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Research Article

PPARα Expression Analysis in a Murine Model of Sepsis-like Inflammation Intestinal Tissue Treated with a Cannabidiol-Enriched Broad Spectrum Cannabis Oil

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Abstract | Background: The *Cannabis sativa* L. plant can modulate numerous physiological and pathological processes in human and non-human animals through its interactions with the Endocannabinoidome (eCBome). Inflammation processes are affected by the eCBome activities, and there are still numerous unknown mechanisms by which this occurs. The peroxisome proliferated-activated receptor α (PPARα) is a nuclear receptor with affinity to cannabinoid ligands and whose transcription activity in the intestine has been linked to anti-inflammatory functions. Aims: To evaluate the effect on morphological changes and PPARα expression of cannabidiol-enriched broad-spectrum cannabis oil (referred to as CBD/BS) in intestinal tissue of a murine model of LPS-induced sepsis-like inflammation. Methods: Murine subjects were randomly localized in different cages (n=3) distributed by treatments. Animals were given a 5-day acclimatation period before experimental procedures. BALB/c mice were treated intraperitoneally with lipopolysaccharide for 5 days and thereafter treated orally for 2 days with CBD/BS (or vehicle) at a dose of 100 mg/kg Intestinal tissue samples were obtained from euthanized animals for histopathology, morphological and immunohistochemistry analysis, and cytokine and PPARα expression analysis at a lamina propria level by RT-qPCR, histology and immunohistochemistry. Results: Our results show that CBD/BS could reduce the intestinal inflammatory process induced by LPS and the modulate PPARα protein staining at a lamina propria level. Conclusions: Our findings support the anti-inflammatory activity of cannabinoids in inflamed intestinal tissue and suggest an implication of PPARα in lamina propria cell infiltration through the course of inflammation induced by lipopolysaccharide. PPARα has a significant role in inflammatory processes, as well as the eCBome, which modulation represents an interesting therapeutic approach for treating inflammation.

Keywords | Histopathology, Murine, Lipopolysaccharide, Sepsis, Endocannabinoid system

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INTRODUCTION

The Cannabis sativa L. plant can modulate numerous physiological and pathological processes in human and non-human animals through its effects on the Endocannabinoidome (eCBome) (Di Marzo and Piscitelli, 2015). This system has been involved in intestinal homeostasis on many levels and has been proposed as a target of a pharmacological approach (Lian *et al*., 2022). The cannabis plant, and its derivates, have been explored for their potential as an adjuvant in treatments for diseases involving chronic inflammation such as inflammatory bowel disease (IBD) (Carvalho *et al*., 2020). Among the shared consequences of chronic intestinal inflammation, we can find alterations in the intestinal epithelial barrier function and the influx of immune cells (Ingersoll *et al*., 2012). Intestinal cellular and molecular organization during sepsis can become dysregulated. Alterations in the intestinal barrier function, as a result and a cause of sepsis, can allow translocation of bacteria, and PAMPS, such as lipopolysaccharide (LPS), and contribute to the inflammatory process. The recruitment of inflammatory cells and the pro-inflammatory response are some clinical signs of local and systemic inflammation (Haussner *et al*., 2019). This, together with compromised absorption, can develop into systemic complications such as fever, weight loss, and delayed growth (Stephens and von der Weid, 2020). Cannabinoids, such as cannabidiol (CBD), tetrahydrocannabinol (THC), and cannabigerol (CBG) had been studied for their anti-inflammatory properties in LPS-induced sepsis-like models and TNBS/DNBS-induced colitis models (Borrelli *et al*., 2013; Schicho and Storr, 2012; Szekely *et al*., 2020). Cannabinoids exert their biological activities through many receptors and target proteins such as the peroxisome proliferation-activated receptors (PPARs) family (Iannotti and Vitale, 2021). PPARα is a nuclear receptor with affinity to cannabinoid ligands and whose transcription activity in the intestine has been linked to anti-inflammatory functions (Decara *et al*., 2020; Peters *et al*., 2012). The use of cannabinoids for treating inflammatory diseases has potential due to their several biological activities and safety. The aim of this study is to evaluate if treatment with cannabidiol oil can modulate PPARα expression in inflamed intestinal tissue. The present study analyzed the expression of PPARα by the stimuli of a cannabidiol enriched broad spectrum cannabis oil (CBD/BS) in the small and large intestines of BALB/c mice treated with lipopolysaccharide. PPARα has a significant role in inflammatory processes, as well as the eCBome, which modulation represents an interesting therapeutic approach for treating inflammation.

MATERIALS AND METHODS

Reagents

The CBD/BS was provided by ICAN and (Botica)n (CDMX, Mexico) at a concentration of 100 mg/mL Cannabidiol (Table 1) (Reference Labs Certificate of Analysis #4146-0011: Report ID: 3842) and grape seed oil (GO) was used as a vehicle.

Table 1: Phytocannabinoids contained in the CBD/BS.

Cannabinoid	% by weight	mg/g
CBDA	LOQ	LOQ
CBD	97.87	978.7
CBDV	0.6	6
THCA	LOQ	LOQ
Δ9-THC	LOQ	LOQ
Δ8-THC	LOQ	LOQ
CBGA	LOQ	LOQ
CBG	1.61	16.1
CBN	LOQ	LOQ
CBC	LOQ	LOQ
Humidity %	0.00	
Total	>99.99	995.40

LOQ is defined as less than 0.2% by weight and 2.0 mg/g.

Salmonella typhimurium lipopolysaccharide (LPS) (Sigma-Aldrich, Missouri, United States of America) was diluted with PBS 1X at a 0.05 mg/mL concentration. Primary rabbit polyclonal antibody to PPARα (ab203043) 1:500, and secondary labeled antibody from Mouse and Rabbit Specific HRP/DAB (ABC) Detection IHC kit (ab64264) 1:3000, were purchased from Abcam (UK). Micrographs obtained of processed tissues by immunohistochemistry (IHC) staining were measured by their Integrated Density (Int Dent) using the Measure Tool of the Fiji/ImageJ software. All primers were purchased from Integrated DNA Technologies (IDT™) and designed using IDT's PrimerQuest Tool for mRNA PPARα and GAPDH, using TaqMan probes and GoTaq® Probe qPCR Master Mix (Promega®, USA) kit; and inflammatory mediators IL-1B, IL-4, IL-6, IL-10, TLR-2, TLR-4, and β-actin using GoTaq Master Mix (Promega®, USA). Finally, mRNA expression was assessed by RT-qPCR and analyzed using the $2^{-\Delta\Delta}$CT method.

Experimental Animals

Twelve mice of the BALB/c strain weighing 25 g, between 8 and 11 weeks, obtained from Tetrarium (NL, Mexico) were used and kept under normal conditions for 5 days in an adaptation period at 25°C with a relative humidity of 40-60 %, and with light/dark cycles of 12 h. The animals were fed standard food for rodents and with free access to purified water. All the experimental procedures were performed at the Faculty of Veterinary Medicine and Zootechnics (FMVZ) of the Autonomous University of Nuevo Leon (UANL) (IACUC code 31/2022-33/2022).

Experimental Design

The animals were divided into 4 groups (n=3) as follows: Group 1 without treatment (WT), Group 2 treated with LPS, Group 3 with LPS + CBD/BS (LPS-CBD/BS), and Group 4 with LPS and GO vehicle (LPS-GO).

Treatments

Treated mice were injected with LPS at a dose of 0.1 mg/kg i.p for 5 days. After LPS treatment, non-control mice were given a dose of CBD/BS at 100 mg/kg CBD or the same volume of GO p.o. for 2 days. After treatments, animals were euthanized by exposing them to high concentrations of diethyl ether for 5-7 seconds and then humanly sacrificed by cardiac puncture according to Mexican law NOM-062-ZOO-1999.

Sampling

Sampling, sample processing and data analysis were done as previowsly described by our research group in (Mar-Solís *et al*., 2021). After euthanasia, small and large intestinal segments were taken and preserved for future use depending on the required technique: 4% paraformaldehyde and Carnoy's solution for tissue preservation for Hematoxylin and Eosin staining and IHC techniques, respectively; and RNAzol® cell lysis reagent, guanidine thiocyanate (Molecular Research Center, Inc., Cincinnati, OH, USA), for mRNA preservation. The small intestine segments corresponded to the jejunum, and the large intestine was entirely used, avoiding the use of the proximal portions (2 cm) for the detection of protein and mRNA.

Histological Analysis

Intestine samples fixed in 4% paraformaldehyde were automatically processed by the conventional histological technique on an automatic tissue processing equipment (KD-TS3B, KEDEE, Zhejiang Jinhua Kedi Instrumental Equipment CO., LDT, ZJ, China). The tissues were dehydrated in alcohols of increasing concentration (60, 70, 80, 96%), and absolute in three changes of this latter) for 1 h each. Subsequently, they were clarified by immersion in xylol for 1 h and finally immersion in hot paraffin for 1 h at 52°C. The samples were embedded in paraffin blocks employing a tissue embedding center (KD-BM, Zhejiang Jinhua Kedi Instrumental Equipment CO., LDT, ZJ, China) to obtain longitudinal sections. The blocks were placed on a cold plate (KD-BL, Zhejiang Jinhua Kedi Instrumental Equipment CO., LDT, ZJ, China) and 4µm thick sections were made in a microtome (LEIICA RM2235, Leica Biosystems, IL, USA), histological sections were mounted on a slide covered with gelatin. The sections analyzed by immunohistochemistry were placed on slides previously treated with a solution of Poly-L-lysine (Sigma-Aldrich) 10% in H20d for 5 minutes and they were left to dry overnight. When the analysis was performed the slides were deparaffinized with xylol and rehydrated with decreasing gradual alcohol from 100% to 70%, and finally in H_2Od water. We employed HandE staining for histopathological changes evaluation in the study groups.

Immunohistochemistry (IHC)

For the immunohistochemical localization of PPARα protein in the small and large intestines, mouse anti-PPARα (ab203043) antibody (Abcam, UK) was used, at a concentration of 1:250 antibody-diluent; and the Mouse and Rabbit Specific HRP/DAB (ABC) Detection IHC Kit (ab64264, Abcam) was also employed.

The immunohistochemistry technique was performed according to (Soto-Domínguez *et al*., 2022). Slides with rehydrated tissues were immersed in Tris Buffer Saline + Tween 20 (TBS-T, Dako®, Agilent, USA) for 5 minutes and the antigen retrieval was done with Tris Retrieval Solution (TRS, Dako®, Agilent, USA) preheated for 20 minutes at 60-65°C. Two washes with TBS-T for 5 minutes were done, and the endogenous peroxidase blocking was done by adding 3% H2O2 directly to the tissue and in a humid chamber for 10 minutes. Washes with TBS-T were done again, and the protein blocking was done with the kit's protein blocker reagent for 30 minutes in a humid chamber and then the slides were incubated with the primary antibody overnight at 4°C in a humid chamber. After this time washes were performed again with TBS-T and 30µL of biotinylated secondary antibody were added for 30 minutes in a humid chamber. Washes were made in TBS-T and incubation with streptavidin in a humeral chamber for 30 minutes was allowed. Finally, washes were made in TBS-T and positivity was identified with the DAB reagent diluted in a 1:20 DAB: substrate ratio, incubating for 2-5 minutes. Washes were performed with H20d water and samples were counterstained with Gill's hematoxylin for 1.5 minutes. Sections were washed with running tap water, rinsed with H20d water, and finally dehydrated with alcohol for 3 minutes at 70%, 96%, absolute ethanol, ethanol-xylene (5 minutes), and xylene (5 minutes). The slides were mounted with Entellan®, observed under a light microscope, and analyzed using the ToupView program.

Quantification of Integrated Density

Photographic images of 8 fields were obtained of both the small and the large intestines sections analyzed with immunohistochemistry at an objective of 100X per slide of everyone. For the quantification, the Measure-Int Dent plugin was used in the Fiji/ImageJ program (Arganda-Carreras *et al*., 2017). The results for everyone were average and the averages were used for statistical analysis.

Total RNA Extraction

For the extraction of RNA from the intestine samples stored in the lysis reagent RNAzol ®, Guanidine Thiocyanate (Molecular Research Center, Inc., Cincinnati, OH,

USA), the tissues were homogenized with 0.5mL of reagent using a tissue homogenizer (Tissue-Tearor Model 985370 BioSpec Products, Inc., USA) and RNA extraction was performed according to the manufacturer's instructions. The RNA pellet was diluted in nuclease-free water and finally quantified (ng/µL) by spectrophotometry (EPOCH, BioTek, Canada), and the yield obtained was evaluated using the 260/280 measurement.

Synthesis of Complementary DNA

After total RNA extraction, complementary DNA synthesis was performed using the commercial reverse transcription kit ImProm-II™ Reverse Transcription System (Promega ®, USA). For each reaction, a mixture of 4µL of MgCl2 (25mM), 1µL of Oligo (dT)15, 1µL of ImProm-II ™ transcriptase, 1500ng of RNA template was made and calibrated to a final volume of 20µL with nuclease-free water. Reactions were performed in 200 µL Eppendorf® microtubes. The samples were incubated in a Veritti® model thermocycler (Applied Biosystem®, USA) for cDNA synthesis. Again, the final sample was quantified by spectrophotometry (EPOCH, BioTek, Canada) and the resulting products were stored at -20°C until use.

Design of Oligonucleotides and PCR

The design of the oligonucleotides for the quantification of mRNA of the protein PPARα, and GAPDH normalizing gene; of the cytokines IL-1β, IL-4, IL-6, IL-10 and IL-4; of TLR2 and TLR4 receptors; and the β-actin normalizing gene was made from the mRNA sequence of each gene, obtained in the Genbank (Table 2).

Table 2: Treatments used in the present study.

Groups	Treatment	Duration
Group 1	WT	
Group 2	0.1 mg/kg †LPS	5 days
Group 3	0.1 mg/kg †LPS and 100 mg/kg ᵠCBD/BS	†LPS: 5 days; ᵠCBD/BS: 2 days.
Group 4*	0.1 mg/kg LPS and ᵈGO	LPS:5 days; ᵈGO: 2 days

†LPS, lipopolysaccharide; ᵠCBD/BS, Cannabidiol-enriched cannabis oil; ᵈGO, Grapeseed oil; *same volume as CBD/BS.

For the qPCR reaction of PEPT1, PPARα, and GADPH, Taqman hydrolysis probes and the GoTaq® Probe qPCR Master Mix commercial kit (Promega ®, USA) was used. The reaction was done by mixing 10 µL of GoTaq® Probe qPCR Master Mix reagent (2X), 0.2 µL of CXR Reference Dye, 1 µL of primers + probe, 1 µL of cDNA (25 ng) and nuclease-free water to obtain a volume of final reaction of 20 µL. For the qPCR reaction of cytokines and TLR receptors, it was performed with SYBR Green fluorochrome with the GoTaq® qPCR Master Mix commercial kit (Promega ®, USA). The reaction was done by mixing 10 µL GoTaq Master Mix (2x), 0.5 µL FWD primer, 0.5 µL REV primer, 1 µL cDNA (25 ng), and nuclease-free water to obtain a final reaction volume of 20 µL. The reactions were performed in 96-well PCR® Microplate plates (Axygen SCIENTIFIC®, California USA), covered with Platemax® UltraClear Sealing Film (Axygen Scientific®, California, USA). Reactions were done in triplicate.

Statistical Analysis Used

Statistical analysis was performed using the GraphPad Prism Version 4.0 program. For the analysis of gene expression and Int Dent, t-tests and one-way ANOVA were used with a comparison of means by the Bonferroni test, a value of P≤0.05 was considered significant.

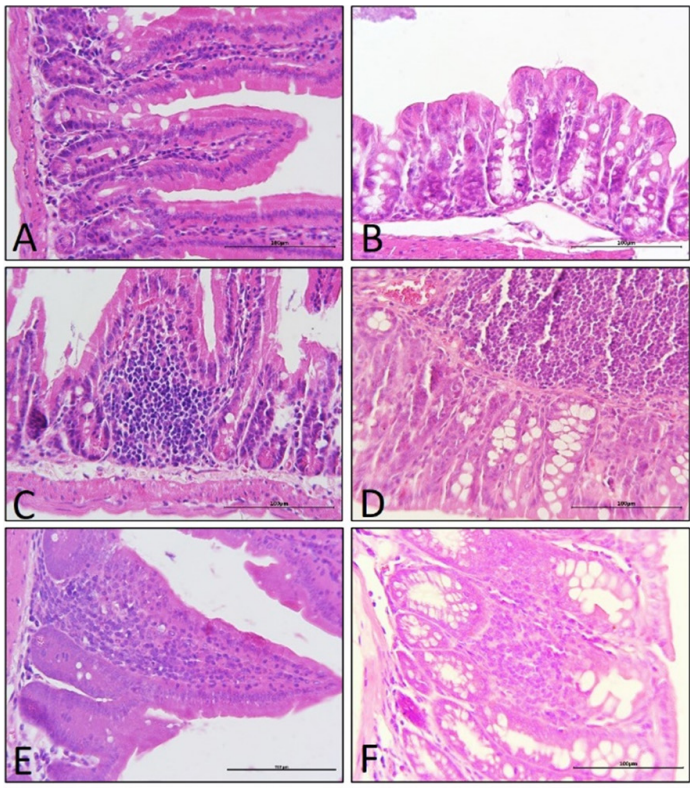

Figure 1: Effect of CBD/BS on the small and large intestinal tissues stained with HandE of LPS-treated BALB/c mice; **A:** Control WT group: small intestine. 100x; **B:** Control WT group: large intestine. 100x. **C:** LPS treated group: small intestine. 400x; **D:** LPS treated group: large intestine. 400x; **E:** LPS-CBD/BS treated group: small intestine. 400x; **F:** LPS-CBD/BS treated group: large intestine. 400x.

RESULTS

Effect of LPS and CBD/BS in Large and Small Intestine

In the small and large intestines, the stimulation of mice with lipopolysaccharide was analyzed at a histological level; and at an mRNA expression level, by RT-qPCR using

the 2-$^{\Delta\Delta}$CT method. The effect of lipopolysaccharide in these tissues was mainly characterized by higher numbers of mononuclear cell infiltrate and lymphoid aggregates in lamina propria, especially in the large intestine and zones of localized hemorrhage (Figure 1); as well as higher mRNA levels of pro-inflammatory cytokines such as IL-6 and IL-1β; and some mainly numerical increments in TLR-2 and TLR-4 (Figure 2).

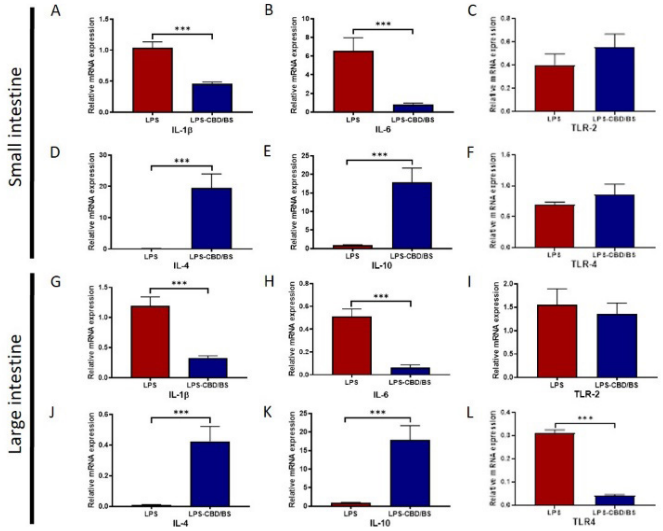

Figure 2: Effect of LPS and CBD/BS on BALB/c mice small and large intestinal tissue relative expression of inflammatory cytokines. RT-qPCR results were analyzed by the 2-$^{\Delta\Delta}$CT method. Statistical differences are marked with * when p<0.05, ** when p<0.01, *** when p<0.001.

Treating LPS-stimulated mice with CBD/BS showed to reduce mononuclear cell infiltrate and reduce the magnitude of lymphoid aggregates (Figures 1E and 1F); as well as reducing mRNA IL-1β and IL-6 (Figures 2A, 2B, 2G and 2H); and to enhance mRNA of IL-4 and IL-10 (Figures 2D, 2E, 2J and 2K). Interestingly, it showed to regulate TLR-4 in the large intestine (Figure 2L), but it did not have a significant effect on the small intestine or mRNA TLR-2 in both tissues (Figures 2C, 2F and 2I).

When analyzing PPARα expression patterns in the intestinal tissue of mice through the immunohistochemistry technique, we observed that the DAB-substrate's oxidation reaction staining linked to PPARα protein was merely restricted to the cytoplasm of lamina propria-associated cells (Figure 3), and an extranuclear localization of PPARα protein (Figures 3C and 3D), that was appreciable in the analyzed intestinal tissues of all experimental groups.

Thereafter, in experimental groups treated with lipopolysaccharide, we observed patterns linkable to the inflammatory process (Figure 4). PPARα protein localization by immunohistochemistry showed cytoplasmatic staining of intestinal lamina propria-associated cells and lymphoid aggregates (Figures 4C and 4E), being more evident in animals treated with LPS (Figure 4A, 4B, 4C and 4F). LPS-treated groups that received CBD/BS treatment showed a considerable decrease in PPARα immunohistochemistry positivity, consistently with the reduction of the inflammatory process in these tissues (Figures 4G, 4H, 4I and 4J).

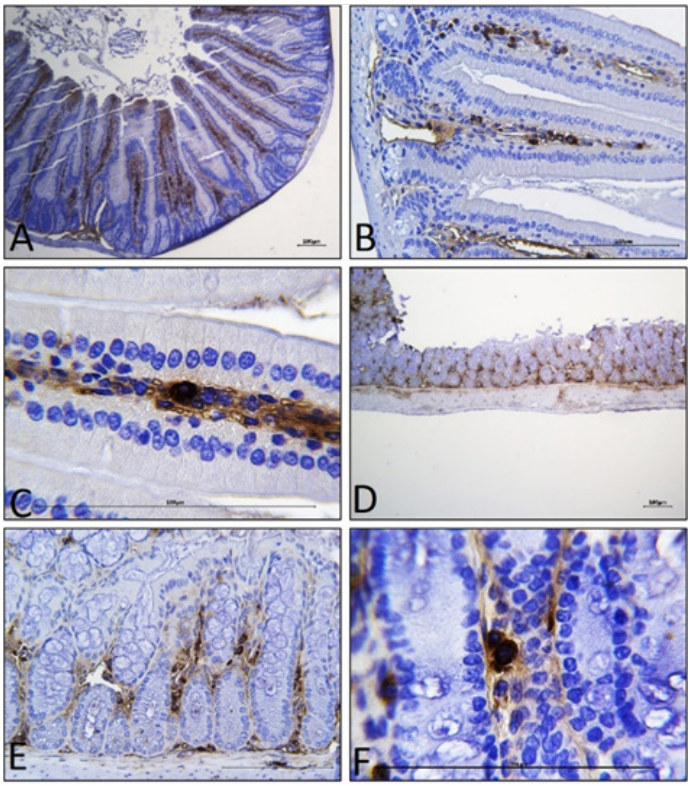

Figure 3: PPARα protein immunolocalization in control groups; **A:** Small intestine: 100x; **B:** Small intestine: 400x; **C:** Small intestine: 1000x. Large intestine: 1000x; **D:** Large intestine: 100x; **E:** Large intestine: 400x; **F:** Large intestine: 1000x.

Analyzing the PPARα mRNA and protein presence by RT-qPCR and immunohistochemistry, respectively, LPS-treated mice showed higher levels of mRNA PPARα and protein staining and quantification levels, compared to mice treated with LPS-CBD/BS or a control group. When treated with CBD/BS, small and large intestines showed no alterations in mRNA PPARα expression, but at a protein level by IHC, it showed to decrease PPARα immunostaining at lamina propria-associated cells, especially in the large intestine (Figure 5).

DISCUSSION

In the present study, we analyzed the effects of a broad-spectrum cannabidiol-enriched cannabis oil (denominated in this work as CBD/BS) on PPARα expression and inflammation in the intestinal tissue of mice treated with lipopolysaccharide. Physiological effects of cannabidiol and other cannabinoids are mediated by several targets such as

members of the superfamilies of the G-protein coupled receptors, transient receptor potential channels, serotonin receptors, PPARs, etc., (Di Marzo and Piscitelli, 2015).

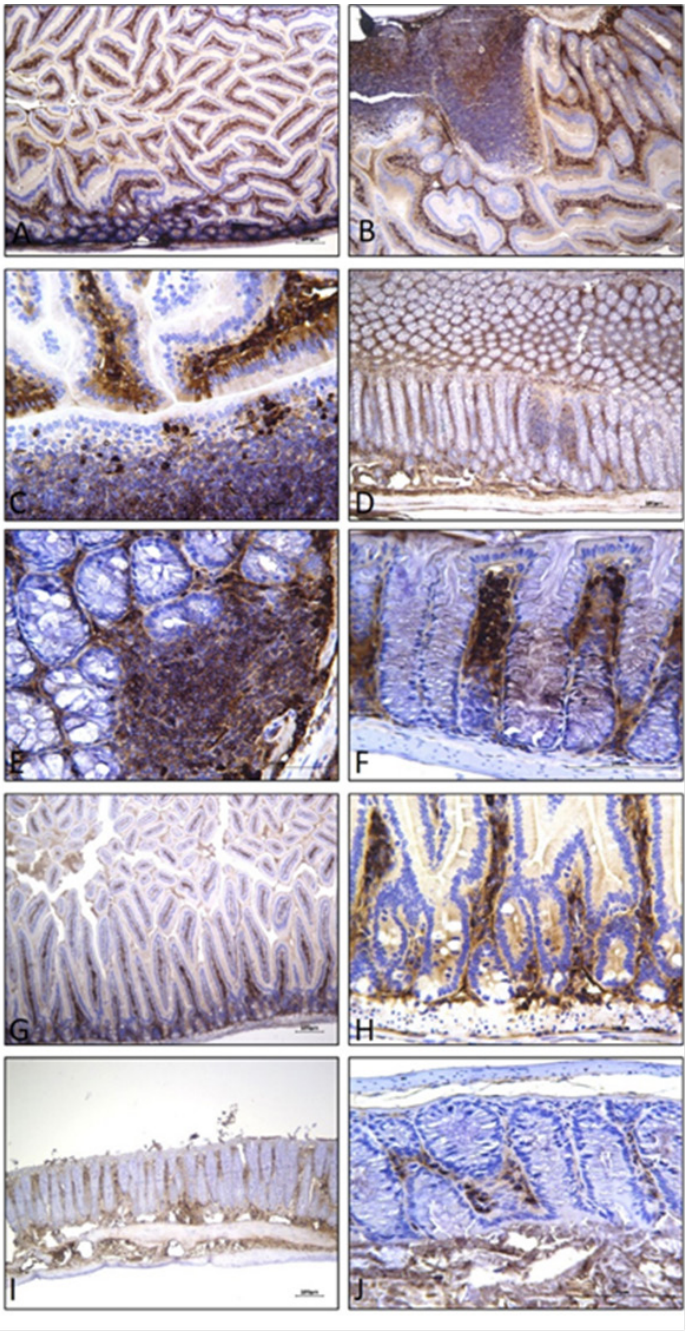

Figure 4: Effect of LPS on BALB/c mice small and large intestinal tissues with anti-PPARα protein immunostaining; **A:** LPS treated group: small intestine. 100x; **B:** LPS treated group: small intestine lymphoid aggregates. 100x. **C:** LPS treated group: small intestine. 400x; **D:** LPS treated group: large intestine. 100x; **E:** LPS treated group: large intestine lymphoid aggregates. 100x; **F:** LPS treated group: large intestine. 400x; **G:** LPS-CBD/BS treated group: small intestine. 100x; **H:** LPS-CBD/BS treated groups: small intestine. 400x; **I:** LPS-CBD/BS treated group: large intestine. 100x; **J:** LPS-CBD/BS treated group: large intestine. 400x.

We found that the administration of CBD/BS helped to reduce an inflammatory stimulus evoked by the intraperitoneal administration of lipopolysaccharide from *Salmonella typhimurium* in intestinal tissue. Cannabinoid capacity to modulate inflammation happens through several mechanisms. Some mechanisms described by other reports involve apoptosis induction, preventing cell proliferation, reducing cytokine production, and enhancing T-regulatory cells for anti-inflammatory activity, among others (Suryavanshi *et al*., 2021). PPARα is a nuclear transcription factor with affinity to cannabinoid ligands (D'Aniello *et al*., 2019) whose transcriptional activity has been linked to inflammatory processes, in which PPARα and PPARγ, as well as other cannabinoid receptors, had shown increment in their levels of expression (Couch *et al*., 2019; D'Aniello *et al*., 2019). We found that after treating mice with lipopolysaccharide, the expression of mRNA PPARα as well as its protein levels in cells associated with lamina propria, increased. In other reports, increments in PPARα's expression had been mentioned when administering lipopolysaccharide. Its role in the inflammatory process becomes evident when the mechanism is blocked with a knockout gene (KO) for PPARα, where inflammatory intensity increases (Korbecki *et al*., 2019). Even though CBD/BS treatment failed to modulate mRNA PPARα, it showed to influence its protein levels in IHC-analyzed intestinal tissues. The three PPAR isoforms (α, β/δ and γ) possess anti-inflammatory activities. PPARα is mainly expressed in CD45+ leukocytes and innate immunity cell populations such as basophils, eosinophils, monocytes, and macrophages, and it also has been described its role in the modulation of inflammatory mediators as well as the endocannabinoid system (Grabacka *et al*., 2021).

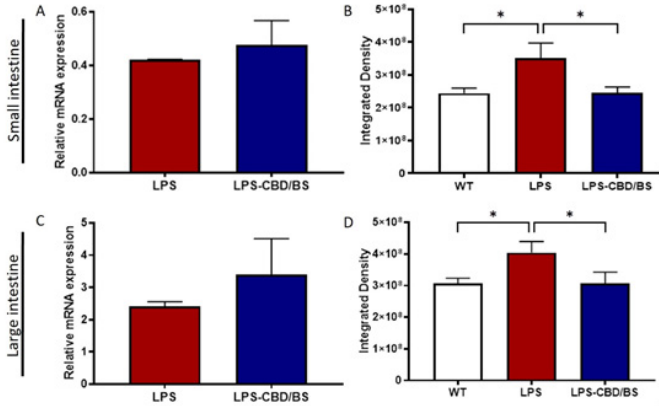

Figure 5: Effect of LPS and CBD/BS on BALB/c mice small and large intestinal tissue mRNA PPARα relative expression and protein integrated density quantification; **A:** Relative mRNA PPARα expression in the small intestine; **B:** PPARα integrated density on the small intestine; **C:** Relative mRNA PPARα expression in the large intestine; **D:** PPARα integrated density on the large intestine. Statistical differences are marked with * when p<0.05, ** when p<0.01, *** when p<0.001.

Table 3: Designed oligonucleotides.

Gen	Accession number	Sequence
PPARα	NM_001113418.1	FWD 5'-CGG TGT GTA TGA AGC CAT CT-3'
		REV 5'-TAA GGA ACT CGC GTG TGA TAA A-3'
GADPH	NM_001289726.1	FWD 5'-CCT ACT GCT GAC CTT TCT TCT-3'
		REV 5'-GCC CTG AGG ACG ATA AAC TAT AA-3'
IL-1B	NM_008361.4	FWD 5'-GGT ACA TCA GCA CCT CAC AA-3'
		REV 5'-TTA GAA ACA GTC CAG CCC ATAC-3'
IL-4	NM_021283.2	FWD 5'- TTG AGA GAG ATC ATC GGC ATT T-3'
		REV 5'-CTC ACT CTC TGT GGT GTT CTT C-3'
IL-6	NM_031168.2	FWD 5'-CTT CCA TCC AGT TGC CTT CT-3'
		REV 5'-CTC CGA CTT GTG AAG TGG TAT AG-3'
IL-10	NM_010548.2	FWD 5'-TTG AAT TCC CTG GGT GAG AAG-3'
		REV 5'-TCC ACT GCC GGT TTA TTT-3'
TLR2	NM_011905.3	FWD 5'-GGA AGA CCT TGC TGT TCT CTA C-3'
		REV 5'-CAC TAT CCG GAG GTT GCA TAT C-3'
TLR4	NM_021297.3	FWD 5'-AGT ATC GAG AGG CTC AGG TAT AG-3'
		REV 5'-TAC AGG ATG CAG GAC AAG TAA TC-3'
β-actin	NM_007393.5	FWD 5'-GAG GTA TCC TGA CCC TGA AGT A-3'
		REV 5'-CAC ACG CAG CTC ATT GTA GA-3'

In the present study, we found PPARα mainly localized in the cytoplasm of lamina propria-associated cells. Although this protein is mainly referred to as localized in cell nuclei, other reports have also found its localization by immunostaining in cells cytoplasm (Shao *et al.*, 2020; Umemoto and Fujiki, 2012; Winczyk and Pawlikowski, 2005). Its compartmentalization in the cytoplasm has been suggested as a mechanism to modulate its activity (Patel *et al.*, 2005). As the immunostaining for PPARα was only observed and measured in lamina propria-associated cells, which showed reduction after the administration of CBD/BS, it could not reflect the total content of PPARα in the intestinal tissue or changes during the inflammation process or due to the CBD/BS in other cell types and associated tissues. Our findings support the anti-inflammatory activity of cannabinoids in inflamed intestinal tissue and show a potential implication of PPARα in lamina propria cell infiltration through the course of inflammation induced by lipopolysaccharide.

CONCLUSIONS AND RECOMMENDATIONS

The present study demonstrates that oral administration of a cannabidiol-enriched broad-spectrum cannabis oil (CBD/BS) exerts anti-inflammatory effects on intestinal tissue in a murine model of LPS-induced sepsis-like inflammation. CBD/BS treatment reduced histological markers of inflammation, such as lymphoid aggregates and mononuclear infiltrates, particularly in the large intestine, and modulated the expression of key inflammatory cytokines—downregulating IL-1β and IL-6, while upregulating IL-4 and IL-10. Although no significant modulation of PPARα mRNA was observed, immunohistochemical analysis revealed a decrease in PPARα protein staining in lamina propria-associated cells, suggesting post-transcriptional regulation or cytoplasmic sequestration as a possible mechanism. These findings reinforce the potential of cannabinoids as therapeutic agents in intestinal inflammatory processes and point toward PPARα as a relevant molecular player in the modulation of intestinal immune responses. Further research should investigate the long-term effects of cannabinoid treatments and explore the precise cellular pathways involved in PPARα regulation and its interaction with the endocannabinoid system

ACKNOWLEDGMENTS

This paper is dedicated to all the medical personnel who fought against SARS-Cov-2 in the past global pandemic.

NOVELTY STATEMENTS

This study provides novel evidence on the effects of a broad-spectrum cannabidiol-enriched cannabis oil (CBD/BS) in modulating intestinal inflammatory processes in a murine model of lipopolysaccharide (LPS)-induced sepsis-like inflammation. Unlike previous reports, this work jointly evaluates morphological alterations, the gene expression of pro- and anti-inflammatory cytokines, and the tissue localization of PPARα protein. Notably, the treatment with CBD/BS significantly reduced PPARα immunostaining in lamina propria-associated cells, despite no changes in its mRNA expression, suggesting a possible post-transcriptional regulatory mechanism. The identification of this modulation highlights the immunomodulatory potential of cannabinoids and offers a new perspective

for therapeutic strategies targeting intestinal inflammatory diseases.

AUTHOR'S CONTRIBUTIONS

Kevin A. Cárdenas-Noriega: Conceptualization, Formal analysis, Resources, Writing - Original Draft, Writing - Review and Editing, Visualization. Adolfo Soto-Domínguez: Conceptualization, Formal analysis, Resources, Writing - Original Draft, Writing - Review and Editing, Visualization. Luis E. Rodríguez-Tovar: Conceptualization, Formal analysis, Visualization. H.R.R: Conceptualization, Formal analysis, Visualization. A.G.G: Conceptualization, Formal analysis, Visualization. **M.C**: Conceptualization, Formal analysis, Visualization. Gloria A. Guillen-Melendez: Writing - Original Draft, Writing - Review and Editing Uziel Castillo-Velázquez: Conceptualization, Formal analysis, Resources, Writing - Original Draft, Writing - Review and Editing, Visualization, funding acquisition.

Abbreviations

Commonly used abbreviations are described in Table 3.

Ethical Approval

This study was conducted on the Faculty of Veterinary Medicine and Zoothecnics at the Universidad Autónoma de Nuevo León, General Mariano Escobedo, Nuevo León, Mexico. Approval was obtained from the Bioethics and Animal Welfare Committee of the Faculty of Veterinary Medicine and Zootechnics at the Universidad Autónoma de Nuevo León (FMVZ/UANL; study number: 31/2022, dictamen 33/2022).

Consent to Publication

Not applicable.

Availability of Data and Materials

All relevant data are within the paper and will be made available on request.

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Conflict of Interest

The authors have declared that no competing interests exist.

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