the P5P-dependent and -independent pathways was increased substantially (to 1080 \pm 341 nmol/g/h and 1337 \pm 249 nmol/g/h, respectively; Fig. 5), consistent with what has been reported previously (19, 30, 31). Treatment with MFF had no significant effect on colonic H₂S synthesis in the rats with colitis, *via* either of the pathways (Fig. 5).

MFF prevents NSAID-induced gastric damage via CB1 receptors

Oral administration of naproxen (30 mg/kg) resulted in the formation of hemorrhagic erosions in the stomach of rats within a few hours of its administration (Fig. 6A). Oral pretreatment with MFF dose-dependently reduced the extent of injury, with complete protection observed with the 10 mg/kg dose. However, no beneficial effect was observed when MFF was given intraperitoneally at 10 mg/kg. Urinary levels of THC were undetectable 3 hours following oral administration of MFF at 1 or 3 mg/kg (<2 ng/ml), but with the 10 mg/kg dose, urinary THC levels averaged 6.2 \pm 1.1 ng/ml.

The protective effects of MFF against naproxen-induced gastric damage were completely blocked by pretreatment with a CB1 antagonist, while the CB2 antagonist had no significant effect (Fig. 6B). Given alone (no MFF), neither of the CB antagonists significantly affected the extent of gastric damage caused by naproxen (data not shown).

Gastric PGE₂ synthesis was inhibited by >90% in all rats treated with naproxen (there were no differences among the treatment groups).

Visceral anti-nociceptive effects of MFF are mediated via CB2 receptors

Gastric distention caused a pressure-dependent reduction of heart rate, a typical response to visceral pain (24, 25) (Fig. 7A).

Treatment with a single dose of MFF at 3 mg/kg completely inhibited this response (Fig. 7B). Pre-treatment with a CB1 or CB2 antagonist alone had no effect on the autonomic response to gastric distention. However, the CB2 antagonist completely blocked the anti-nociceptive effect of MFF (Fig. 7B).

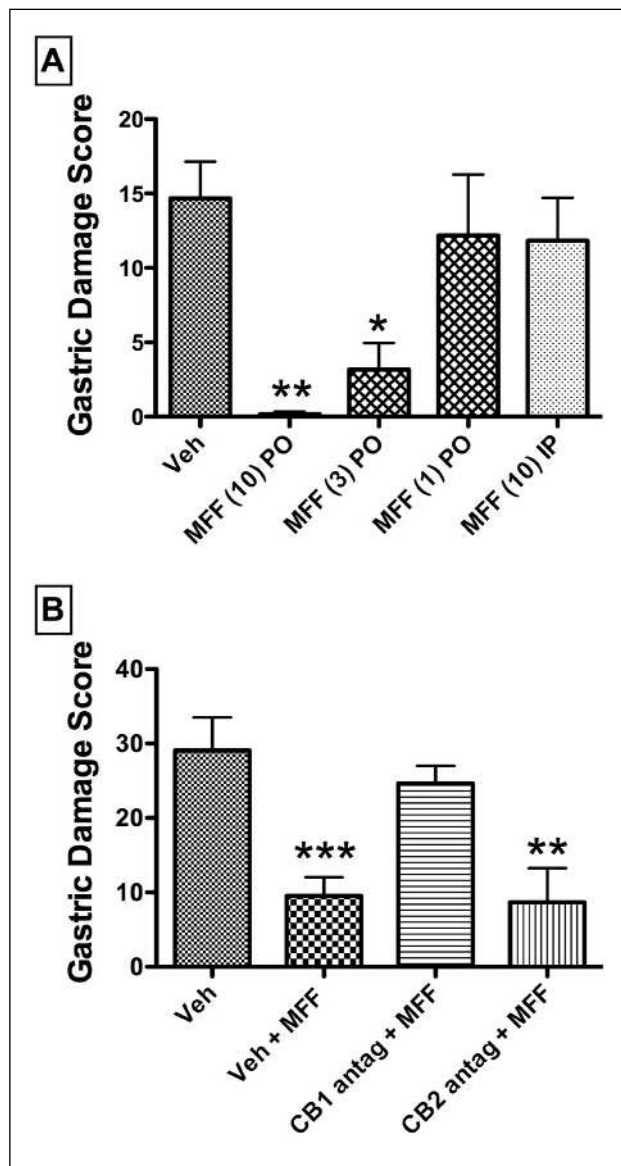


Fig. 6. Reduction of naproxen-induced gastric hemorrhagic damage by the cannabis extract MFF.

Panel A: MFF was effective at dose-dependently reducing damage when administered orally (PO), but not when administered intraperitoneally (i.p.). Naproxen was administered orally at a dose of 30 mg/kg. * $p < 0.05$ versus the vehicle-treated group.

Panel B: The gastroprotective effects of the cannabis extract, MFF (10 mg/kg p.o.), were CB1-dependent. MFF significantly reduced the extent of naproxen-induced gastric damage, but the effect was reversed by pretreatment with a CB1 receptor antagonist (AM251; 3.3 mg/kg i.p.), but not by a CB2 receptor antagonist (AM630; 3 mg/kg i.p.). Naproxen was administered orally (60 mg/kg) 3 h prior to blind scoring of damage.

Data are shown as the mean \pm S.E.M. ($n \geq 5$ per group; ** $p < 0.01$, *** $p < 0.001$ versus the vehicle-treated group; ANOVA and Dunnett's test).

DISCUSSION

Cannabis and cannabinoid-based agents or extracts are commonly used for treating a wide range of ailments, particularly those associated with chronic pain, and often those involving the digestive system (1, 2, 6-8). Cannabis has well-characterized adverse effects, including an increased risk of schizophrenia and psychosis, but also additional adverse effects specifically related to ingestion through smoking (13). Thus, many patients seek cannabis-based therapies that can be used *via* other routes. In the present study, we examined the effects of a simple extract of cannabis (MFF) in models of inflammatory bowel disease, NSAID-induced gastric damage and visceral pain. MFF dose-dependently reduced the severity of experimental colitis and NSAID-induced gastric damage, and

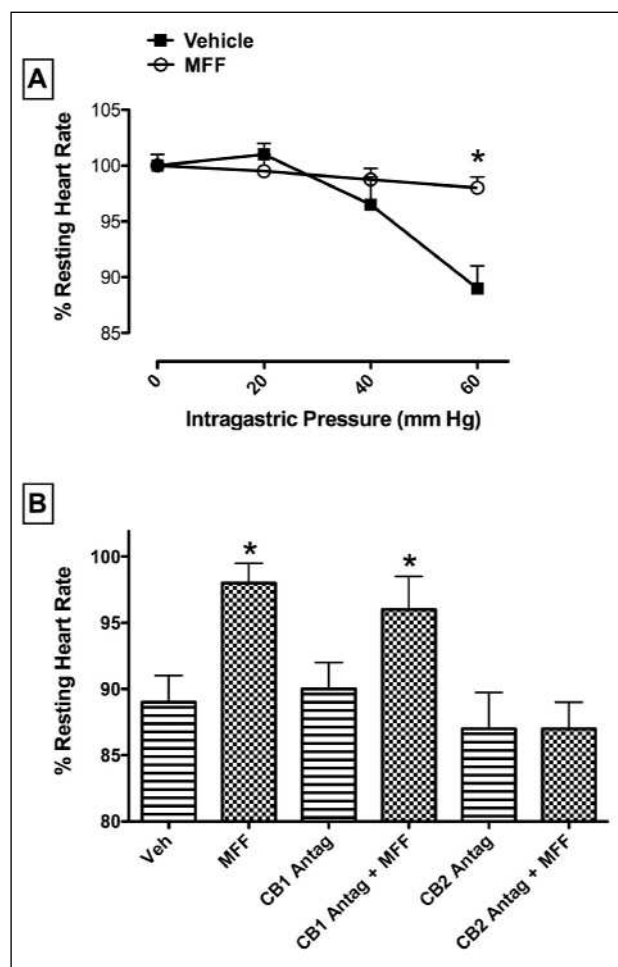


Fig. 7. Visceral anti-nociceptive effects of MFF are CB2-dependent.

Panel A: In vehicle-treated rats, an increase in intra-gastric pressure produced a decrease in resting heart rate, indicative of visceral pain. However, in rats treated with MFF (3 mg/kg i.p.), the gastric distention did not induce any pain response.

Panel B: The gastric distention-induced visceral pain response (decrease in heart rate) was blocked by MFF (3 mg/kg), but was not affected by pretreatment with a CB1 (AM 251; 3.3 mg/kg i.p.) or CB2 (AM 630; 3 mg/kg i.p.) antagonist. However, the anti-nociceptive effect of MFF was prevented by pretreatment with the CB2 antagonist.

Data are shown as the mean \pm S.E.M. ($n \geq 5$ per group; * $p < 0.05$ versus the vehicle-treated group; ANOVA and Dunnett's test).

Table 2. Effects of MFF on food intake and body weight.

(A) Food intake (g)

Day	Healthy Vehicle	Healthy MFF	Colitis Vehicle	Colitis MFF
0	31.3 ± 1.4	32.2 ± 1.4	31.1 ± 1.3	31.9 ± 1.2
1	27.5 ± 1.1	23.7 ± 0.9	2.3 ± 1.5*	2.7 ± 1.3*
2	32.0 ± 1.1	27.5 ± 0.5	8.8 ± 1.8*	10.2 ± 1.9*
3	30.3 ± 1.2	28.8 ± 0.6	8.7 ± 2.7*	22.0 ± 6.2
4	23.8 ± 0.6	22.8 ± 0.7	5.8 ± 2.0*	8.7 ± 1.0*

(B) Body weight (g)

Day	Healthy Vehicle	Healthy MFF	Colitis Vehicle	Colitis MFF
0	223.2 ± 5.1	220.7 ± 6.1	220.7 ± 2.6	219.2 ± 3.5
1	230.0 ± 5.2	226.3 ± 5.6	190.2 ± 6.7	189.7 ± 5.6
2	241.5 ± 5.8	234.3 ± 5.9	190.5 ± 7.4	198.2 ± 5.4
3	250.7 ± 6.3	242.5 ± 6.2	192.0 ± 3.5	204.8 ± 4.2 ^ψ
4	262.7 ± 5.9	256.3 ± 7.0	194.5 ± 4.1	210.0 ± 4.3 ^ψ

Data are shown as the mean ± S.E.M. for 6 rats per group. In the Colitis groups, DNBS was administered at the end of day 0. Treatment with MFF at 10 mg/kg twice-daily (i.c.) or vehicle was initiated at the end of day 1.

* $p < 0.05$ versus the same group on day 0; $^{\psi}p < 0.05$ versus the corresponding Colitis Vehicle group; There were no significant differences in food intake between the Colitis-Vehicle and Colitis-MFF groups on any day.

was also effective in reducing gastric distention-induced pain. However, there were clear differences in terms of mechanism of action in the different models. The anti-nociceptive effects of MFF were mediated *via* CB2 receptors, while the gastro-protective effects were mediated *via* CB1 receptors. The beneficial effects in colitis may have been mediated partially *via* CB2 receptors, and appeared to be independent of effects on mucosal synthesis of prostaglandins and hydrogen sulfide. It is also noteworthy that the beneficial effects in experimental colitis were produced by doses of MFF, given intracolonic, that did not produce detectable levels of THC in the urine.

There is considerable evidence suggesting a role for cannabinoids and cannabinoid receptors in gastrointestinal injury and inflammation. CB1 receptors are located throughout the GI tract, particularly on intrinsic and extrinsic neurons (3). On the other hand, CB2 is expressed on lamina propria plasma cells and activated macrophages, as well as on epithelial cells and enteric neurons (3). Increased expression of CB1 and CB2 has been observed in biopsies of the gut from patients with inflammatory bowel disease (8), as well as in experimental colitis (9, 10). Cannabinoids have been shown to promote epithelial/mucosal healing (8, 32) and reduce functional disturbances associated with colitis (32), while CB1 and CB2 agonists, given systemically, reduced colonic damage and incidence of diarrhea in a mouse model of colitis (11). Mice genetically deficient of CB1 are more susceptible to colitis induced by dextran sulfate sodium, and administration of a CB1 antagonist similarly worsened colitis in that model (9) (note that dextran sulfate sodium produces a pan-gastroenteritis rather inflammation specific to the colon (33)). In the present study, the significant beneficial effects of MFF in rats with DNBS-induced colitis were not affected by co-administration of a CB1 antagonist or a CB2 antagonist. Administration of the CB2 antagonist alone (no MFF) caused an exacerbation of colitis, which is consistent with observations in a mouse model (10). As discussed below, the CB2

antagonist also significantly reduced colonic PGE₂ synthesis and increased colonic MPO activity in rats with colitis, but these effects were not seen when MFF was co-administered. Thus, the actions of MFF that contribute to resolution of colitis could be mediated to some extent *via* CB2 (since we cannot be sure of complete receptor blockade), or MFF may produce effects that override the detrimental effects of CB2 receptor blockade.

In the studies of the stomach, a clear role for CB1 receptors in the actions of MFF was apparent. MFF, given orally by not intraperitoneally, was a potent protective agent against naproxen-induced hemorrhagic erosion formation in rats. The protective effect of MFF was completely blocked by the CB1 receptor antagonist, but not the CB2 receptor antagonist. These results are consistent with previous reports that a CB1 antagonist could reduce the severity of gastric damage induced in rats by aspirin (34) or by cold-restraint stress (35). The mechanism underlying the protective effects CB1 receptor activation against naproxen-induced gastric damage is unclear. However, given that gastric PG synthesis was inhibited by >90% in the rats receiving naproxen, effects of CB1 activation on PG synthesis, which have been reported to occur in the amnion (12), are unlikely to have been significant in this gastric damage model.

The visceral pain studies also demonstrated a clear beneficial effect of MFF that was mediated *via* a CB receptor. MFF markedly reduced the pain response (bradycardia) associated with gastric distention, and this effect was completely blocked by the CB2 antagonist, but not by the CB1 antagonist. Previous studies of cannabinoid receptors in models of visceral pain have implicated both CB1 and CB2 (36) in the anti-nociceptive mechanism, though a study of bradykinin-induced visceral pain showed a clear role for CB2 receptors (37), and CB2 receptors have been identified as playing an important role in limiting visceral hypersensitivity and pain (4). Unlike the actions of MFF in promoting resolution of colitis and gastric protection against naproxen-induced damage, the anti-nociceptive effects of MFF were observed after systemic administration (oral administration of MFF was not tested in this model because of the presence of the balloon catheter in the stomach of the rats).

We investigated the possibility that the beneficial effects of MFF in experimental colitis may have been to some extent mediated by effects of this extract on mucosal PG synthesis. Krowicki (38) demonstrated that the gastric motor and cardiovascular effects of THC were dependent upon activation of cyclooxygenase. PG synthesis is markedly elevated during colitis and plays an important role in promoting resolution of inflammation and healing of ulcerated tissue (39). In this setting, the PGs are derived primarily from cyclooxygenase-2 (39). We observed that treatment with the CB2 antagonist significantly reduced PG colonic synthesis, which is consistent with its observed exacerbation of colitis by the antagonist (significantly increased colonic MPO activity and colonic damage score). Co-administration of MFF with the CB2 antagonist restored colonic PG synthesis to normal levels in the rats with colitis, but MFF did not elevate colonic PG synthesis in any of the other groups of rats, so it seems unlikely that such an effect played a significant role in the actions of MFF.

H₂S is another mediator produced by the colonic mucosa that can influence a wide range of gastrointestinal functions (40, 41), and can exert anti-inflammatory effects *via* a number of mechanisms (42, 43). The synthesis of H₂S has been shown to be markedly up-regulated in experimental colitis (19, 30, 31). Moreover, like prostaglandins, H₂S plays an important role in promoting the resolution of inflammation and healing of ulcers in the GI tract. (19, 28, 31, 44). A significant increase in colonic H₂S synthesis was observed in healthy rats treated with MFF, but not in rats with colitis. Based on these data, it would appear that the pro-resolution effects of MFF are not H₂S-mediated.

The precise composition of MFF, a simple ethanolic extract, is not known. MFF was deliberately produced as a simple extract based on the concept that it may represent the starting point for an inexpensive alternative to cannabis, and particularly its use *via* smoking, for treatment of certain GI conditions, and to do so with a lower incidence of systemic, particularly psychoactive, effects. Other studies have demonstrated significant beneficial effects of components of cannabis (*i.e.*, THC, cannabidiol) in experimental colitis, when given systemically (32, 45, 46). It is unlikely that beneficial effects of MFF in colitis can be attributed to either of these components, given that urinary THC levels were not elevated in rats receiving MFF intracolonicly, MFF did not reduce the severity of colitis when given orally (which did result in significant urinary THC levels) and since cannabidiol does not activate CB1 or CB2 receptors (32). However, given that cannabidiol is a major component (~40%) of cannabis extracts (32), one cannot exclude the possibility that this substance made some contribution to the beneficial effects of MFF in colitis. Of course, THC may have contributed to the gastrointestinal effects of MFF when it was given orally, which resulted in significant levels of THC in the urine.

In summary, we have demonstrated that a very simple extract of medicinal cannabis can exert marked beneficial effects in a rat model of colitis when administered intracolonicly. This extract also exhibited significant anti-nociceptive actions in a gastric distention-induced visceral pain model, and, when given orally, it significantly protected the stomach against damage induced by an NSAID. The involvement of CB1 and CB2 receptors in the actions of MFF differed from model to model, and in the case of colitis, did not fully account for the observed beneficial effects. Use of cannabis for treatment of inflammatory bowel disease is widespread, but because of its systemic administration, usually *via* smoking, there are significant adverse effects. MFF may serve as the basis for a simple preparation of medicinal cannabis that would produce beneficial effects in the GI tract with reduced systemic toxicity.

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