Received: 15 May 2019 | Revised: 17 April 2020 | Accepted: 3 May 2020

DOI: 10.1111/odi.13413

ORIGINAL ARTICLE

ORAL DISEASES WILEY

Cannabidiol on 5-FU-induced oral mucositis in mice

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Funding information
Coordenação de Aperfeiçoamento de Pessoal de Nivel Superior –Brasil (CAPES)

Abstract

Purpose: The aim of this study was to evaluate the clinical, histological, hematological, and oxidative stress effects of cannabidiol (CBD) in mice with induced oral mucositis.

Methods: We used 90 mice of the CF-1 strain in which oral mucositis was induced using a protocol with 5-fluorouracil (5-FU) chemotherapy. The animals were divided randomly into 10 study groups. Three groups were treated with different doses of CBD (3, 10, and 30 mg/kg), while 2 were control groups (positive control: 5-FU + mechanical trauma + placebo; and negative control: mechanical trauma + placebo), and 2 experimental times were studied (4 and 7 days). All treatments were by intraperitoneal administration.

Results: In the clinical evaluation, the groups treated with CBD showed less severity of oral lesions compared with the positive control at both experimental times. The intensity of the inflammatory response was also lower in the groups treated with this drug, but there was no statistically significant difference when compared with the positive control. With regard to erythrocyte, leukocyte, and platelet counts and anti-oxidant enzyme activity, the groups treated with CBD showed better results, but only some of these variables showed statistically significant differences.

Conclusions: CBD seems to exert an anti-inflammatory and anti-oxidant activity favoring a faster resolution of oral mucositis in this animal model.

KEYWORDS

5-FU chemotherapy, anti-oxidant enzymes, cannabidiol, CAT, erythrocyte count, GSH, inflammatory score, mice, mucositis classification, oral mucositis, platelet count, white blood cell count

1 | INTRODUCTION

Oral mucositis (OM) is a common complication of chemotherapy (CT). Its occurrence affects 60%–80% of patients under this anti-neoplastic treatment (Peng et al., 2017; Reyes-Gibby et al., 2017; Sonis & Villa, 2018). It is a debilitating condition that sets in around 5–7 days after administration of CT and persists for days to weeks. It is an inflammatory response and presents clinically with areas of ulceration of the mucosa, in varying degrees of severity. It is accompanied by painful symptomatology and may result in dysphagia, weight

loss, malnutrition, and susceptibility to opportunistic infections. It affects the patient's quality of life and may become a dose-limiting factor in treatment (Koohi-Hosseinabadi et al., 2017; Reyes-Gibby et al., 2017; Sonis & Villa, 2018).

5-Fluorouracil belongs to the class of anti-metabolite anti-cancer drugs capable of interfering with essential biochemical processes. Generally, it induces cell death during the S phase of cell growth. Its main mechanism of action is inhibition of thymidylate synthase, which leads to depletion of dTTP pools and subsequent DNA synthesis (Yin et al., 2018). It is widely used in the treatment of various solid malignancies such as those of the gastrointestinal tract, head and neck, and other anatomical sites. 5-FU therapy is associated with a wide range of adverse effects, including gastrointestinal mucositis, and is one of the chemotherapeutic agents with the greatest association with the incidence of OM (Al-Dasooqi et al., 2013; Focaccetti et al., 2015; Kalpadakis et al., 2007; Lalla et al., 2014; Rtibi et al., 2018).

Lesions begin when the oral mucosa is exposed to chemotherapeutic drugs, resulting in DNA damage and cell death, mainly through the production of reactive oxygen species and oxidative stress (OS). Various reports demonstrate that oral mucosal lesions can be attenuated and even prevented by agents able to limit OS caused by free radicals. These agents are called anti-oxidants, which act by neutralizing ROS by blocking their formation or by eliminating them from the body. Their role is to protect healthy tissue cells against the oxidizing action of free radicals (Al-Dasooqi et al., 2013; Sonis, 2004, 2010, 2011; Sonis & Villa, 2018; Villa & Sonis, 2016; Yuan & Sonis, 2014).

Cannabidiol (CBD) is a component of *Cannabis sativa* (marijuana), a plant that exerts potent anti-inflammatory, immunomodulatory, anti-oxidant, anti-emetic, and analgesic effects through the activation of cannabinoid receptors (CB1 and CB2) located in the central nervous system and immune cells (Lafaye, Karila, Blecha, & Benyamina, 2017; MacCallum & Russo, 2018). Studies have reported that CBD does not have psychotropic activity and exerts beneficial effects in neuropsychiatric diseases and inflammatory disorders (Booz, 2011; Burstein, 2015; Cassol-Jr et al., 2010; Cuba, Salum, Cherubini, & Figueiredo, 2017; Elbaz et al., 2015; Pan et al., 2009; Rajesh et al., 2010; Yang et al., 2014). In Wistar male rats, CBD showed an anti-inflammatory action in the healing process in trauma-induced lesions (Klein et al., 2018) and a decrease in local bone loss and lower NF-kB expression in induced periodontitis (Napimoga et al., 2009). These results suggest that CBD can be useful in the control of inflammation caused by induced periodontal disease in rats. The possibility of obtaining similar results in study models of OM can advance research in this area, aimed at obtaining favorable clinical effects. On the basis of these premises, it is believed that CBD can be considered an alternative in the treatment of CT-induced OM.

In view of the biological complexity of the pathogenesis of OM and considering its severity and consequences in the cancer patient, the objective of this study was to evaluate the clinical, histological, hematological, and oxidative stress effects of CBD in mice with OM in the ventral tongue induced by 5-FU.

2 | MATERIALS AND METHODS

2.1 | Animal model

The sample consisted of 90 male mice of the CF-1 strain, 10 weeks old, weighing 30–40 g, obtained from the Center for Experimental Biological Models of the Pontifical Catholic University of Rio Grande do Sul (CeMBE, PUCRS). The animals were housed and acclimatized in the CeMBE, where they were kept in plastic cages identified according to their respective groups (9/group; 5/cage), which were randomly selected. During the experiment period, they were provided with feed and filtered water ad libitum. The cages were lined with autoclaved wood shavings and placed in micro-isolators with constant temperature of 23 ± 1°C, relative humidity of 50 ± 5%, and 12-hr light–dark cycle (lights on at 0700 hr, lights off at 1900 hr).

This research was carried out in accordance with the ethical principles in force for the use of laboratory animals, established by the National Council for Control of Animal Experimentation, and its protocol was approved by the Scientific Committee of the Dentistry Course of PUCRS and the Ethics Committee on the Use of Animals of PUCRS (CEUA No. 1500488).

2.2 | Study groups and experimental times

The animals were identified by their tail as to which group they belonged. The distribution of study groups was according to product used and dose and experimental time, according to the following scheme (Figure 1).

2.3 | Products used

- 5-Fluorouracil (5-FU) (Fauldfluor, 2.5 g/50 ml; Libbs Farmacêutica Ltda, São Paulo, Brazil)

Time:	Groups:
(a) 4 days	1A: 5-FU + MECHANICAL TRAUMA + 3 mg/kg CBD
	2A: 5-FU + MECHANICAL TRAUMA + 10 mg/kg CBD
	3A: 5-FU + MECHANICAL TRAUMA + 30 mg/kg CBD
	4A: 5-FU + MECHANICAL TRAUMA + placebo*#
	5A: MECHANICAL TRAUMA + placebo*##
(b) 7 days	1B: 5-FU + MECHANICAL TRAUMA + 3 mg/kg CBD
	2B: 5-FU + MECHANICAL TRAUMA + 10 mg/kg CBD
	3B: 5-FU + MECHANICAL TRAUMA + 30 mg/kg CBD
	4B: 5-FU + MECHANICAL TRAUMA + placebo*#
	5B: MECHANICAL TRAUMA + placebo*##

FIGURE 1 Distribution of study groups according to the product used, dose, and experimental time

- Ketamine (Dopalen, 1 g/1 ml; Ceva Saúde Animal Ltda, São Paulo, Brazil)
- Xylazine (Anasedan, 2 g/100 ml; Ceva Saúde Animal Ltda, São Paulo, Brazil)
- Cannabidiol, 99.9% (THC Pharm GmbH, Frankfurt, Germany)
- Tween 80 (oleic acid, ≥58%—Sigma, São Paulo, Brazil): used as solvent for CBD.
- Saline (0.9% sodium chloride): used as vehicle.

*Placebo: Tween 80 in saline (0.2 ml)

2.4 | Induction of OM and treatments

The OM induction protocol consisted of two intraperitoneal (IP) injections of 5-FU (60 mg kg^{-1} day^{-1}) on days zero and 2 only, based on previously published studies (Aras, Sezer, Erkilic, Demir, & Dagli, 2013; Ottaviani et al., 2013; Skeff et al., 2014). Groups 5A and 5B were not subjected to this 5-FU administration. However, the entire sample of this group was handled in a similar way as the others and received IP injection of saline.

On the 3rd and 4th days of the experiment, the animals were anesthetized with 100 mg/ml ketamine hydrochloride (100 mg/kg) and 20 mg/ml xylazine hydrochloride (10 mg/kg). Mechanical trauma was induced in the middle third of the ventral surface of the tongue with the use of an 18G needle, scraping twice with a linear movement of 5 mm each, to induce OM. This technique, with some modifications, has been extensively used to induce OM similar to that occurring in humans (Aras et al., 2013; Ottaviani et al., 2013; Skeff et al., 2014). Immediately after the mechanical trauma on the 4th day, the animals began to receive CBD treatment determined for each group, IP every 24 hr. Euthanasia in the respective groups and times of the study was performed by means of deep anesthesia with isoflurane on the 8th and 11th days of the experiment, that is, after 4 and 7 days of treatment (Figure 2).

2.5 | Hematological analysis

Prior to euthanasia, the animals were anesthetized by inhalation of isoflurane and blood samples were then collected by cardiac puncture (0.25 ml). Whole blood samples were placed in glass vials containing 0.5 μl of EDTA, which were then sealed and labeled.

Hematological analyses were performed at CeMBE with whole blood samples placed in tubes containing 10% sodium EDTA, using a veterinary automated hematological analyzer (Sysmex pocH-100iV Diff; Roche, São Paulo, Brazil). Erythrocyte, total leukocyte, and platelet counts were performed.

2.6 | Clinical and histological evaluation

Immediately after euthanasia, each animal was clinically evaluated by 2 blinded examiners in the region of the tongue subjected to trauma, to determine the absence or presence of the induced lesion, to measure the extent of the lesion with a periodontal probe, and to classify the presence and severity of OM according to the following adapted classification (Ottaviani et al., 2013; Patel, Biswas, Shoja, Ramalingayya, & Nandakumar, 2014; Skeff et al., 2014):

1. normal
2. erythema
3. epithelial desquamation
4. ulcer on up to 25% of surface
5. ulcer on more than 25% up to 50% of surface
6. ulcer on more than 50% up to 75% of surface
7. ulcer on more than 75% of surface

After the clinical analysis, the tongue was surgically removed from each animal. It was fixed in 10% formalin for 24 hr, where the longitudinal section of the ulcer area was in the central portion of the tongue. The specimens were embedded in paraffin, and three consecutive 3-μm-thick sections were cut for each specimen. Slides were prepared and stained with hematoxylin and eosin (HE).

The analysis was performed using an Olympus binocular microscope (Model BX50). A calibrated and blinded examiner evaluated entirely all the sections obtained at magnifications ranging from 100× to 400×. Intra-examiner calibration was done by reanalysis of 20 slides with a 7-day interval between observations (kappa = 0.889 ± 0.061, $p < .001$). Next, the field with the highest

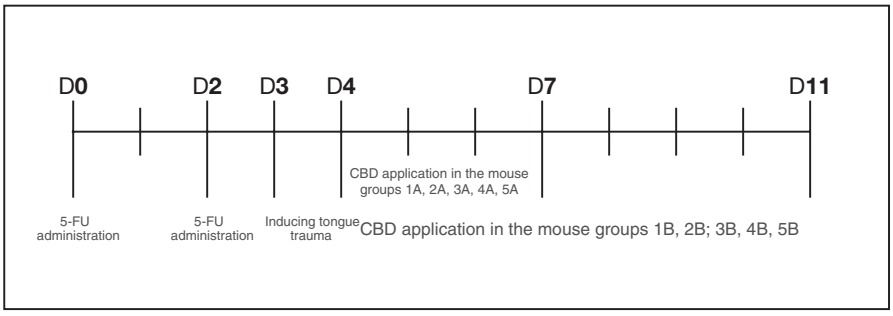


FIGURE 2 Time line of study procedures from day 0 (D0) until day 11 (D11). The 4 days represent the period from D4 to D7 and 7 days from D4 to D11

intensity of inflammatory response was chosen to determine the score, according to the criteria below (Figueiredo, Pesce, Gioso, & Figueiredo, 2001; de Freitas Cuba, Braga Filho, Cherubini, Salum, & Fig ueiredo, 2016; Klein et al., 2018):

1. Absent: absence of inflammation
2. Mild: sparse mononuclear cells
3. Moderate: mononuclear infiltrate and/or sparse neutrophils and eosinophils
4. Intense: polymorphonuclear infiltrate of neutrophils and eosinophils

2.7 | Biochemical analysis of oxidative stress

For OS evaluation, the liver was surgically removed from each selected animal in the day 4 or day 7 groups. The organs were placed in an Eppendorf tube and stored at −80°C. The biochemical analysis was performed at the Toxicology and Pharmacology Research Center, College of Health Sciences, Pontifical Catholic University of Rio Grande do Sul, where the anti-oxidant enzyme catalase (CAT) and anti-oxidant reduced glutathione (GSH) were determined using a Cary 100 spectrophotometer.

2.8 | Statistical analysis

The data were tabulated and evaluated using SPSS 17.0 software. ANOVA followed by Tukey's test was used for comparisons regarding weight loss, erythrocytes, leukocytes, platelets, CAT, and GSH. The Kruskal–Wallis test was used for analysis of the inflammatory response and classification of OM between the groups, complemented by the Student–Newman–Keuls test. The Mann–Whitney test was used in the analysis of inflammatory response and comparison of OM between the study times. The significance level was set at 5% for all analyses.

3 | RESULTS

3.1 | Weight alterations in mice

During the experiment, 11 animals died. Therefore, 79 mice were included in the study with the following distribution:

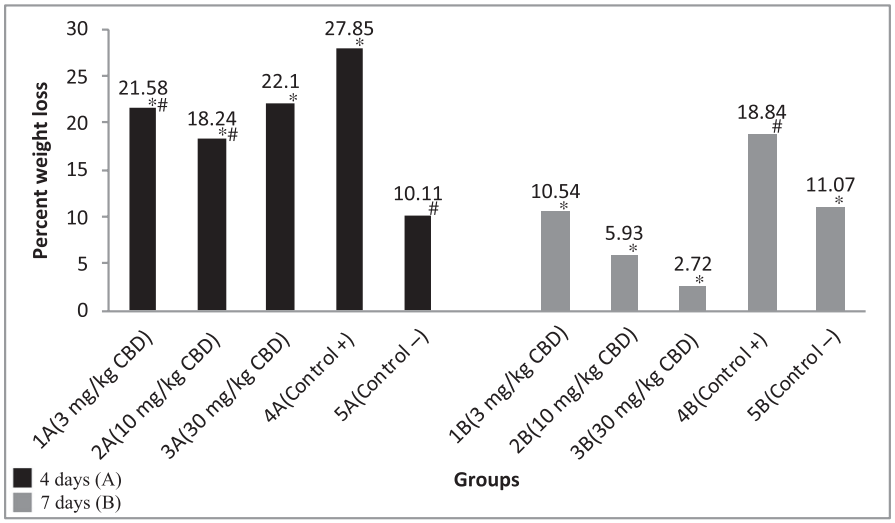
4 days: 1A—3 mg/kg CBD (n = 8), 2A—10 mg/kg CBD (n = 7); 3A—30 mg/kg CBD (n = 9); 4A—positive control (n = 9); and 5A—negative control (n = 7)

7 days: 1B—3 mg/kg CBD (n = 9); 2B—10 mg/kg CBD (n = 7); 3B—30 mg/kg CBD (n = 5); 4B—positive control (n = 9); and 5B—negative control (n = 9).

All animals lost weight during the experiment, as shown in Figure 3.

Among the groups that received CT, there was no statistical difference in the experimental time of 4 days (mean weight loss (g) ± *SD*: 1A = 8.25 ± 2.49; 2A = 7.14 ± 3.72; 3A = 8.11 ± 3.06; 4A = 11.11 ± 1.36) (p = .135). However, the greatest weight loss was observed in group 4A animals (positive control; mean weight loss (g) ± *SD* = 11.11 ± 1.36), which differed significantly from group 5A (negative control; mean weight loss (g) ± *SD* = 3.71 ± 3.86) (p = .001). Among the groups treated with CBD, 2A (10 mg/kg CBD; mean weight loss (g) ± *SD* = 7.14 ± 3.72) showed less weight loss, being statistically similar to group 5A (negative control; mean weight loss (g) ± *SD* = 3.71 ± 3.86) (p = .326).

In the 7-day evaluation, all groups treated with CBD showed lower weight loss than the control groups (mean weight loss (g) ± *SD*: 1B = 4.14 ± 1.9; 2B = 2.43 ± 2,23; 3B = 1.00 ± 0.71; 4B = 7.56 ± 1.94; 5B = 3.11 ± 2.15), although there was a statistically significant difference only in relation to group 4B (positive control; mean weight loss (g) ± *SD* = 7.56 ± 1.94) (p = .000), showing no significant difference compared with group 5B (negative control; mean weight loss (g) ± *SD* = 3.11 ± 2.15) (p = .371). The least weight loss was observed in group 3B (30 mg/kg CBD; mean weight loss (g) ± *SD* = 1.00 ± 0.71).



*#: Different symbols indicate statistically significant difference between the groups

FIGURE 3 Percent weight loss of study groups at 4 and 7 days

In the comparison between times, it was observed that the greatest weight loss occurred in the 4-day analysis. But at 7 days, there was considerable weight gain, in which only the animals of groups 5A and 5B (negative control: 5A = 3.71 ± 3.86; 5B = 3.11 ± 2.15) (p = .655) did not show a statistically significant difference.

3.2 | Clinical evaluation of OM

The specimens from the negative control groups (5A and 5B) were excluded since they did not receive 5-FU, so the lesions present could not be considered OM. The induced lesion in the negative control groups was found in all animals at 4 days and was completely healed in the day 7 analysis (Figure 4). The classification of the OM lesions is described in Table 1.

In the day 4 clinical analysis, all animals displayed some degree of OM, ranging from grade 3 to grade 5. However, the severity of the lesions was greater in group 4A (positive control; mean rank = 25.6), differing statistically from the others (mean rank: 1A = 15.1; 2A = 12.35; 3A = 13.6) (p = .006). When comparing the groups treated with CBD, there was no statistical difference between them (1A and 2A; p = .580; 1A and 3A; p = .756; 2A and 3A p = .788). In the 7-day experimental period, there was a decrease in the severity of OM in all groups that received CBD treatment (mean rank: 1B = 9.8; 2B = 13.3; 3B = 12.0), ranging from grade 0 to grade 3, but no statistically significant difference was found between these groups (1B and 2B; p = .419; 1B and 3B p = .659; 2B and 3B p = .792), but differing statistically from the positive control group (positive control; mean rank = 24.8) (p = .001). In animals from groups 4A and 4B (positive control), lesion severity remained unchanged or worsened in most animals, with slight improvement in two animals, varying from grade 3 to grade 6; however, no statistical significance was observed between experimental times in this group (mean rank: 4A = 25.6; 4B 24.8) (p = .611) (Figure 4). We observed an improvement in the lesions over time in all the groups that received CBD treatment, but this was statistically significant only between groups 1A and 1B (3 mg/kg CBD) (p = .002) and 3A and 3B (30 mg/kg CBD) (p = .029).

3.3 | Histological analysis of inflammatory response

The intensity of the inflammatory response varied from mild to severe (Figure 5) in all groups that received 5-FU in the 4-day analysis, with no statistically significant difference between these groups (mean rank: 1A = 23.3; 2A = 23.7; 3A = 18.9; 4A = 20.3) (p = .763). However, at the same experimental time, the negative control group (mean rank: 5A = 16.0) displayed mild-to-moderate inflammation but not significantly different compared with the other study groups (p = .643).

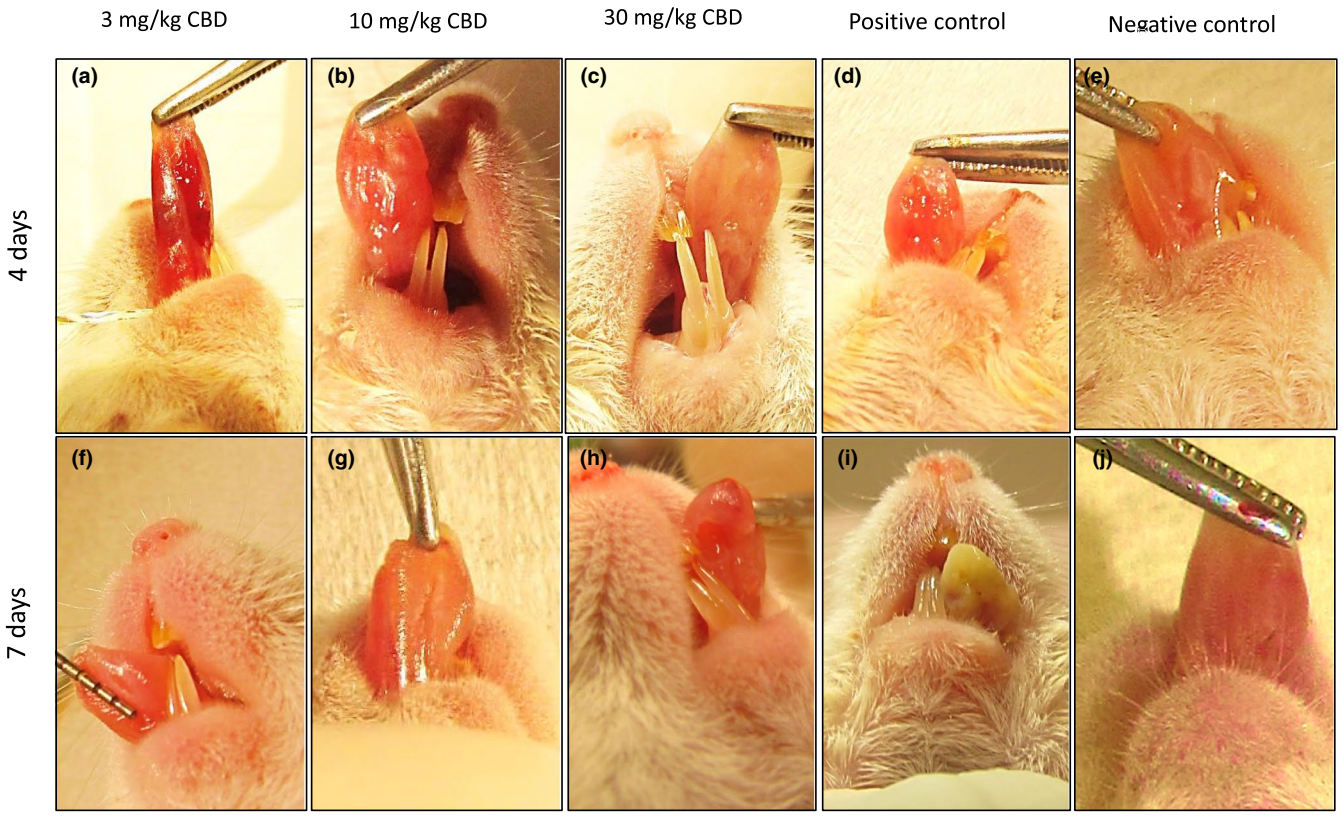


FIGURE 4 Clinical evaluation of OM. (a) Animal in group 1A immediately after mechanical trauma. (b) Animal in group 2A showing grade 3 OM. (c) Animal in group 3A showing grade 4 OM. (d) Animal in group 4A showing grade 5 OM. (e) Animal in group 5A. (f) Animal in group 1B showing a discrete ulcerated lesion. (g) Animal in group 2B showing grade 3 OM. (h) Animal in group 3B showing grade 3 OM. (i) Animal in group 4B showing grade 6 OM. (j) Animal in group 5B [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Comparison of classification of induced OM in relation to study group that received chemotherapy (Kruskal–Wallis and Student–Newman–Keuls test)

	Time							
	4 days				7 days			
Classification of OM	1A (*n* = 8)	2A (*n* = 7)	3A (*n* = 9)	4A (*n* = 9)	1B (*n* = 9)	2B (*n* = 7)	3B (*n* = 5)	4B (*n* = 9)
0	–	–	–	–	7	3	3	–
1	–	–	–	–	–	1	–	–
2	–	–	–	–	–	–	–	–
3	5	6	6	1	2	3	2	3
4	2	–	3	2	–	–	–	1
5	1	1	–	6	–	–	–	4
6	–	–	–	–	–	–	–	1
p value*			.006				.001	
*Mean rank***	15.1^{B}	12.3^{B}	13.6^{B}	25.6^{A}	9.8^{B}	13.3^{B}	12.0^{B}	24.8^{A}

Note: 1 = 3 mg/kg CBD; 2 = 10 mg/kg CBD; 3 = 30 mg/kg CBD; 4 = placebo.

Abbreviations: CBD, cannabidiol; OM, oral mucositis.

*Comparison between treatment and control.

**Rank means followed by different letters are significantly different.

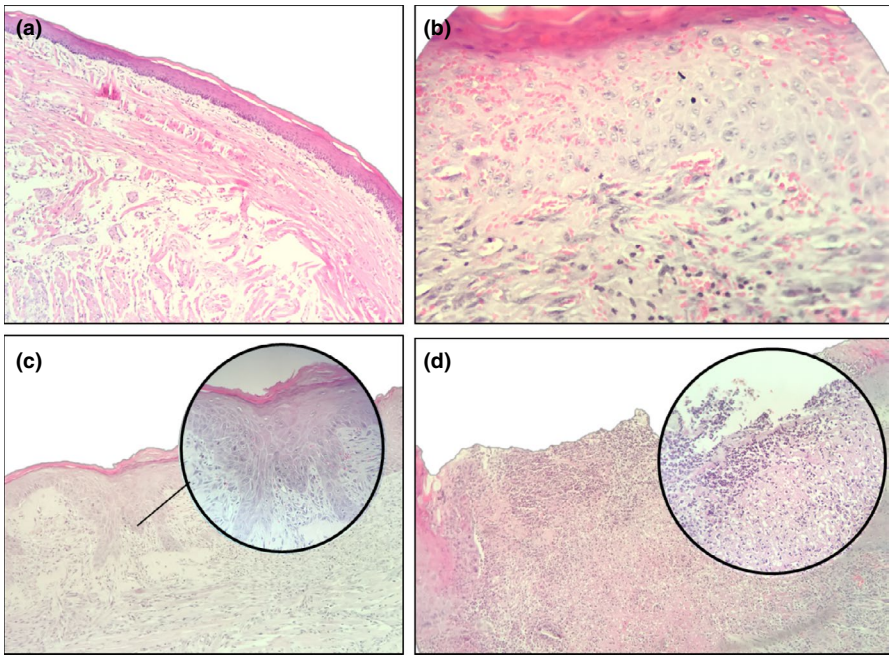


FIGURE 5 (a) Photomicrograph showing sparse mononuclear cells, characterizing mild inflammation (HE, 100×). (b) Photomicrograph showing the presence of inflammatory infiltrate of mononuclear cells with neutrophils and sparse eosinophils, characterizing moderate inflammation with the presence of epithelial ulceration (HE, 400×). (c) Photomicrograph showing remodeling of the epithelium in the ulcer area (HE, 100×). (d) Photomicrograph showing the presence of inflammatory infiltrate of polymorphonuclear cells, characterizing intense inflammation, edema, and ulceration (HE, 100×) [Colour figure can be viewed at wileyonlinelibrary.com]

In the 7-day analysis, the inflammatory response scores remained between mild and intense in groups 1B (3 mg/kg CBD), 2B (10 mg/kg CBD), 3B(30 mg/kg CBD), and 4B (positive control) with no significant difference (mean rank: 1B = 22.6; 2B = 18.2; 3B = 30.5; 4B = 25.5) (p = .095), while most of group 5B (negative control) animals showed scores reduced to mild (mean rank: 5A = 7.3) (p = .003).

Progression of the severity of the inflammatory response was observed in all groups that received 5-FU in the 7-day period in relation to the 4-day analysis. However, this difference was statistically significant only between groups 3A and 3B (30 mg/kg CBD) (p = .004) and 4A and 4B (positive control) (p = .05). The classification of the inflammatory response is described in Table 2.

3.4 | Hematological and biochemical analysis

Table 3 presents the results of the erythrocyte, leukocyte, and platelet counts and CAT activity and GSH levels according to the study times and groups. In the 4-day period, the positive control group (4A) showed lower values than the other groups for all variables; however, there was a statistically significant difference in erythrocyte count in relation to group 1A (3 mg/kg CBD) (mean ± *SD*: 1A = 10.29 ± 1.84; 4A = 7.69 ± 0.26) (p = .001), in leukocyte count in relation to groups 2A (10 mg/kg CBD) (mean ± *SD*: 2A = 5.68 ± 2.28; 4A = 2.84 ± 0.59) (p = .001) and 5A (negative control) (mean ± *SD*: 5A = 6.85 ± 1.23; 4A = 2.84 ± 0.59) (p = .000), and in platelet count only in relation to group 5A (mean ± *SD*: 5A = 1,283.43 ± 139.1; 4A = 830.11 ± 99.0)

TABLE 2 Comparison of inflammatory response in relation to study group (Kruskal–Wallis and Student–Newman–Keuls test)

	4 days					7 days				
Inflammatory response	1A (*n* = 8)	2A (*n* = 7)	3A (*n* = 9)	4A (*n* = 9)	5A (*n* = 7)	1B (*n* = 9)	2B (*n* = 7)	3B (*n* = 5)	4B (*n* = 9)	5A (*n* = 9)
Mild	3	2	3	3	3	1	1	–	–	7
Moderate	1	2	5	4	4	3	4	–	3	2
Intense	4	3	1	2	–	5	2	5	6	–
p value*				.643					.003	
*Mean rank***	23.3^{A}	23.7^{A}	18.9^{A}	20.3^{A}	16.0^{A}	22.6^{A}	18.2^{AB}	30.5^{A}	25.5^{A}	7.3^{B}

Note: 1 = 3 mg/kg CBD; 2 = 10 mg/kg CBD; 3 = 30 mg/kg CBD; 4 = placebo; 5 = without CT.

Abbreviations: CBD, cannabidiol; CT, chemotherapy; OM, oral mucositis.

*Comparison between groups

**Rank means followed by different letters are significantly different

TABLE 3 Comparison of hematological and oxidative stress response in relation to study group and time (ANOVA and Tukey's test)

Groups/times	1 (Mean ± *SD*)**	2 (Mean ± *SD*)**	3 (Mean ± *SD*)**	4 (Mean ± *SD*)**	5 (Mean ± *SD*)**
Erythrocytes					
A (*n* = 8)	10.24 ± 1.84^{Aa}	9.4 ± 0.69^{ABa}	8.09 ± 1.65^{Ba}	7.69 ± 0.26^{Ba}	9.31 ± 0.75^{ABa}
B (*n* = 9)	7.32 ± 1.72^{Ab}	7.85 ± 0.12^{ABb}	6.44 ± 0.78^{Aa}	7.11 ± 0.30^{Ab}	8.50 ± 0.47^{Ba}
p value*	.004	.002	.06	.000	.072
Leukocytes					
A (*n* = 7)	4.15 ± 1.18^{ABa}	5.68 ± 2.28^{ACa}	4.06 ± 0.62^{ABa}	2.84 ± 0.59^{Ba}	6.85 ± 1.23^{Ca}
B (*n* = 7)	7.47 ± 3.01^{Ab}	8.07 ± 2.06^{Aa}	7.36 ± 7.36^{Ab}	5.45 ± 1.59^{Ab}	5.92 ± 0.39^{Aa}
p value*	.011	.075	.017	.001	.174
Platelets					
A (*n* = 9)	994.38 ± 213.7^{ABa}	1,068.71 ± 524.7^{ABa}	1,055.11 ± 236.9^{ABa}	830.11 ± 99.0^{Aa}	1,283.43 ± 139.1^{Ba}
B (*n* = 5)	1,614.11 ± 306.8^{Ab}	1,593.86 ± 328.1^{Ab}	1,684.8 ± 373.6^{Ab}	1,643.11 ± 428.5^{Ab}	1,460.25 ± 105.8^{Aa}
p value*	.000	.044	.002	.000	.096
CAT					
A (*n* = 9)	1.41 ± 0.41^{Aa}	1.48 ± 0.45^{Aa}	1.45 ± 0.24^{Aa}	1.11 ± 0.28^{Aa}	1.32 ± 0.11^{Aa}
B (*n* = 9)	0.77 ± 0.20^{Ab}	0.72 ± 0.28^{Ab}	0.98 ± 0.25^{ABb}	1.27 ± 0.21^{BCa}	1.48 ± 0.05^{Ca}
p value*	.001	.003	.005	.206	.028
GSH					
A (*n* = 7)	12.03 ± 2.40^{Aa}	12.91 ± 1.39^{Aa}	12.19 ± 1.83^{Aa}	9.31 ± 2.09^{Ba}	11.46 ± 0.79^{ABa}
B (*n* = 9)	4.26 ± 1.24^{Ab}	3.82 ± 1.19^{Ab}	8.09 ± 3.07^{Bb}	10.67 ± 1.40^{BCa}	12.38 ± 1.64^{Ca}
p value*	.000	.000	.008	.124	.235

Note: 1 = 3 mg/kg CBD; 2 = 10 mg/kg CBD; 3 = 30 mg/kg CBD; 4 = placebo; 5 = without CT.

Abbreviations: CAT, catalase; CBD, cannabidiol; CT, chemotherapy; GSH, anti-oxidant reduced glutathione; OM, oral mucositis.

*Comparison between times.

**Means ± *SD* followed by different upper case letters differ significantly in relation to groups, while different lower case letters differ with regard to times.

(*p* = .018). For the OS variables, there were no statistically significant differences between groups in relation to CAT, but group 4A (positive control; mean ± *SD*: 1.11 ± 0.28) differed from all groups treated with CBD (mean ± *SD*: 1A = 1.41 ± 0.41; 2A = 1.48 ± 0.45; 3A = 1.45 ± 0.24) (*p* = .03; *p* = .004; *p* = .016).

In the 7-day experimental period, the analysis of hematological variables showed statistical difference only in relation to erythrocytes in comparison between controls 4B (positive) and 5B (negative) (mean ± *SD*: 5B = 8.50 ± 0.47; 4B = 7.11 ± 0.30) (*p* = .008). In the OS evaluation, significant differences were found between the positive controls (4B) and groups 1B and 2B for both variables (CAT mean ± *SD*: 4B = 1.27 ± 0.21; 1B = 0.77 ± 0.20; 2B = 0.72 ± 0.28) (*p* = .000; *p* = .000) (GSH mean ± *SD*: 4B = 10.67 ± 1.40; 1B = 4.26 ± 1.24; 2B – 3.82 ± 1.19) (*p* = .000; *p* = .000).

4 | DISCUSSION

OM is a frequent and highly debilitating side effect in patients undergoing anti-neoplastic CT, capable of compromising the prognosis and affecting the patient's quality of life. Moreover, to date, there are no effective treatments for OM, only palliative strategies (Koohi-Hosseinabadi et al., 2017; Peng et al., 2017).

The anti-oxidant, anti-inflammatory, and analgesic properties of CBD, along with its safety, were decisive factors in stirring interest in its use against OM. The effects of the administration of different concentrations of CBD were evaluated to establish a dose–response relationship, since the literature does not provide a consensus on the ideal dose for anti-oxidant and anti-inflammatory action. In previous studies, the doses tested varied from 1 to 30 mg, but none of them stood out as advantageous in relation to the others in this study (Aviello et al., 2012; Callejas et al., 2018; Cassol-Jr et al., 2010; Chen et al., 2016; Elbaz et al., 2015; Klein et al., 2018; Napimoga et al., 2009; Pan et al., 2009; Yang et al., 2014).

The induction of mucositis in animal models is technically difficult since the protocols already described in the scientific literature vary with the species, type of drug, and dose used. The time of development of the lesions and their intensity depend directly on the protocol used, which can compromise the reliability of the analyses. Thus, based on previously published studies, a protocol with 5-FU and mechanical trauma in the ventral tongue was chosen (Ottaviani et al., 2013; Skeff et al., 2014). The experimental times in this induction protocol were also determined from the existing literature; in mice, there is a peak severity of OM lesions at 4 days, followed by the beginning of the healing process at 7 days (Aras et al., 2013; Ottaviani et al., 2013; Patel et al., 2014; Skeff et al., 2014).

The choice of the animal model as well as the methods used in this study was based on the need for a rigid standardization of the analysis criteria. Thus, we decide to use the classification of OM including criteria according to percentage of ulcerated area, since macroscopic analysis does not give any dimension of symptomatology, where this limitation is minimized by the standardization of induced trauma and of clinical analysis (Ottaviani et al., 2013; Patel et al., 2014; Skeff et al., 2014).

Regarding weight loss during the experiment, this was observed mainly at 4 days rather than 7 days, where the animals gained considerable body weight again. The positive control groups showed greater weight loss at both experimental times, where this was significant in relation to the other groups at 7 days. Weight loss is a frequent consequence of anti-neoplastic treatments (Arribas et al., 2017; Lin et al., 2018).

According to Gorter, the use of CBD in cancer patients is able to stimulate appetite, reduce nausea caused by chemotherapy, and promote weight maintenance and even gain (Gorter, 1999). The anti-emetic action of this compound has been reported by several authors and related to the indirect activation of receptors associated with these stimuli (Rock et al., 2012; Rock & Parker, 2015). In a recent study published by Callejas et al., where the anti-inflammatory potential of CBD in rat intestinal disease was tested, the authors demonstrated a significant increase in body weight in the group treated with CBD compared with the others due to the reversal of the inflammatory process present (Callejas et al., 2018).

The weight loss in mice at 4 days could be attributed to feeding problems due to the severity of oral lesions seen at those days.

Considering the 11 deaths recorded, it was observed that 4 of these occurred shortly after the anesthetic procedure due to mechanical trauma. The others occurred between 6 and 9 days after QT. Therefore, it is believed that they may be related to the toxicity of the drug used and not to CBD, since in a recent systematic literature review it was found that CBD is not cytotoxic to normal cells and is considered safe for use in animals and humans. It should also be noted that the administration of CBD seems to be safe at the doses used. The literature suggests that this drug has a low adverse effect profile and that its controlled administration is safe and well-tolerated in animals and humans, as well as being non-cytotoxic to normal cells (Iffland & Grotenhermen, 2017).

In the macroscopic analysis of induced OM, the groups treated with CBD showed degrees of severity significantly lower than those of the control group at both experimental times. There was a significant decrease in the classification of the lesions in the groups that received CBD in relation to the time, while the control group in contrast did not show the positive development of OM. On the other hand, although the control groups showed higher scores at both times, the histological evaluation did not display a statistically significant difference between the groups. The intensity of the inflammatory response was inverse to the clinical findings in the comparison between the experimental times, still leaving a question to be elucidated. Some additional investigations, such as immunohistochemistry for inflammatory markers (TNF-α, IL-1-β, IL-6), histomorphometric analysis of epithelialization, and picrosirius staining, could be interesting to enrich the analysis of the effects of CBD on tissue repair.

Recent studies that have tested the use of CBD for other diseases of inflammatory origin discuss the ability of the product to modulate the immune response by decreasing the production of pro-inflammatory cytokines such as TNF-alpha, Nf-Kb, and interleukins, and the migration of neutrophils to the periphery of the damaged tissues, which would reduce the tissue inflammatory reaction (Klein et al., 2018; Napimoga et al., 2009; Rajan et al., 2016; Rajesh et al., 2010).

Interestingly, the leukopenia observed in the groups that received 5-FU in the 4-day period can explain the tissue inflammatory response being lower at the beginning of the experiment and more intense at 7 days with leukocyte recovery. Hematological analysis suggests that CBD may help reduce pancytopenia, which often occurs in patients undergoing CT. Supporting this idea, a literature review by MacCallum and Russo revealed that CBD is able to reduce the cytotoxic effects of CT (MacCallum & Russo, 2018).

CAT and GSH are endogenous anti-oxidant agents capable of eliminating free radicals. This enzyme activity and metabolite show high levels when the body is under OS (Booz, 2011; Cruz et al., 2015). The results of the analysis of these biochemical parameters indicate

that in the evaluation at 4 days, both agents were increased by CBD treatment compared with the control group. In the day 7 analysis, the activity of the markers was significantly lower in the groups treated with CBD and remained high in the positive control group. These results support clinical findings and suggest that CBD was able to modulate the OS induced by CT.

Studies have shown that CBD is capable of suppressing the production of pro-inflammatory mediators by suppressing the cellular immune response, which may be important in the treatment of several diseases of inflammatory origin. Inhibition of adenosine uptake and decreased production of some inflammatory mediators such as IFN-γ, TNF-α, IL-1β, and IL-10 appear to be crucial in the anti-inflammatory action of CBD (Borges et al., 2013; Cassol-Jr et al., 2010; Chen et al., 2016; Elbaz et al., 2015; Kozela et al., 2016; Rajan et al., 2016; Rajesh et al., 2010).

In a recent study conducted by Klein et al., CBD was tested in the treatment of ulcerative lesions of traumatic origin in an animal model with Wistar rats, in which the animals received a daily intraperitoneal injection of CBD (5 or 10 mg/kg body weight) or vehicle alone. The data suggested that CBD exerted an anti-inflammatory action in the early stages of the healing process (Klein et al., 2018).

Many investigators have noted that the action of CBD on OS may be more potent than with classic anti-oxidants, such as α-tocopherol (Cuba et al., 2017; Klein et al., 2018; Koohi-Hosseinabadi et al., 2017). Considering that the increase in free radicals, evidenced by cellular damage caused by CT, is capable of generating an imbalance in OS and the production of pro-inflammatory mediators responsible for the tissue damage in OM, suggest that CBD is capable of acting both in OS control and in the suppression of the inflammatory response, playing a protective role in relation to OM.

Recent studies point to CBD as a promising alternative to conventional cancer treatment due to its ability to inhibit the proliferation, adhesion, migration, invasion, and angiogenesis of neoplastic cells. However, the molecular mechanisms involved in these properties are not well established and they depend on which neoplasms are to be treated and their drug (Cuba et al., 2017).

The serious consequences of OM call for new investigations into new therapeutic targets. The understanding of its biopathology and initiation from the formation of free radicals have promoted a growing interest in studies relating the role of the most varied types of anti-oxidant agents in the prevention of OM. Given the promising results in several studies, the applications of CBD in this process should be highlighted as an emerging research niche considering the potential innovative effects that this substance seems to have in a variety of health conditions.

5 | CONCLUSION

The results of this study suggest the use of CBD as an alternative for the prevention and treatment of OM, since CBD was found to reduce the inflammatory process and the severity of the lesions and OS, favoring tissue repair in induced OM. It is believed that the findings obtained may support studies involving and favoring the use of CBD in different fields of medicine and dentistry.

ACKNOWLEDGMENTS

This study was funded in part by the Coordenação de Aperfeiçoamento de Pessoal de Nivel Superior—Brasil (CAPES)—Finance Code 001 and the Pontifical Catholic University of Rio Grande do Sul (PUCRS). The authors thank Dr. A. Leyva (USA) for English editing of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Letícia de Freitas Cuba: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; software; validation; visualization; writing–original draft; writing–review and editing. **Fernanda Gonçalves Salum:** Visualization; writing–original draft. **Francisco Silveira Guimarães:** Resources; visualization; writing–original draft. **Karen Cherubini:** Visualization; writing–original draft. **Ruchielli Loureiro Borghetti:** Data curation; investigation; resources. **Maria Antonia Zancanaro de Figueiredo:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; supervision; validation; visualization; writing–original draft; writing–review and editing.

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How to cite this article: Cuba LDF, Salum FG, Guimarães FS, Cherubini K, Borghetti RL, de Figueiredo MAZ. Cannabidiol on 5-FU-induced oral mucositis in mice. *Oral Dis*. 2020;26:1483–1493. https://doi.org/10.1111/odi.13413