ESCOLA DE CIÊNCIAS DA SAÚDE
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MARIANA KLEIN

EFEITO DO CANABIDIOL NO REPARO DE ÚLCERAS MECANICAMENTE INDUZIDAS NA MUCOSA ORAL DE RATOS: AVALIAÇÃO CLÍNICA E HISTOLÓGICA

Porto Alegre
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Pontifícia Universidade Católica do Rio Grande do Sul
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Orientadora: Profa. Dra. Maria Antonia Zancanaro de Figueiredo

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Porto Alegre

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“A verdadeira viagem de descobrimento não consiste em procurar novas paisagens, mas em ter novos olhos”.

(Marcel Proust)

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RESUMO

As lesões ulceradas correspondem às enfermidades mais frequentes da mucosa bucal. O dano à barreira epitelial resulta em desconforto, podendo interferir na higiene oral, mastigação, deglutição e fala. O manejo dessas lesões objetiva basicamente otimizar e acelerar o reparo tecidual, além de diminuir a sintomatologia dolorosa. No entanto, não está disponível atualmente uma alternativa considerada o padrão-ouro para o tratamento das úlceras traumáticas bucais. O canabidiol (CBD) é o principal componente não-psicomimético da *Cannabis sativa* e desempenha potentes efeitos anti-inflamatórios, antioxidantes e analgésicos em diversas condições patológicas. A presente dissertação está estruturada na forma de 2 artigos científicos. O primeiro consiste em uma revisão de literatura, cujo objetivo foi avaliar os diferentes mecanismos de ação do CBD que possam estar envolvidos no reparo de lesões ulceradas traumáticas, sugerindo o caráter promissor desta droga na terapia de distúrbios inflamatórios orais. O segundo trata de um estudo experimental desenvolvido em modelo animal, com objetivo de avaliar clínica e histologicamente o efeito da administração intraperitoneal do CBD, nas doses de 5 e 10 mg/kg, por 3 e 7 dias, no reparo de lesão ulcerada induzida no ventre lingual de 60 ratos Wistar. O tratamento com CBD foi capaz de diminuir os escores inflamatórios das lesões após 3 dias ($p < 0,05$), contudo não foi suficiente para interferir no tempo de cicatrização das úlceras. Dessa forma, concluiu-se que o CBD é capaz de modular o processo inflamatório em lesões ulceradas orais, podendo representar uma alternativa promissora no manejo dessa condição.

Palavras-chave: Canabidiol; Canabinoides; Úlceras Orais; Cicatrização; Cannabis sativa.


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ABSTRACT

Ulcerative lesions are some of the most common injuries of the oral mucosa. Damage to the epithelial barrier results in discomfort, which may interfere with oral hygiene, chewing, swallowing and speech. The management of these lesions basically aims to optimize and accelerate tissue repair, in addition to reducing its symptomatology. However, there is no option considered gold standard for the treatment of traumatic mouth ulcers. Cannabidiol (CBD) is the main non-psychomimetic component of *Cannabis sativa*. It exerts potent antiinflammatory, antioxidant and analgesic effects when tested in various pathological conditions. The present dissertation is structured in the form of 2 scientific papers. The first article consists of a literature review to evaluate the different mechanisms of action of CBD that may be involved in the repair of ulcerative lesions, suggesting that this drug is promising for the treatment of oral inflammatory disorders. The second article is a report of an experimental study conducted in an animal model, to assess the effect of 5 and 10 mg/kg CBD on the repair of an ulcerative lesion induced on the ventral tongue of 60 Wistar rats after 3 and 7 days. Treatment with CBD decreased inflammatory scores after 3 days ($p < 0.05$), but was unable to clinically influence the size of ulcerative lesions. Thus, it is concluded that CBD has an inflammation modulating effect and may represent a promising alternative in the management of oral wounds.

Keywords: Cannabidiol; Cannabinoids; Oral Ulcer; Wound Healing; Cannabis sativa

SUMÁRIO

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1. INTRODUÇÃO

As propriedades terapêuticas da *Cannabis sativa* (popularmente chamada de maconha) são conhecidas desde os tempos medievais. Preparações à base dessa planta eram usadas principalmente pelas civilizações indianas e árabes para tratamento de dor, constipação e febre (FASINU et al., 2016; PISANTI et al., 2017). Na década de 80, a descoberta de receptores específicos, capazes de interagir com os compostos químicos da planta no organismo de mamíferos, resultou no aumento do interesse científico pela *Cannabis* e na busca pela elucidação de suas propriedades farmacológicas (IANNOTTI; DI MARZO; PETROSINO, 2016).

Os receptores canabinoides tipo 1 (CB1) e tipo 2 (CB2) estão presentes na membrana celular, acoplados à proteína G, de células do sistema nervoso e do sistema imune, respectivamente (IANNOTTI; DI MARZO; PETROSINO, 2016; LU; MACKIE, 2016). A partir do reconhecimento dessas estruturas, alguns pesquisadores se voltaram para a busca de seus ligantes endógenos. Assim, foram identificadas as principais substâncias endocanabinoides, que são a anandamida e o 2-araquidonil glicerol (2AG) (IANNOTTI; DI MARZO; PETROSINO, 2016; LU; MACKIE, 2016; ZUARDI, 2008). O sistema endocanabinoide (sEC) é constituído pela tríade formada pelos receptores, substâncias endocanabinoides e suas enzimas de síntese e degradação (LU; MACKIE, 2016; RUSSO, 2016).

O sEC é considerado um regulador fisiológico homeostático único e difundido (RUSSO, 2016), capaz de modular a atividade neurotransmissora e diversos outros sistemas do organismo. Suas funções podem ser sintetizadas em “relaxar, comer, dormir, esquecer e proteger”, tendo efeitos que alteram a percepção da dor, fome, ansiedade, aprendizado e memória (MCPARTLAND et al., 2015). Além disso, influencia no controle motor, na imunidade, na proliferação de células tumorais e, inclusive, na inflamação (FASINU et al., 2016).

Quimicamente similares aos endocanabinoides e com potencial de mimetizar os seus efeitos, os fitocanabinoides são os principais constituintes bioativos da *Cannabis sativa*. Essas substâncias são capazes de interagir, direta ou indiretamente, com os

receptores CB1 e CB2 (KATCHAN; DAVID; SHOENFELD, 2016). Dentre cerca de 104 fitocanabinoides já identificados na planta, os mais conhecidos são o delta-9-tetra-hidro-canabinol (THC) e o canabidiol (CBD) (FASINU et al., 2016). Esses agentes apresentam diferentes propriedades farmacológicas, determinadas por suas distintas afinidades pelos receptores canabinoides (BURSTEIN, 2015; PISANTI et al., 2017).

O THC é o principal componente da planta e foi o primeiro a ser descoberto e estudado. É um agonista parcial do CB1 e do CB2 (KATCHAN; DAVID; SHOENFELD, 2016; PISANTI et al., 2017), utilizado em medicações para o tratamento de náusea e vômitos induzidos por quimioterapia e da anorexia associada à AIDS (BURSTEIN; ZURIER, 2009). No entanto, o THC apresenta importantes efeitos psicotrópicos, que limitam o seu uso medicinal (KATCHAN; DAVID; SHOENFELD, 2016).

O CBD é o segundo maior componente da *Cannabis sativa*. Foi isolado pela primeira vez em 1940, mas sua estrutura e configuração química foram determinadas somente em 1963 por Mechoulam e Shvo (BURSTEIN, 2015; FASINU et al., 2016; MECHOULAM et al., 1970; ZUARDI, 2008). Quando comparado ao THC, o CBD demonstra menor afinidade pelos receptores canabinoides (MCPARTLAND et al., 2015; PISANTI et al., 2017). Assim, se destaca por ser o principal constituinte não psicomimético da *Cannabis*. Além de não estar associada à psicoatividade, sua administração não influencia a função motora, memória e temperatura corporal (BERGAMASCHI et al., 2011; IFFLAND; GROTENHERMEN, 2017). Ele desempenha importantes efeitos, seja neuroprotetor (GIACOPPO et al., 2015), analgésico (COSTA et al., 2004a), anti-inflamatório (BURSTEIN, 2015), e imunomodulador (PISANTI et al., 2017; RUSSO, 2016). A revisão de literatura conduzida por Bergamaschi e colaboradores (2011), e atualizada por Iffland e Grotenhermen (2017), sugere que a utilização do CBD é segura e bem tolerada tanto em animais quanto em humanos. No entanto, a farmacodinâmica desse composto ainda permanece pouco esclarecida (PISANTI et al., 2017). Os estudos indicam que o CBD atua através de diferentes mecanismos de ação, incluindo atividades mediadas por receptores não-canabinoides ou, ainda, funções independentes da interação com receptores (BOOZ, 2011; BURSTEIN, 2015; MCPARTLAND et al., 2015; PISANTI et al., 2017).

Dentre esses mecanismos propostos, o CBD é considerado um agonista inverso do receptor CB2 (MCPARTLAND et al., 2015; THOMAS et al., 2009), podendo atuar na migração de células imunes, especialmente neutrófilos e macrófagos (KATCHAN; DAVID; SHOENFELD, 2016). Malfait e colaboradores (2000), utilizando um modelo de artrite reumatóide induzido em animais, publicaram um dos primeiros estudos científicos a destacar o efeito anti-inflamatório do CBD. Além disso, suas potentes propriedades já foram descritas em diversos outros modelos de doença inflamatória, tanto agudos quanto crônicos, incluindo neuroinflamação (ESPOSITO et al., 2009), doença renal (PAN et al., 2009), doença inflamatória intestinal (BORRELLI et al., 2009), asma (VUOLO et al., 2015) e lesão pulmonar aguda (RIBEIRO et al., 2015). A literatura indica que o CBD tem potencial de modular a inflamação, regulando a liberação de citocinas pró-inflamatórias, principalmente interleucina-1 beta (IL-1β) e fator de necrose tumoral alfa (TNF-α) (BORRELLI et al., 2009; ESPOSITO et al., 2009; PAN et al., 2009; RIBEIRO et al., 2015; VUOLO et al., 2015).

Na área odontológica, ainda são escassos os estudos utilizando o CBD. Em um modelo de doença periodontal, o uso de 5 mg/kg de CBD foi capaz de reduzir a reabsorção óssea, atuando na migração de neutrófilos bem como na produção de IL-1β e de TNF-α (NAPIMOGA et al., 2009). Uma revisão de literatura publicada recentemente por nosso grupo de pesquisa sugere que o CBD possa ser uma alternativa terapêutica da mucosite oral quimio e/ou radioinduzida, considerando seu potencial anti-inflamatório e antioxidante (CUBA et al., 2017).

Sabe-se que o processo inflamatório representa uma etapa determinante do reparo tissular. O recrutamento de células inflamatórias estimulado pelo dano tecidual, juntamente com a produção de citocinas e fatores de crescimento, é responsável por desencadear a cascata de eventos que resultam na cicatrização (ENOCH; LEAPER, 2007; KASUYA; TOKURA, 2014; YAMANO; KUO; SUKOTJO, 2013; YOUNG; MCNAUGHT, 2011). No entanto, já foi demonstrado que a inflamação excessiva, induzida pela superexpressão de IL-1β e TNF-α, pode estar vinculada ao atraso no reparo de lesões orais. Acredita-se que medidas capazes de modular essas citocinas possam otimizar a cicatrização tecidual (BRIZENO et al., 2016; WAGNER et al., 2016).

Embora ainda não haja uma alternativa amplamente aceita ou reconhecida como padrão-ouro para o tratamento de úlceras traumáticas orais, diversos protocolos têm sido propostos, associando o uso de corticoides (OLIVEIRA et al., 2016), laserterapia (WAGNER et al., 2016), antissépticos, antibióticos (MARIANO et al., 2015) e fitoterápicos (COELHO et al., 2015; DUARTE et al., 2011; HAMEDI et al., 2016; LIM et al., 2016). No entanto, essas estratégias muitas vezes não têm o desempenho esperado, ou ainda estão associadas a efeitos colaterais indesejados (COELHO et al., 2015; OLIVEIRA et al., 2016).

Considerando os estudos que evidenciam as propriedades farmacológicas do CBD e a fisiologia do reparo tecidual, propõe-se que esta substância possa ser indicada no manejo de úlceras traumáticas bucais. Assim sendo, o objetivo deste estudo foi avaliar o efeito do CBD, em distintas doses, como uma alternativa terapêutica para essas lesões.

A presente dissertação foi estruturada na forma de 2 artigos científicos. Apresenta-se, inicialmente, uma revisão de literatura, destacando o potencial uso do CBD como agente anti-inflamatório para lesões bucais. No segundo artigo, foi feito um estudo experimental, analisando-se clínica e histologicamente o processo cicatricial de úlceras induzidas na língua de ratos tratados com distintas doses de CBD.

2. OBJETIVOS

2.1. Objetivo geral

- Avaliar o efeito da administração intraperitoneal de distintas doses de canabidiol no reparo de úlceras orais.

2.2. Objetivos específicos

- Avaliar clinicamente o efeito da administração intraperitoneal de distintas doses de canabidiol (5 mg/kg e 10 mg/kg) no reparo de úlceras mecanicamente induzidas em língua de ratos;

- Avaliar histologicamente o efeito da administração intraperitoneal de distintas doses de canabidiol (5 mg/kg e 10 mg/kg) no reparo de úlceras mecanicamente induzidas em língua de ratos.

3. ARTIGO CIENTÍFICO I

O artigo de revisão, intitulado “**Cannabidiol as a novel therapeutic strategy for oral inflammatory diseases: a review of current knowledge and future perspectives**" foi formatado de acordo com as normas do periódico *Alternative Therapies in Health and Medicine* (ISSN: 1078-6791) para o qual foi submetido (Anexo 1). Este periódico apresenta fator de impacto 1.24 e Qualis B1, na Área de Odontologia, segundo a Classificação de Periódicos da CAPES Quadriênio 2013-2016.

Title: Cannabidiol as a novel therapeutic strategy for oral inflammatory diseases: a review of current knowledge and future perspectives

Running title: Cannabidiol and oral inflammatory diseases

Keywords: cannabidiol; cannabinoids; oral diseases

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Cannabidiol as a novel therapeutic strategy for oral inflammatory diseases: a review of current knowledge and future perspectives

ABSTRACT:

The high frequency and painful profile of inflammatory oral lesions and the lack of an effective drug protocol for their management stimulate the search for pharmacological alternatives for the treatment of these conditions. Cannabidiol is the major non-psychotropic constituent of *Cannabis sativa,* receiving lately scientific interest because of its potential in the treatment of inflammatory disorders such as asthma, colitis and arthritis. There is little published in the current literature about the use of cannabidiol in oral health. Among its many protective functions, the ability to attenuate inflammation through the modulation of cytokines and its antiedema and analgesic effects may be important features in the treatment of oral lesions. In this review, we suggest that cannabidiol can be useful in the management of oral inflammatory disorders.

INTRODUCTION

Inflammatory lesions affect the oral tissues with high frequency. These injuries may have traumatic, autoimmune, neoplastic or infectious etiologies and are often capable of causing a break in the mucosal barrier[1–4]. Rupture of the epithelial structure induces the development of an inflammatory response and becomes a potential source of infection, resulting in pain and discomfort[4]. Thus, restoring the integrity of mucosal tissue is essential to re-establish oral function.

Wound healing is a complex series of events involving well-defined phases of hemostasis, inflammation, proliferation, and remodeling[5,6]. This dynamic process is coordinated by several mediators including inflammatory cells, cytokines and growth factors[6]. Modulation of the inflammatory response during the healing process of oral lesions is a key factor for tissue repair to occur properly and in a shorter time. This may be especially necessary when considering chronic wounds, which may be induced by conditions such as diabetes and local infection[7,8].

The management of oral mucosal lesions is mainly supportive and often consists in relieving pain and symptoms besides attempts to accelerate tissue repair. Despite the variety of medications available, there is no consensus on the ideal treatment modality. Currently, pharmacological approaches include topical and systemic corticosteroids, antibiotics, local anesthetics, antiseptics, immunomodulatory and non-steroidal antiinflammatory agents[4,9]. Among the strategies to induce and modulate oral healing, the use of phytotherapeutic agents or natural compounds has shown potential[10–15].

Cannabidiol (CBD) is the major non-psychoactive component of *Cannabis sativa*. The therapeutic properties of CBD have been widely studied, and antiinflammatory, analgesic, antioxidant and immunomodulatory effects have been identified[16–18]. In recent years, CBD has demonstrated effectiveness in treating a number of conditions involving both inflammation and oxidative stress, such as rheumatoid arthritis[19], inflammatory bowel disease[20], diabetes[21] and neurodegenerative disorders[22]. Furthermore, it is considered well tolerated, without significant side effects when chronically administered[23,24].

Despite the vast literature addressing the therapeutic effects of CBD in various pathologies, there are still few studies in oral medicine evaluating its health benefits. CDB has antiinflammatory properties in the treatment of periodontitis[25], and recently, a review indicated CBD as a promising therapeutic agent in the management of chemo- and radio-induced oral mucositis[26]. A variety of mechanisms appear to be involved in the beneficial effects of CBD. One in particular is its ability to modulate the production of inflammatory mediators, which are also involved in the oral healing process, allowing its use in oral medicine[25-27]. In addition, its analgesic and antiedema properties[17] may aid to the management of different oral conditions.

The purpose of this study was to review the literature on the effect of CBD in oral disorders and the therapeutic viability of its use in the repair of oral lesions, considering the scientific evidences and its main characteristics. Accordingly, the authors surveyed the literature related to this subject in the PubMed database, using as search criterion complete articles published in English. Selection involved current studies covering the physiology of wound healing and CBD effects in inflammatory diseases.

Cannabidiol

In history, *Cannabis sativa* (marijuana) have been used for medical purpose for centuries. Among its numerous constituents, this plant contains more than 100 active components termed cannabinoids. The 2 most important phytocannabinoids are Δ9-tetrahydrocannabinol (THC) and CBD[28,29]. These substances are capable of interacting with specific cannabinoid receptors - CB1 and CB2. While CB1 receptors are primarily expressed in the central and peripheral nervous system and can modulate excitatory and inhibitory neurotransmission, CB2 receptors are localized on immune cells, acting as immunomodulators[16,30–32].

THC is the most abundant phytocannabinoid in marijuana and has good affinity for CB1 and CB2 receptors. However, its undesirable psychoactivity limits the therapeutic use of this substance[16]. CBD, first isolated in 1940, is the main non-psychotropic constituent of *Cannabis sativa*[16,18,21]. Its use has been recognized in the treatment of several pathologies like Alzheimer's disease[16], sepsis[23], inflammatory

bowel diseases[20] and also some types of cancer[21]. The exact mechanism of action of CBD has not been fully elucidated, but appears to include functions independently of CB receptors[21]. Its beneficial effects such as immune and inflammatory modulation[16], antioxidation[18] and analgesia[17,33], combined with its lack of psychoactive, good safety and a tolerability profile, encourage the use of CBD for numerous therapeutic applications.

CBD and oral medicine

Although widely explored in several pathologies and showing promising effects regarding different organs, few studies have evaluated CBD properties in oral tissues. In the area of oral medicine, we found only two scientific publications regarding the effects of CBD for oral diseases (Table 1). These studies indicated that CBD may be promising in the treatment of periodontitis[25], and chemo- and radioinduced oral mucositis[26].

Napimoga et al.[25] conducted an experimental study with induced periodontitis in rats and found that animals treated with daily intraperitoneal injection of 5 mg/kg CBD for 30 days exhibited decreased alveolar bone loss and a lower expression of RANKL/RANK. Furthermore, in gingival tissue, CBD administration decreased neutrophil migration and reduced interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) production. The authors concluded that these antiinflammatory effects of CBD could be useful in controlling the development of periodontitis.

Marijuana components have already been used in oncology patients due to their benefits in relieving symptoms such as nausea and also controlling appetite, sleep and anxiety problems. Recently, a literature review evaluated the therapeutic viability of CBD in managing oral mucositis induced by anticancer treatment[26]. On the basis of its mechanism of action, besides its analgesic and antioxidant effects, authors have proposed that CBD may be used in the treatment of oral mucositis, a serious condition that affects the quality of life of cancer patients.

Therapeutic potential of CBD in inflammatory oral lesions

CBD therapy has been explored in the management of various inflammatory conditions[16]. Its positive effects in chronic conditions, such as autoimmune encephalomyelitis, have been observed even when administered topically[34]. Previous studies found that the antiinflammatory effect of CBD frequently occurs in a dose-dependent manner, resulting in a bell-shaped curve[19,35]. Although the mechanism of action of CBD is still not fully elucidated, its effect on inflammatory diseases may be explained by various biological systems, involving both cellular and molecular events[16].

Considering the pathogenesis of inflammatory oral lesions, several CBD mechanisms of action could be related to an improvement of tissue repair in oral wounds[16,36]. Previous publications point to various CBD properties in different diseases that may enhance its potential use in the management of various oral conditions (Figure 1). Each mechanism will be explained individually below.

a) Modulation of neutrophil infiltration

Neutrophils play a critical role in tissue repair, acting mainly in the early stages of the wound healing process[5,6]. They are responsible for initiating an inflammatory cascade, attracting fibroblasts and macrophages to the injured region. In the late inflammation period, macrophages and lymphocytes are the main cells at the ulcer site, by increasing the production of cytokines and growth factors[5,8].

Neutrophil infiltration may contribute to the pathogenesis of various diseases, acting on the amplification and progression of inflammation. It is known that prolonged inflammation may delay wound healing, since excess edema, proinflammatory cytokines and toxic free radicals can be damaging to tissues and slow the progression of tissue repair[7,37]. The literature suggests that CBD has the ability to act on neutrophil proliferation and chemotaxis[36]. *In vivo* studies demonstrated that CBD was able to influence neutrophil infiltration when used in the treatment of periodontitis[25], Crohn's disease[35] and also when administered topically for encephalomyelitis[34].

b) Pain and edema reduction

Pain and edema are common complaints of patients with inflammatory oral lesions[4,13]. According to the literature, CBD may be effective in controlling both symptoms[17]. In rats with induced colitis, daily CBD at 5 mg/kg for 6 days reduced the signs of colon injury, decreasing edema in the intestinal mucosa and submucosa and helping with tissue regeneration[38]. This would agree with the data reported by Costa et al.[17] who determined CBD effects in a model of carrageenan-induced acute rat paw inflammation. These authors found a substantial reduction in hyperalgesia and edema after daily oral administration of CBD for 3 days. The effects were dose-dependent and evident within a few hours after a single application of the medication.

Another study observed that CBD had antihyperalgesic properties also in rats with chronic inflammatory pain[33]. Daily oral treatment with CBD reduced chronic pain in a dose-dependent manner, which was associated with a reduction of proinflammatory mediators.

c) Modulation of the release of proinflammatory cytokines

Some conditions or genetic and clinical variables, such as diabetes, smoking, and inflammatory/immunological pathologies, interfere in wound healing and may delay tissue repair[8,27]. Previous literature shows that deficient tissue repair of ulcerated oral mucosal lesions can be observed in diabetic rats, and this may be associated with increased inflammatory response and cytokine expression[7]. Diabetes causes a prolonged inflammatory phase of healing, observed by an increase in polymorphonuclear neutrophil infiltration and higher expression levels of IL-1β and TNF-α in oral ulcers of these animals. Therefore, the use of drugs that modulate inflammatory response can be effective when adequate tissue repair of oral lesions is desired, especially if conditions that impair oral healing are present.

Malfait et al.[19] reported one of the first studies to evaluate the potential of CBD as an antiinflammatory agent. Using a murine model of collagen-induced arthritis, CBD exerted a potent immunosuppressive effect both *in vivo* and *in vitro*, in a dose-dependent manner. The authors suggested that CBD has an immunosuppressive and

antiinflammatory action, reducing IFN (interferon)-γ release and suppressing the proliferation of lymphocytes.

In agreement with this study, different inflammatory markers were associated with the antiinflammatory effect of CBD. In cisplatin-induced nephrotoxicity in mice[39] and in rat experimental periodontitis[25], treatment with CBD attenuated inflammation by modulating TNF-α and IL-1β levels. Similarly, in mice with induced acute lung injury, a single dose of 20 mg/kg CBD modulated neutrophil, macrophage and lymphocyte migration and also decreased TNF and IL-6 production[40].

Thus, in different inflammatory pathologies, CBD appears to modulate a variety of markers and cytokines. Even in a topical formulation, it was able to attenuate IL-1β, TNF-α, IL-6, IFN-γ and transforming growth factor-beta (TGF-β) expression in experimental autoimmune encephalomyelitis[34]. Additionally, in a model of asthma[41], CBD treatment reduced levels of IL-4, IL-5, IL-13, IL-6, and TNF-α.

d) CBD as an antioxidant

Some studies have pointed out that CBD can be considered promising in the treatment of various conditions due to its ability to influence the immune system and oxidative stress[17,18,23]. Its antioxidant activity was reported to be effective in treating cisplatin-induced nephrotoxicity[39] and inflammatory bowel disease[38]. Considering that substances with antioxidant potential are known to improve wound healing and protect tissues from oxidative damage[10], CBD may have a regenerative effect in the treatment of oral ulcers, including oral mucositis[26].

e) Modulation of MMPs

Matrix metalloproteinases (MMPs) are a group of enzymes responsible for the degradation of extracellular matrix proteins and which participate in inflammation and repair processes. The overexpression of these molecules in inflammatory diseases and other pathological conditions may result in unwanted tissue destruction. Previous studies have found that MMP-2 may be associated with delayed healing and increased inflammation in the pathogenesis of oral diseases such as lichen planus[42], periodontitis[43] and mucositis[44].

Rawal et al.[45], encouraged by gingival enlargement seen in marijuana users, assessed the effects of CBD on human gingival fibroblast metabolism. These authors showed that CBD was not cytotoxic to fibroblasts and, depending on the dose, exerted a biphasic effect on the production of TGF-β and MMP in these cells. Lower CBD concentrations increased TGF-β, MMP-1 and MMP-2 production, but higher CBD concentrations decreased TGF-β and MMP levels and lowered MMP-2 activity. It was suggested that altered inflammatory responses caused by CBD may contribute to drug-induced gingival overgrowth. Thus, the ability of CBD to modulate the production and activity of these enzymes supports the use of this drug in the treatment of inflammatory oral diseases.

CONCLUSION

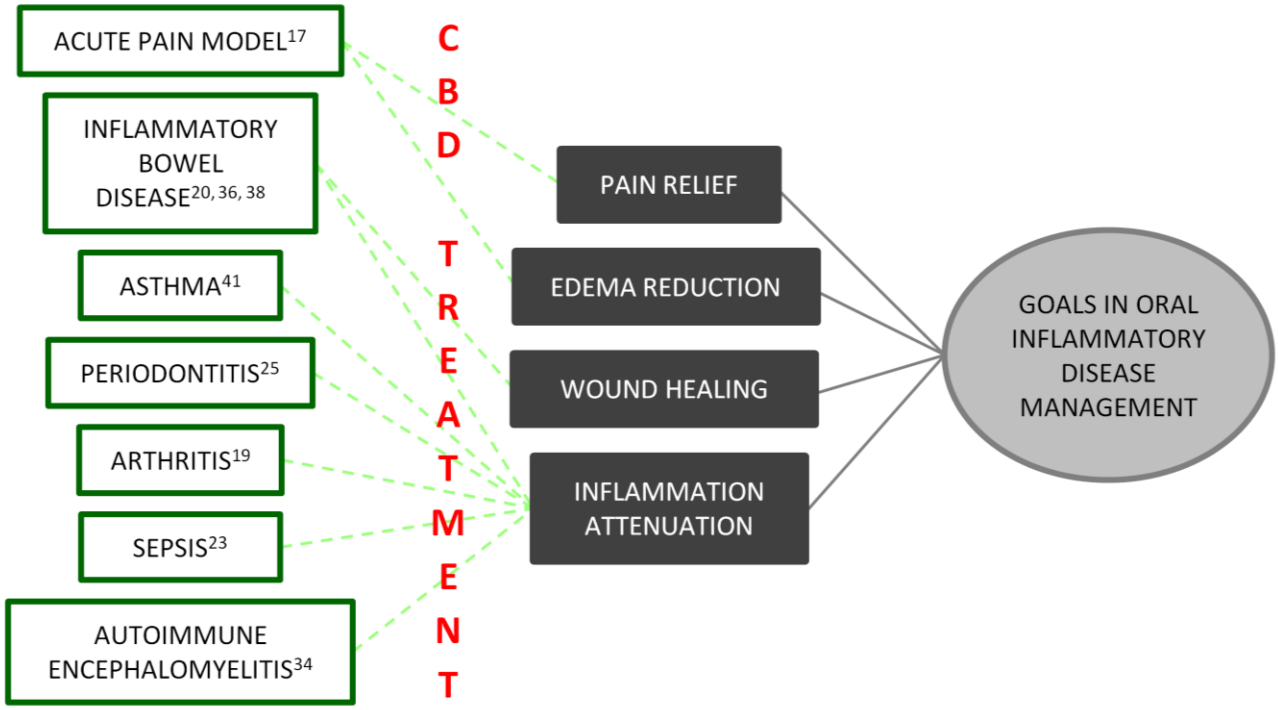
Although still sparse, available studies of CBD effects on the oral cavity have shown promising results. To date, CBD pharmacodynamics is yet to be defined, and further studies are required to elucidate the mechanisms of action and potential adverse effects of CBD. This review may be useful in providing awareness of the importance of this theme and highlights the potential use of CBD as a safe pharmacological approach for the treatment of oral inflammatory lesions. CBD may be a starting point for the development of new drugs in oral medicine.

Table 1: Effects of CBD Treatment in Oral Diseases

Disorder	Results	Reference
Periodontitis	In this experimental study in rats, CBD treatment decreased alveolar bone loss, reduced the expression of receptor activator of nuclear factor-κB ligand RANKL/RANK and decreased neutrophil migration and IL-1β and TNF-α production.	[25]
Oral mucositis	This literature review concluded that CBD may have a potential antimucositis effect, both in the control of oxidative stress and suppression of the inflammatory response	[26]

Abbreviations: CBD, Cannabidiol; IL-1β, interleukin-1 beta; TNF-α, tumor necrosis factor-alpha.

Figure 1: Why CBD Can Be Considered A Therapeutic Strategy For Inflammatory Oral Lesions? Many beneficial effects of CBD when tested in different disorders indicate its potential in treating a number of inflammatory oral diseases.



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ARTIGO CIENTÍFICO II

4. ARTIGO CIENTÍFICO II

O artigo de pesquisa, intitulado "**Cannabidiol as an alternative for management of oral traumatic ulcers in rats**" foi formatado de acordo com as normas do periódico *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology* (ISSN 2212-4403), para o qual será submetido. Esse periódico apresenta fator de impacto 1.416 e Qualis A2 na Área de Odontologia, segundo a Classificação de Periódicos da CAPES Quadriênio 2013-2016.

CANNABIDIOL AS AN ALTERNATIVE FOR MANAGEMENT OF ORAL TRAUMATIC ULCERS IN RATS

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STATEMENT OF CLINICAL RELEVANCE:

In this study, CBD treatment determined antiinflammatory effects in oral ulcerative lesions. These results may guide the design of future research using CBD for the treatment of oral lesions and also contribute to the development of new alternatives antiinflammatory drugs.

ABSTRACT

Objective. To evaluate the effect of cannabidiol (CBD) on the healing of traumatic ulcers induced on the ventral tongue of rats.

Study Design. Standardized ulcers were induced in 60 Wistar rats using a 5-mm biopsy punch on the midline of the ventral tongue. Animals received intraperitoneal (ip) injections of CBD at doses of 0 (control), 5 and 10 mg/kg daily. Animals were weighed daily and wound healing was clinically and histologically evaluated after 3 and 7 days of treatment.

Results. CBD treatment did not influence the wound area of ulcerative lesions at either observation time. Conversely, microscopic findings revealed that at day 3 post-wounding, CBD treated lesions exhibited significantly lower inflammatory scores than those in the control group. However, this difference was not observed at day 7.

Conclusion. In this study, the administration of 5 and 10 mg/kg CBD exerted an antiinflammatory effect in oral ulcerative lesions although it was not sufficient to accelerate clinical wound healing at 3 and 7 days.

Keywords: cannabidiol; cannabinoids; oral ulcer; mouth diseases; wound healing.

INTRODUCTION

Ulcerative lesions often affect the oral mucosa, and the painful symptomatology associated with them may interfere with chewing, speech, and even oral hygiene[1]. Such functional limitations directly influence the quality of life of patients, and therefore, the prompt reestablishment of the protective barrier formed by the epithelial tissue represents a crucial step. Wound repair is a complex and dynamic process, organized in well-defined overlapping phases of hemostasis, inflammation, proliferation and tissue remodeling[2–4]. The intrinsic coordination of this sequence of events is well orchestrated by an intense intercellular communication network, including cellular and molecular responses and the release of a variety of cytokines and growth factors[2,5].

Inflammation plays a critical role in the repair process. However, it is known that, when in excess, it can delay healing[6,7] and result in a greater production of free radicals that may have toxic effects on tissues[2,3]. Thus, the modulation of the release of proinflammatory cytokines is a challenging goal when the aim is to optimize healing time and to reduce pain symptoms of ulcerative lesions[7,8]. Several studies have investigated the development of an ideal healing agent, although there is still no widely accepted or gold standard treatment for the management of oral ulcers. Different strategies are often combined and involve the use of corticosteroids[7], antibiotics[9], laser therapy[8,10] and natural compound formulations[11,12]. However, these alternatives may not have significant benefits or can even lead to unwanted side effects[7,12].

Cannabis sativa, popularly known as marijuana, has been used for centuries for medicinal purposes. Its bioactive compounds, called phytocannabinoids, are currently the subject of intense medical research. Cannabidiol (CBD) is the main non-

psychomimetic cannabinoid derived from this plant[13,14]. The growing interest in the therapeutic exploration of CBD is due mainly to its potent antiinflammatory[15], antioxidant[16] and analgesic[17] properties. Previous studies have pointed out that CBD is able to suppress the production of a wide range of proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α)[18], and interleukin-1 beta (IL-1β)[19], chemokines and growth factors, and also may inhibit immune cell proliferation, activation, maturation and migration and antigen presentation[15,16].

Furthermore, literature reviews point out that CBD has a low adverse-effect profile, even when chronically administered, and its use is safe and well tolerated by both animals and humans[13,20]. Recent advances have been made in the understanding of its exact mechanism of action. However, its pharmacokinetics has not been fully elucidated. This compound has already proved to exert beneficial effects in a number of inflammatory conditions such as arthritis[21], inflammatory bowel disease[22] and asthma[23]. In the dental area, we found only one *in vivo* experimental study using CBD, which concluded that it may be a promising candidate for the treatment of periodontitis[24]. Recently, a literature review highlighted CBD as an alternative option in the management of chemo- and radioinduced oral mucositis[25].

Thus, in view of the physiology of oral wound healing and CBD antiinflammatory properties, the present study aimed to evaluate the clinical and histological effects of CBD administration on the repair of traumatic ulcers on the tongue of rats.

MATERIALS AND METHODS

Animals

All animals were treated and handled according to the principles established by the Guide for the Care and Use of Laboratory Animals. The experimental protocol (Project No. 7661) was approved by the Research Ethics Committee and the Ethics Committee on the Use of Animals of Pontifical Catholic University of Rio Grande do Sul (PUCRS, Brazil).

Sixty male Wistar rats (*Rattus novergicus)*, about 90 days old and weighing approximately 300 g each, were obtained from the Center for Experimental Biological Models (CeMBE/PUCRS), and they were housed in a temperature-controlled (23 ± 1°C) and artificially lit room on a 12-h light-dark cycle. Rats were kept in labeled plastic boxes, which were lined with wood shavings and placed on ventilated racks, and they received food (Nuvilab chow - Nuvital Nutrientes S.A, Colombo, PR, Brazil) and filtered water *ad libitum*. All animals were acclimated for at least 7 days before experimentation.

Rats were randomly selected and divided into 3 groups (1 control and 2 test groups) of 20 animals each. These groups were further divided into 2 subgroups, according to the duration of treatment (3 or 7 days).

1: Ulcer production and treatment with 5 mg/kg CBD + vehicle for 3 days (*n* = 10)

2: Ulcer production and treatment with 10 mg/kg CBD + vehicle for 3 days (*n* = 10)

3: Ulcer production and treatment with vehicle (control group) for 3 days (*n* = 10)

4: Ulcer production and treatment with 5 mg/kg CBD + vehicle for 7 days (*n* = 10)

5: Ulcer production and treatment with 10 mg/kg CBD + vehicle for 7 days (*n* = 10)

6: Ulcer production and treatment with vehicle (control group) for 7 days (*n* = 10)

Each rat was identified by its tail according to the group to which it belonged.

Surgical procedure

Prior to any intervention, animals were weighed for drug administration adjustment. Rats were anesthetized with an intraperitoneal (ip) injection of 10% ketamine (75 mg/kg body weight) and 2% xylazine (3 mg/kg body weight). An ulcer was then produced on the ventral tongue of each rat at the midline, up to 3 mm from the apex. All lesions were performed by the same operator, using disposable punches of 5 mm in diameter (Bleymed Equipamentos Médicos, Curitiba, PR, Brazil) and penetrating 1 mm deep in the tissue, to standardize wound size[26] (Figure 1). Hemostasis was achieved by pressing sterile gauze on the surgical wound.

Treatment

Immediately after producing the lesions, animals started receiving the designated treatment for each group. Synthetic CBD (≥99 purity - kindly provided by THC Pharm GmbH, Frankfurt, Germany) was dissolved in 2% polyoxyethylenesorbitan monooleate (Tween 80) in saline. The solutions were immediately prepared before administration in a volume of 1 ml/kg and protected from light[24,27,28] .

According to each study group, the animals received a daily ip injection of CBD (5 or 10 mg/kg body weight) or vehicle alone (2% Tween 80 in saline). The CBD doses were selected according to the previous studies[21,24,29,30] and animals were weighed daily for dose adjustment. Half of the sample (10 animals of each group) were euthanized at 3 days and the remaining animals at 7 days after the surgical procedure, using a carbon dioxide chamber.

Clinical evaluation

After euthanasia, each animal was weighed and the clinical evaluation was immediately performed. The ulcers were measured using a digital pachymeter, and all measurements were performed by the same examiner. The largest (D) and smallest (d) diameters of the lesion were recorded and the area of the ulcer was calculated as previously described in the literature[6,7,11,31], using the following formula: $A = \pi \times D/2 \times d/2$.

Sample processing and histological evaluation

After clinical analysis, the tongue of each animal was surgically removed and fixed for 24 hours in 10% formalin. Three sections 4 µm thick were obtained from the ulcer area of each specimen, perpendicular to the long axis of the tongue. The slides were stained with hematoxylin and eosin (HE) as part of the conventional technique for microscopic analysis.

All sections were examined by a single blinded examiner with an Olympus binocular microscope (model BX50). Previous intra-examiner calibration was achieved by duplicate analysis of 20 slides with 7 days interval between the two observations (Kappa= 0.765; $p<0.001$). After blinding and calibration, all specimens obtained were fully evaluated. The presence of edema, hyperemia, inflammatory infiltrate and vascularization was determined. The field showing the most intense inflammatory response for each specimen was selected and then classified into 4 different scores[32–34].

0. Absent: absence of inflammation

1. Mild: sparse mononuclear cells

2. Moderate: mononuclear infiltrate and/or sparse neutrophils and eosinophils

3. Intense: polymorphonuclear infiltrate of neutrophils and eosinophils

Statistical analysis

The analyses were performed using SPSS version 21.0. Qualitative variables were expressed as absolute frequencies and quantitative variables were expressed as mean and standard deviation or median and interquartile range. Inflammatory scores and body weight variation were analyzed using the Kruskal-Wallis test and Mann-Whitney test. Analysis of variance (ANOVA) was used to evaluate the interaction between times and groups in relation to the wound area. Results were considered statistically significant when $p < 0.05$.

RESULTS

Surgical procedures were performed without any anesthetic or transoperative complications. All animals remained alive during the experimental period. However, two animals from the control group were excluded from the study because they developed ulcers not within the standard size previously stipulated - punch was accidentally transfixed in the tongues.

Clinical evaluation

All animals had clinically visible ulcers at both experimental periods of 3 and 7 days. Concerning the macroscopic appearance, lesions at 3 days seemed edematous, with local inflammatory signs, and were covered by a yellowish pseudomembrane (Figure 2). After 7 days, wounds had more defined contours and regular borders. It was

possible to observe a whitish halo around the ulcerated area, which appeared to be more evident in the groups treated with CBD.

The average areas of remaining wounds are given in Table 1. The lesions were significantly smaller at 7 days compared to 3 days ($p < 0.0001$). However, no significant differences in clinical wound healing were observed between rats treated with CBD compared with control group on the 3rd and 7th days post-wounding ($p = 0.596$).

Histological evaluation

On day 3 post-wounding, histological analysis (Figure 3) revealed a wide ulcerative lesion, covered by a fibrinopurulent necrotic membrane and surrounded by a thin epithelium. The connective tissue showed edema and a moderate (score 2) to intense (score 3) inflammatory infiltrate mainly composed of polymorphonuclear cells. At this time, the groups treated with CBD showed significantly lower inflammation scores compared to animals that only received vehicle administration ($p < 0.05$)

On the 7th day post-ulceration (Figure 4), the pattern of the inflammatory infiltrate remained nearly unchanged compared with day 3 in all groups with no statistical difference regarding inflammation scores between 3 and 7 days ($p > 0.05$). Inflammation profile ranged from mild (score 1) to severe (score 3), and no significant difference was observed between groups ($p = 0.725$). Nevertheless, it was possible to observe an increased tissue organization at this time. Wounds exhibited newly formed blood vessels and fibroplasia, with collagen fibers deposited in a well-arranged manner. Marginal epithelium was thicker, with acanthosis, exuberant epithelial projections and hyperkeratinization of its surface. These epithelial changes seemed to be more evident in the CBD-treated groups.

Weight analysis

All animals had their body weight measured and its variation was observed. All groups showed a decrease in body weight on the first days after wounding. However, there was no difference in weight loss between the different treatments on days 3 and 7 post-wounding ($p > 0.05$).

DISCUSSION

Wound healing is a complex and dynamic process coordinated by the release of various cytokines and inflammatory cells[2,4]. This study revealed that CBD treatment did not promote clinical wound healing, although it did exert antiinflammatory effects in oral lesions.

Inflammation plays a crucial role in tissue repair. Prolonged inflammation inhibits the proliferation and differentiation of epithelial cells due to reactive oxygen species production[3]. Moreover, wounds that take longer to heal cause more pain and are susceptible to infection[35]. CBD effects on inflammation have already been described using both *in vivo* and *in vitro* models of chronic and acute diseases[14–16]. A recent literature review pointed out that further studies are needed to clarify the exact mechanism by which CBD displays these properties and to determine which receptors are related to them[14]. However, several previous studies have shown that these effects involve the inhibition or modulation of the production of proinflammatory cytokines, chemokines and growth factors, along with interference with the migration of macrophages and neutrophils and decrease in oxidative and nitrosative stress[14,16].

Neutrophils are the first cells to be recruited to an injury site, acting more markedly in the first 48 hours after tissue damage[2,4]. The ability of CBD to inhibit chemotaxia and proliferation of these cells[14,15,24] may explain why in the present study its antiinflammatory effect was observed in the early stage of healing (Day 3 post-wounding). Besides that, proinflammatory cytokines released by neutrophils during the initial stage of repair, such as TNF-α and IL-1β, are responsible for increasing vascular permeability, edema, and neutrophil chemotaxia[4,6,8]. Its overexpression, as in diabetic patients, is related to increased inflammation and delayed wound healing, while the modulation of these cytokines leads to optimization of tissue repair[6–8]. Although we did not evaluate the effect of CBD on these specific inflammatory markers, the effect of CBD on the expression of TNF-α and IL-1β has been previously described in experimental animal models of neuroinflammation[36], renal[19] and lung[18] injuries, inflammatory bowel disease[22] and asthma[23]. Nevertheless, further studies are needed to clarify the exact mechanism of action by which CBD reduces inflammation in mouth ulcers.

In our study, CBD treatment was unable to accelerate wound healing despite its antiinflammatory effect. An important point that may influence this tissue response is the possible ability of CBD to induce effects on keratinocytes. Cannabinoid receptors are expressed in epithelial tissue and may represent a potential therapeutic target for the management of various skin diseases such as psoriasis[37]. This was proposed by Wilkinson et al., who found that CBD inhibited the proliferation of cultured transformed (HPV-16 E6/E7) human epidermal keratinocytes in a concentration-dependent manner, using a keratinocyte proliferation assay[38]. On the other hand, Styrczewska et al.[35] developed a wound dressing composed of flax fiber containing CBD and concluded that this resource showed promising results in healing and activating cell proliferation and

migration *in vitro*. CBD did not alter the morphology or proliferation of fibroblasts and keratinocytes and was also responsible for exerting an antiinflammatory effect and increased the activity of matrix metalloproteinases[35]. Thus, the influence of CBD on keratinocytes is still controversial, and further studies are needed to investigate its effects and identify the exact mechanisms of action.

The literature reveals that CBD has the ability to inhibit angiogenesis by several mechanisms, involving endothelial cell inhibition and the modulation of specific regulatory factors[39]. This feature favors its antitumor effect, widely explored for cancer therapy. However, considering that angiogenesis is an important step in wound healing, this antiangiogenic effect may have a negative impact on this process, since the stimulation of vascular neoformation is desirable when the intention is to accelerate tissue repair[8].

In regard to body weight, a decrease was observed during the first days after wounding for all groups but there was no difference in weight loss in the groups treated with CBD *versus* control after the experimental periods of 3 and 7 days. Ulcerative lesions in the oral cavity are often painful and may thereby hinder eating, leading to malnutrition and dehydration due to dysphagia[11,31]. An analgesic property has been reported for CBD[14]. Its administration was shown to induce an antihyperalgesic effect in a model of chronic[40] and acute[17] inflammation, and it was proposed that this effect may be mediated by the modulation of TRPV1 (transient receptor potential vanilloid type 1), also known as the capsaicin receptor[40,41]. However, we did not find significant differences in body weight variation in CBD-treated animals *versus* control. These findings are in agreement with the results of Jamontt et al., who found that CBD administration for colitis treatment had no influence on rats' body weight[29]. On the

contrary, in an experimental study with Wistar rats, it was observed that daily administration of CBD at doses of 2.5 and 5 mg/kg for 13 days significantly slowed body weight gain[42]. Considering that there are still controversies over whether weight gain/loss in fact represents a CBD side effect[13,20], Iffland et al. proposed that this influence may be very small, since appetite and weight changes are complex and multifactorial outcomes[13].

Regarding the CBD doses used in this study, it is necessary to emphasize that they were chosen on the basis of the results in the available literature. Previous experimental studies with rats used CBD at doses that ranged from 1 to 480 mg/kg for ip administration[20] and reported studies suggest that CBD properties have an inverted U-shaped dose-response curve[21,29,43]. When used at a dose of 5 mg/kg daily for ip administration, CBD exerts antiinflammatory and antioxidant effects in rats with rheumatoid arthritis[21], colitis[22], asthma[23], periodontitis[24] and renal injury[27]. Esposito et al. tested CBD (2.5 and 10 mg/kg, ip, daily for 7 days) in rats with induced neuroinflammation and observed dose-dependent antioxidant and antiinflammatory effects, including the modulation of IL-1β release[36]. CBD treatment (2.5 to 10 mg/kg, ip, daily for 3 days) was also used by Pan et al., who found that this substance attenuated, in a dose-dependent manner, renal injury induced by cisplatin, decreasing expression of TNF-α and IL-1β[19]. In the present study, CBD at both 5 and 10 mg/kg had an antiinflammatory effect in oral ulcers.

Finally, it should also be noted that no side effects of CBD were observed and that its administration seems to be safe at the doses used. A literature review conducted by Bergamaschi et al.[20] and recently updated by Iffland et al.[13] 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. This would agree with data reported by Naftali et al.[44], who conducted a randomized clinical trial with 20 patients with Crohn's disease. Patients treated with a daily dose of 20 mg CBD for 8 weeks showed high tolerability and no side effects. This is particularly encouraging for the development of new clinical trials aimed at assessing the benefits and adverse effects of CBD.

CONCLUSION

The present study demonstrated that CBD treatment in rats exerted antiinflammatory effects but was unable to induce faster healing of oral ulcers. CBD may be a promising alternative for treating oral lesions in situations where inflammatory response is exacerbated and healing impaired, such as in diabetic patients. These results encourage further studies to investigate CBD’s exact mechanisms and effects on epithelial tissue repair.

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FIGURE LEGENDS

Figure 1. Induction of oral ulcers. **A.** A 5-mm disposable biopsy punch was used to produce a standardized ulcer on the ventral tongue of rats. **B.** Clinical appearance of lesions immediately after ulceration.

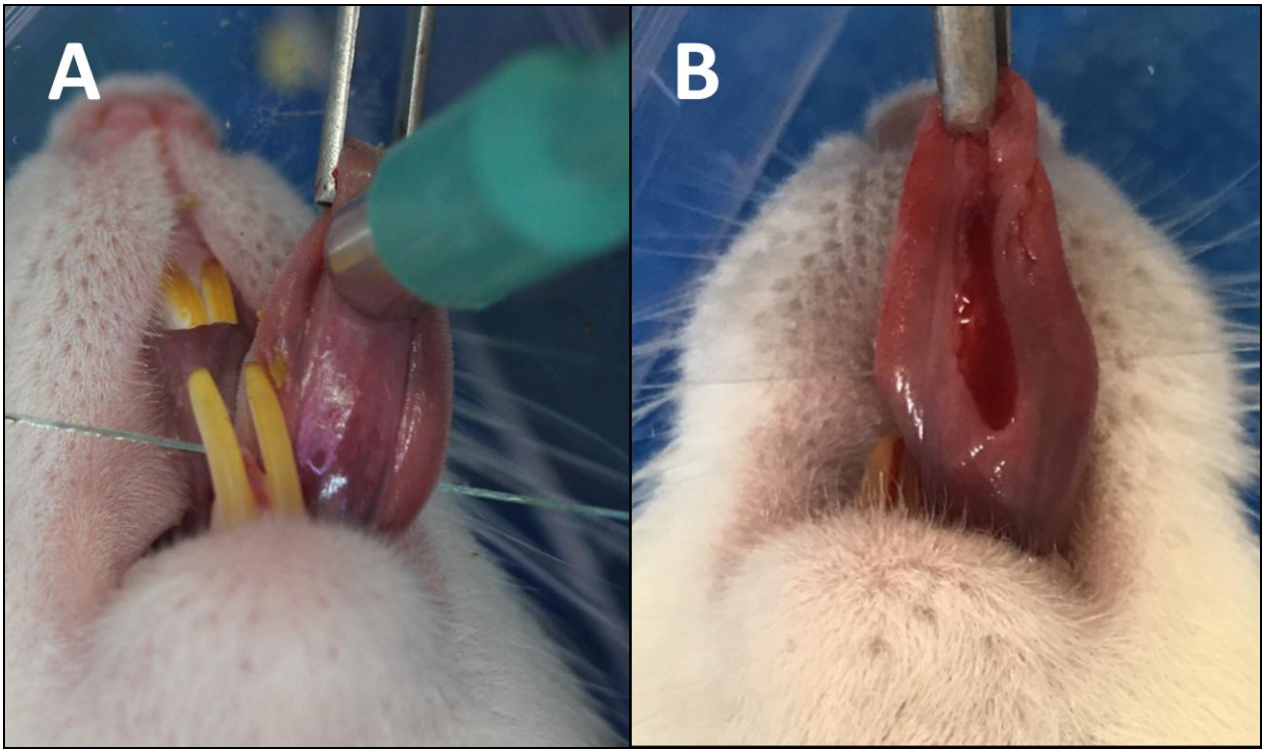

Figure 2. Macroscopic evaluation of oral ulcerative lesions after 3 days (A, B and C) and 7 days (D, E and F) of treatment. **A.** Treated with vehicle (control group) for 3 days; **B.** Treated with 5 mg/kg CBD for 3 days; **C.** Treated with 10 mg/kg CBD for 3 days; **D.** Treated with vehicle (control group) for 7 days; **E.** Treated with 5 mg/kg CBD for 7 days; **F.** Treated with 10 mg/kg CBD for 7 days.

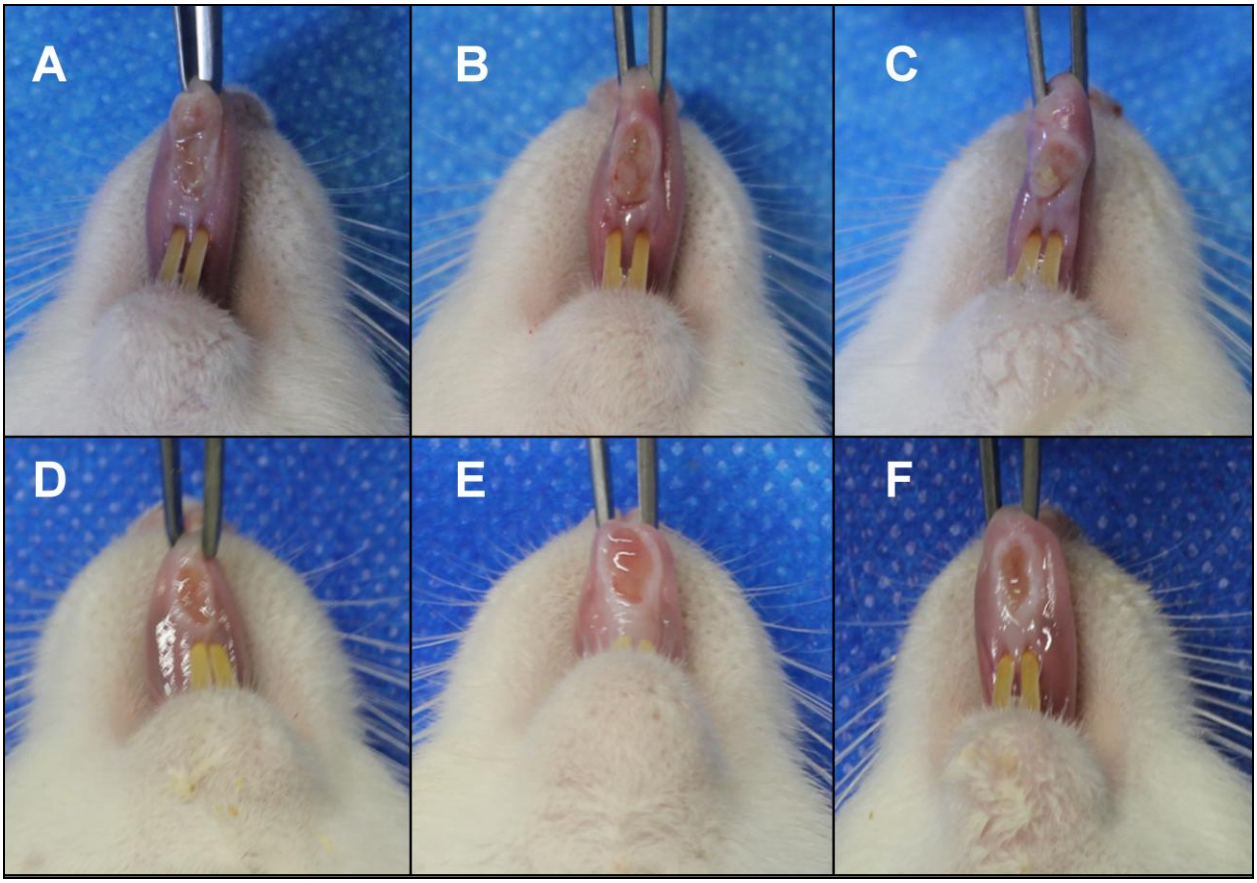

Figure 3. Histological features at 3 days post-wounding (photomicrographs, HE staining)**. A.** Control group. It is possible to observe an ulcerated area covered by necrotic membrane, with edema and some areas of an intense and diffuse inflammatory infiltrate (approximate magnification x40). **B.** Control group. Intense inflammatory infiltrate on the ulcer's surface, composed by neutrophils and lymphocytes and areas of edema (approximate magnification x400). **C.** CBD-treated group (5 mg/kg). Ulcerated area exhibited a moderate inflammatory infiltrate (approximate magnification x40). **D.** CBD-treated group (5 mg/kg). A moderate and diffuse inflammatory infiltrate (approximate magnification x200).

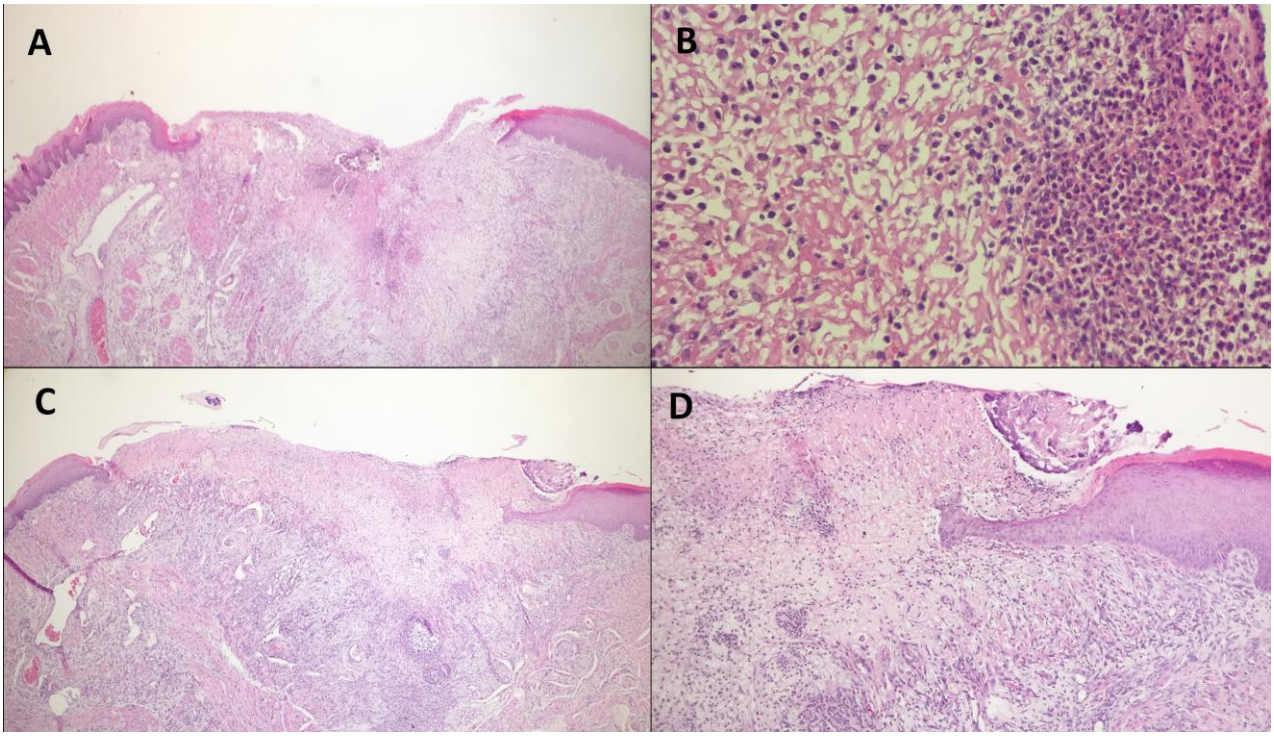

Figure 4. Histopathological aspects of 7th day post-ulceration (photomicrographs, HE staining). **A.** Control group. Lesions showed improved tissue organization with collagen fibers deposited in a well-arranged manner. Inflammatory infiltrate was more evident at superficial layer from ulcer area (approximate magnification x40). **B.** CBD-treated group (10 mg/kg). Lesions exhibited the same inflammation profile as the control group. Marginal epithelium seemed to be thicker, with exuberant epithelial projections and hyperkeratinization of its surface (approximate magnification x40). **C.** CBD-treated group (10 mg/kg). Connective tissue richly cellularized with sparse inflammatory infiltrate (approximate magnification x400).

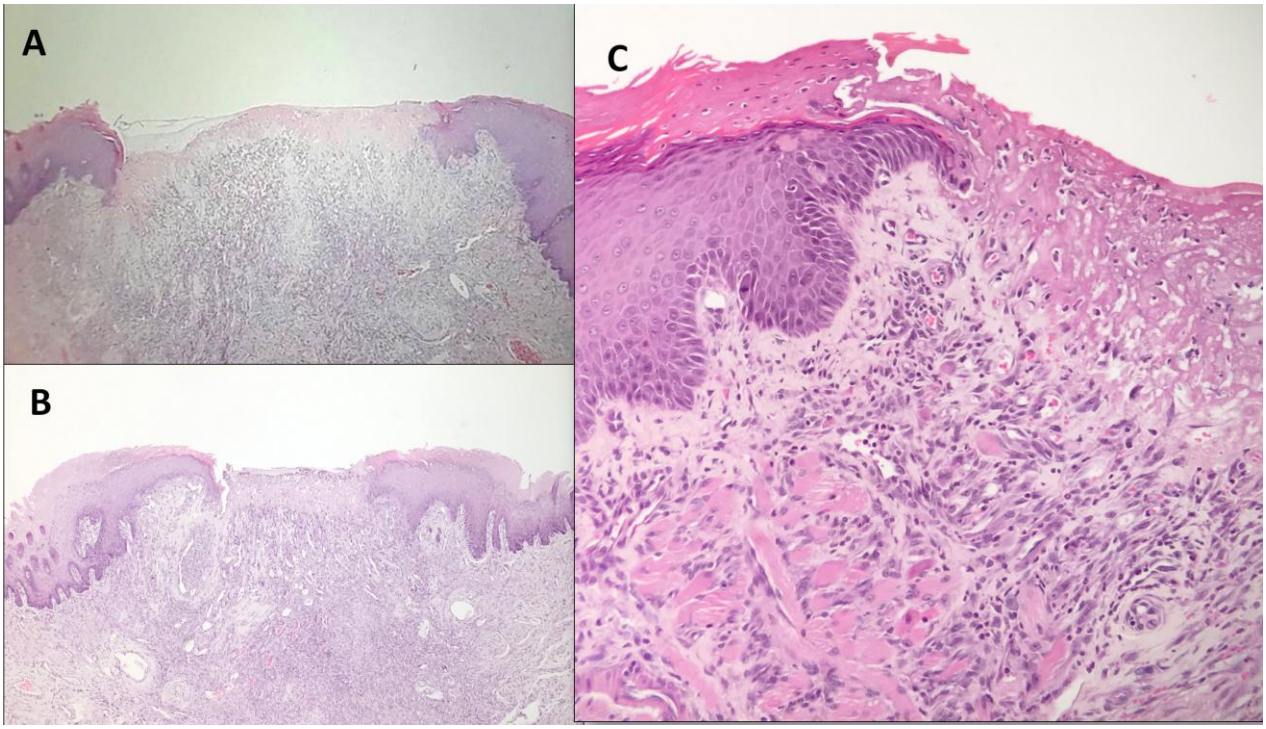

Table 1. Clinical analysis of ulcerative lesions after 3 and 7 days. Ulcer area was measured and expressed in mm².

Experimental time	Control	CBD 5 mg/kg	CBD 10 mg/kg
3 days	16,586 ± 4,701^{A}	19,647 ± 5,982^{A}	17,106 ± 5,315^{A}
7 days	5,971 ± 2,002^{B}	6,570 ± 2,936^{B}	6,376 ± 2,952^{B}

Data are expressed as mean ± standard deviation. Values with different superscript letters are significantly different ($p < 0,05$, ANOVA test).

5. DISCUSSÃO COMPLEMENTAR

O advento da utilização da maconha para fins medicinais ainda é bastante polêmico e tem sido pauta de inúmeras discussões em diversos países ao redor do mundo. Em janeiro de 2017, foi registrado no Brasil o primeiro medicamento a base de *Cannabis sativa*, denominado Mevatyl - também conhecido em alguns países europeus como Sativex. Este produto apresenta-se sob forma de *spray* e contém na sua formulação os fitocanabinoides THC e CBD (ANVISA, 2017a). É utilizado para o tratamento sintomático da espasticidade relacionada à esclerose múltipla, sendo indicado para pacientes que não respondem às terapias convencionais (ANVISA, 2017a). Desde 2015, era permitida no Brasil exclusivamente a importação de medicamentos contendo derivados da maconha. Para isso, era necessária a prescrição médica por profissional legalmente habilitado, além da avaliação e aprovação prévia da Agência Nacional de Vigilância Sanitária. Em 2016, os medicamentos derivados da *Cannabis sativa* foram excluídos da lista de compostos proibidos no país e passaram a ser incluídos na de medicamentos psicotrópicos sujeitos a controle especial (ANVISA, 2016).

Essas recentes medidas reforçam a necessidade de um amplo debate sobre a utilização médica dos derivados da *Cannabis* no país, uma vez que ainda é vista de forma preconceituosa pela sociedade (MOREIRA et al., 2016). Sabe-se que a criminalização da maconha teve início mundialmente há décadas, sendo motivada não somente pela saúde pública, mas também por pressões políticas e sociais. No Brasil, a efetiva repressão ao uso da droga ganhou força a partir de 1930 (MARTINS et al., 2016).

Em 1961, a Organização das Nações Unidas (ONU), através da Convenção sobre Substâncias Entorpecentes, proibiu o cultivo da planta e a colocou sob controle e supervisão, exceto para fins médicos e científicos. Em 1971 e 1988, respectivamente, as convenções da ONU sobre Substâncias Psicotrópicas e Contra o Tráfico Ilícito de Entorpecentes e Substâncias Psicotrópicas, também regulamentaram a utilização da *Cannabis* e dos seus derivados (ANVISA, 2017b). No entanto, nas últimas décadas, o crescente desenvolvimento de pesquisas acerca dos benefícios da maconha para o

tratamento de diversas doenças pressionou medidas para a sua regulamentação. Graças à quantidade cada vez maior de estudos apontando possíveis efeitos benéficos dos fitocanabinoides em diferentes áreas da saúde (PISANTI et al., 2017), governos de distintos países passaram a criar leis específicas que aprovam a utilização de *Cannabis sativa* para finalidades medicinais.

O potencial terapêutico da droga para o tratamento de doenças incuráveis encorajou famílias brasileiras a utilizarem formulações clandestinas de medicamentos à base de CBD e THC. Entretanto, a falta de controle na produção e no uso dessas substâncias é considerada perigosa, uma vez que pode comprometer a saúde dos pacientes, que desconhecem a dosagem adequada bem como a pureza do produto fabricado (SABÓIA, 2017). Esse fato tornou inadiável a regulamentação do uso terapêutico da *Cannabis*, visando favorecer a segurança, a qualidade e a eficácia desses medicamentos.

Apesar do crescente progresso das pesquisas na área médica, a Odontologia ainda participa timidamente da discussão vinculada aos benefícios terapêuticos da droga (CUBA et al., 2017; NAPIMOGA et al., 2009). Assim sendo, o presente estudo visa também à gradativa inserção da Odontologia neste debate. Mesmo com os entraves burocráticos existentes no país, é expressiva a participação de pesquisadores brasileiros nos estudos científicos com os canabinoides. Os investimentos de cunho científico nesta temática contemplam a criação de um Centro de Pesquisa em Canabinoides vinculado à Faculdade de Medicina de Ribeirão Preto (STELLA, 2017). Esse passo representa um importante avanço nacional para pesquisa e desenvolvimento de drogas contendo derivados da *Cannabis*.

O projeto executado e exposto nesta dissertação apresenta, ainda, alguns tópicos a serem discutidos. O desenho experimental, incluindo o sítio e o tamanho da lesão ulcerada, foi conduzido de acordo com achados da literatura e a vivência prévia do grupo de pesquisa referida em publicações semelhantes (DE FREITAS CUBA et al., 2016; JASPER et al., 2016).

Estudos que avaliam a mucosa oral frequentemente utilizam ratos como modelo *in vivo* (CAVALCANTE et al., 2011), por terem este tecido estruturalmente semelhante ao dos humanos e processo cicatricial similar. Outras vantagens incluem o baixo custo

e a facilidade na manipulação desses animais (CAVALCANTE et al., 2011; PEPLOW; CHUNG; BAXTER, 2010). Ademais, os ratos machos são preferidos na literatura, possivelmente pelo preço mais acessível, além da menor influência de alterações hormonais que, nas fêmeas, poderiam interferir na cicatrização de feridas (PEPLOW; CHUNG; BAXTER, 2010).

Uma revisão de literatura sobre o efeito da laserterapia na cicatrização de feridas em ratos e camundongos concluiu que há grande variabilidade nos desenhos experimentais desses estudos, incluindo diferenças no local, tamanho e quantidade de lesões avaliadas em cada publicação (PEPLOW; CHUNG; BAXTER, 2010). No presente estudo, o ventre lingual foi escolhido por ser de fácil acesso e boa visualização. Por outro lado, Cavalcante e colaboradores (2011) avaliaram um modelo experimental de lesão ulcerada na mucosa jugal de ratos e concluíram que este sítio apresenta boa reprodutibilidade para pesquisas *in vivo*. Essa localização também foi utilizada por Brizeno e colaboradores (2016) e Deyhimi e colaboradores (2016) para avaliar a cicatrização oral. Outras regiões como palato (HASHEMIPOUR et al., 2017; KIM et al., 2013; VILLA et al., 2015), gengiva (DE CARVALHO et al., 2014; LIM et al., 2016) e dorso da língua (COELHO et al., 2015; WAGNER et al., 2013, 2017) também costumam ser utilizadas para este fim. No entanto, sabe-se pela prática clínica que a mucosa não-ceratinizada é mais favoravelmente acometida por lesões ulceradas. Tal característica não é contemplada nos estudos que avaliam lesões em palato, dorso lingual e gengiva, sendo o ventre lingual um sítio representativo desta realidade. Outra vantagem do sítio utilizado neste estudo é o maior acesso e visibilidade do campo cirúrgico quando comparado à mucosa jugal, que requer dispositivos para abertura de boca e distensão deste tecido. Além disso, o ventre lingual parece representar um local mais protegido do constante traumatismo dentário e da alimentação dos roedores do que os demais.

O tipo de lesão produzido para avaliação do reparo também é muito variável na literatura, incluindo técnicas de abrasão com lâmina de bisturi (CAVALCANTE et al., 2011), queimaduras químicas (DE CARVALHO et al., 2014; LIM et al., 2016) ou térmicas e feridas isquêmicas (PEPLOW; CHUNG; BAXTER, 2010). Lesões traumáticas induzidas por *punch* são amplamente utilizadas (COELHO et al., 2015; DEYHIMI et al.,

2016; DUARTE et al., 2011; HASHEMIPOUR et al., 2017; LIM et al., 2016; MARIANO et al., 2015; WAGNER et al., 2013, 2017) por ser uma metodologia simples e de fácil reprodutibilidade. Ademais, o reparo por segunda intenção exigido nesta técnica favorece a avaliação histológica do processo cicatricial, quando comparado a lesões que cicatrizam por primeira intenção (PEPLOW; CHUNG; BAXTER, 2010). O *punch* permite adequada padronização do diâmetro da úlcera produzida, embora a delicadeza e a espessura delgada da língua exijam cautela na determinação da profundidade da úlcera. No presente estudo, o *punch* foi acidentalmente transfixado na língua de dois animais, que foram excluídos da amostra por não contemplarem as medidas de análise pré-estabelecidas para as lesões. A utilização de um anteparo fixo - lâmina de vidro com apoios (Apêndice 3) - facilitou o controle da pressão ao penetrar o *punch* e permitiu melhor padronização da profundidade das úlceras.

O diâmetro do *punch* é amplamente variável na literatura, assim como os tempos experimentais utilizados para avaliar a cicatrização. Segundo Cavalcante e colaboradores (2011), que avaliaram o reparo de lesões em mucosa jugal de ratos, uma redução significativa do tamanho da úlcera só foi percebida a partir do terceiro dia de sua indução. Considerando os efeitos esperados do CBD em fases precoces da inflamação, optou-se por um período observacional de 3 dias, conforme a literatura prévia (MARIANO et al., 2015). O período de 3 e 7 dias também foi usado por Duarte e colaboradores (2011) e Villa e colaboradores (2015).

No presente estudo, a avaliação clínica do reparo tecidual deu-se pela mensuração da área ulcerada, através dos diâmetros registrados com paquímetro digital. Essa técnica é bastante empregada na literatura (BRIZENO et al., 2016; CAVALCANTE et al., 2011; LIM et al., 2016; OLIVEIRA et al., 2016), embora outras diferentes estratégias possam ser utilizadas, incluindo o uso de programas como o ImageJ (WAGNER et al., 2017), cálculo de porcentagem de cicatrização (COELHO et al., 2015; WAGNER et al., 2013, 2017) e de contração da úlcera (BRIZENO et al., 2016). O cálculo da área da lesão, utilizado neste estudo, parece ser mais reprodutível do que a técnica utilizada previamente pelo nosso grupo de pesquisa (JASPER et al., 2016), em que o diâmetro da lesão era mensurado com régua endodôntica, e a sua profundidade, com sonda milimetrada. Na metodologia empregada no presente estudo,

o paquímetro digital oferece maior acurácia no registro das medidas. Além disso, o cálculo de área, considerando o maior e o menor diâmetros da lesão, contempla mais precisamente a forma elíptica da úlcera formada.

Em relação à análise histológica, diversos escores inflamatórios e de reparo tem sido propostos na literatura (COELHO et al., 2015; DEYHIMI et al., 2016; DUARTE et al., 2011; HASHEMIPOUR et al., 2017; WAGNER et al., 2013). Dentre eles, optou-se pela classificação proposta por Figueiredo e colaboradores (2011), conforme a experiência do nosso grupo de pesquisa (DE FREITAS CUBA et al., 2016; JASPER et al., 2016). Além da avaliação por escores, alguns estudos utilizam métodos como contagem de células (DUARTE et al., 2011; LIM et al., 2016) ou, ainda, análise histológica descritiva (WAGNER et al., 2017). No presente estudo, investigações adicionais como, por exemplo, imunoistoquímica para marcadores inflamatórios (TNF-α e IL1-β), análise histomorfométrica da epitelização e coloração picrosirius também poderiam ser interessantes, visando enriquecer a análise dos efeitos do CBD no reparo tecidual.

A diluição do CBD com *Tween* 80 e a solução salina foi utilizada em diversos estudos prévios (CASSOL-JR et al., 2010; LIU et al., 2010; MUKHOPADHYAY et al., 2011; NAPIMOGA et al., 2009; SOARES et al., 2015; VUOLO et al., 2015). Contudo, existem autores que adicionam etanol, juntamente com *Tween* e solução salina, como veículo para o CBD (BORRELLI et al., 2009; COSTA et al., 2004b; PAGANO et al., 2016; RIBEIRO et al., 2015). O processo de diluição do CBD é bastante delicado, uma vez que esta substância apresenta-se na forma de um pó de baixa solubilidade aquosa. Para evitar formação de grânulos, que poderiam obstruir o lúmen da seringa durante a administração intraperitoneal, incorporou-se lentamente o pó ao líquido, com posterior auxílio de uma cuba ultrassônica (Apêndice 4).

A escolha da administração do CBD por via intraperitoneal parece ser limitante quando se deseja transpor os resultados obtidos para a prática clínica. No entanto, é a via mais comumente empregada e citada na literatura para avaliação do efeito do CBD em roedores, devido às características da substância e à conveniência da sua utilização (IFFLAND; GROTENHERMEN, 2017). Sabe-se que o CBD, quando administrado por via oral, apresenta uma baixa biodisponibilidade. Tal característica ocorre devido ao

metabolismo de primeira passagem ser hepático, além de sua instabilidade em pH gástrico e baixa solubilidade aquosa, que levam à absorção incompleta da medicação (HAMMELL et al., 2016; PISANTI et al., 2017). Considerando-se o ineditismo do estudo apresentado e a fim de facilitar a comparação das doses utilizadas e dos resultados obtidos com as evidências já disponíveis, optou-se pela já consagrada via intraperitoneal.

Tendo em vista tais limitações, a forma tópica parece ser a via mais adequada para o tratamento de lesões orais. O Metavyl/Sativex, comercialmente disponível, é um *spray* oromucoso administrado topicamente na região sublingual. Cada mililitro da solução contem 27 mg de THC e 25 mg de CBD (ANVISA, 2017a). Sua administração tem sido bem tolerada, contudo seus efeitos colaterais incluem dor e desconforto no sítio da aplicação (KEATING, 2017). Supõe-se que tais queixas estejam associadas ao conteúdo alcoólico do medicamento, necessário para a diluição dos fitocanabinoides, e não especificamente com o CBD (SCULLY, 2007). Além disso, estudos anteriores em diferentes modelos de doença sugerem que, mesmo quando administrado topicamente, o CBD é capaz de apresentar propriedades terapêuticas significativas. Hammell e colaboradores (2016) observaram que a aplicação tópica de um gel à base de CBD em um modelo de monoartrite induzida em ratos melhorou a dor e produziu efeito anti-inflamatório nos animais tratados. Giacoppo e colaboradores (2015) testaram o uso tópico de um creme à base de CBD em camundongos após indução de encefalomielite autoimune. Os resultados demonstraram que o tratamento reduziu a expressão de citocinas pró-inflamatórias, incluindo IL-10, TGF-β e IFN-Ɣ, produzindo também um efeito antioxidante. Dessa forma, o uso de uma formulação tópica de CBD no manejo de lesões ulceradas orais pode ser uma alternativa interessante no delineamento de futuras pesquisas.

De acordo com o que foi exposto nesta dissertação, acredita-se que esta pesquisa possa subsidiar estudos que envolvam e favoreçam o uso do CBD em diferentes campos da área médica e odontológica.

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PUCRS

ANEXO 1 - Submissão do artigo 1 ao periódico *Alternative Therapies in Health and Medicine* (ISSN: 1078-6791)

De: Maria Antonia Z de Figueiredo
Enviado: terça-feira, 26 de dezembro de 2017 17:16
Para: athmsubmissions@innovisionhm.com
Assunto: Submission article on CBD

Dear editor of *Alternative Therapies in Health and Medicine,*
Attached please find a copy of the manuscript entitled *Cannabidiol as a novel therapeutic strategy for oral inflammatory diseases: a review of current knowledge and future perspectives.* We are submitting solely to this journal and have tried to follow the requisite steps for submission. Please let us know if there is anything missing.
Thank you.
Best wishes,
Maria Antonia Zancanaro de Figueiredo

ANEXO 2 - Aprovação do Projeto de Pesquisa pela Comissão Científica da Faculdade de Odontologia da PUCRS

SIPESQ

Sistema de Pesquisas da PUCRS

Código SIPESQ: 7661 Porto Alegre, 9 de novembro de 2016.

Prezado(a) Pesquisador(a),

A Comissão Científica da FACULDADE DE ODONTOLOGIA da PUCRS apreciou e aprovou o Projeto de Pesquisa "Efeito do canabidiol no reparo de úlceras mecanicamente induzidas na mucosa oral de ratos : avaliação clínica e histológica". Este projeto necessita da apreciação da Comissão de Ética no Uso de Animais (CEUA). Toda a documentação anexa deve ser idêntica à documentação enviada ao CEUA, juntamente com o Documento Unificado gerado pelo SIPESQ.

Atenciosamente,

Comissão Científica da FACULDADE DE ODONTOLOGIA

ANEXO 3 - Aprovação do Projeto de Pesquisa pela Comissão de Ética no Uso de Animais (CEUA) da PUCRS

S I P E S Q

Sistema de Pesquisas da PUCRS

Código SIPESQ: 7661 Porto Alegre,8 de dezembro de 2016

Prezado(a) Pesquisador(a),

A Comissão de Ética no Uso de Animais da PUCRS apreciou e aprovou o Projeto de Pesquisa "Efeito do canabidiol no reparo de úlceras mecanicamente induzidas na mucosa oral de ratos : avaliação clínica e histológica" coordenado por MARIA ANTONIA Z DE FIGUEIREDO.

Sua investigação, respeitando com detalhe as descrições contidas no projeto e formulários avaliados pela CEUA, está autorizada a partir da presente data.

Informamos que é necessário o encaminhamento de relatório final quando finalizar esta investigação. Adicionalmente, ressaltamos que conforme previsto na Lei no. 11.794, de 08 de outubro de 2008 (Lei Arouca), que regulamenta os procedimentos para o uso científico de animais, é função da CEUA zelar pelo cumprimento dos procedimentos informados, realizando inspeções periódicas nos locais de pesquisa.

Duração do Projeto: 08/12/2016 - 08/01/2017

Nº de Animais	Espécie
60	Rattus norvegicus
Total de Animais: 60	

Atenciosamente,

Comissão de Ética no Uso de Animais(CEUA)

APÊNDICES

APÊNDICE 1 – Ficha de avaliação clínica utilizada para o estudo.

PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL
FACULDADE DE ODONTOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA
ÁREA DE CONCENTRAÇÃO EM ESTOMATOLOGIA CLÍNICA

EFEITO DO CANABIDIOL NO REPARO DE ÚLCERAS MECANICAMENTE INDUZIDAS NA MUCOSA ORAL DE RATOS: AVALIAÇÃO CLÍNICA E HISTOLÓGICA

FICHA DE AVALIAÇÃO CLÍNICA

IDENTIFICAÇÃO

Rato nº: _____ **Peso inicial:** _____ g **Peso final:** _____ g

TRATAMENTO:

[ ] GRUPO 1 – CBD 5 mg/kg
[ ] GRUPO 2 – CBD 10 mg/kg
[ ] GRUPO 3 – VEÍCULO

TEMPO:

[ ] GRUPO A - 3 DIAS
[ ] GRUPO B - 7 DIAS

AVALIAÇÃO CLÍNICA LOCAL

Presença de úlcera: [ ] Sim [ ] Não **Tamanho da ulceração:**_____

Área da lesão: _____

Sinais inflamatórios: [ ] Eritema [ ] Edema

Sinais secundários: [ ] Sim [ ] Não

Outras áreas de ulceração:

[ ] Sim [ ] Não Localização:_____

Outras informações (sangramento, supuração, abscesso...):

_____
_____
_____

Fotos: _____ **Data da avaliação:** ____/____/_____

APÊNDICE 2 – Ficha de avaliação histológica utilizada no estudo.

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EFEITO DO CANABIDIOL NO REPARO DE ÚLCERAS MECANICAMENTE INDUZIDAS NA MUCOSA ORAL DE RATOS: AVALIAÇÃO CLÍNICA E HISTOLÓGICA

FICHA DE AVALIAÇÃO HISTOLÓGICA

IDENTIFICAÇÃO

Rato nº: _____ **Peso inicial:** _____ g **Peso final:** _____ g

TRATAMENTO:

[ ] GRUPO 1 – CBD 5 mg/kg
[ ] GRUPO 2 – CBD 10 mg/kg
[ ] GRUPO 3 – VEÍCULO

TEMPO:

[ ] GRUPO A - 3 DIAS
[ ] GRUPO B - 7 DIAS

AVALIAÇÃO HISTOLÓGICA (HE) DA ÁREA COM MAIOR RESPOSTA CELULAR

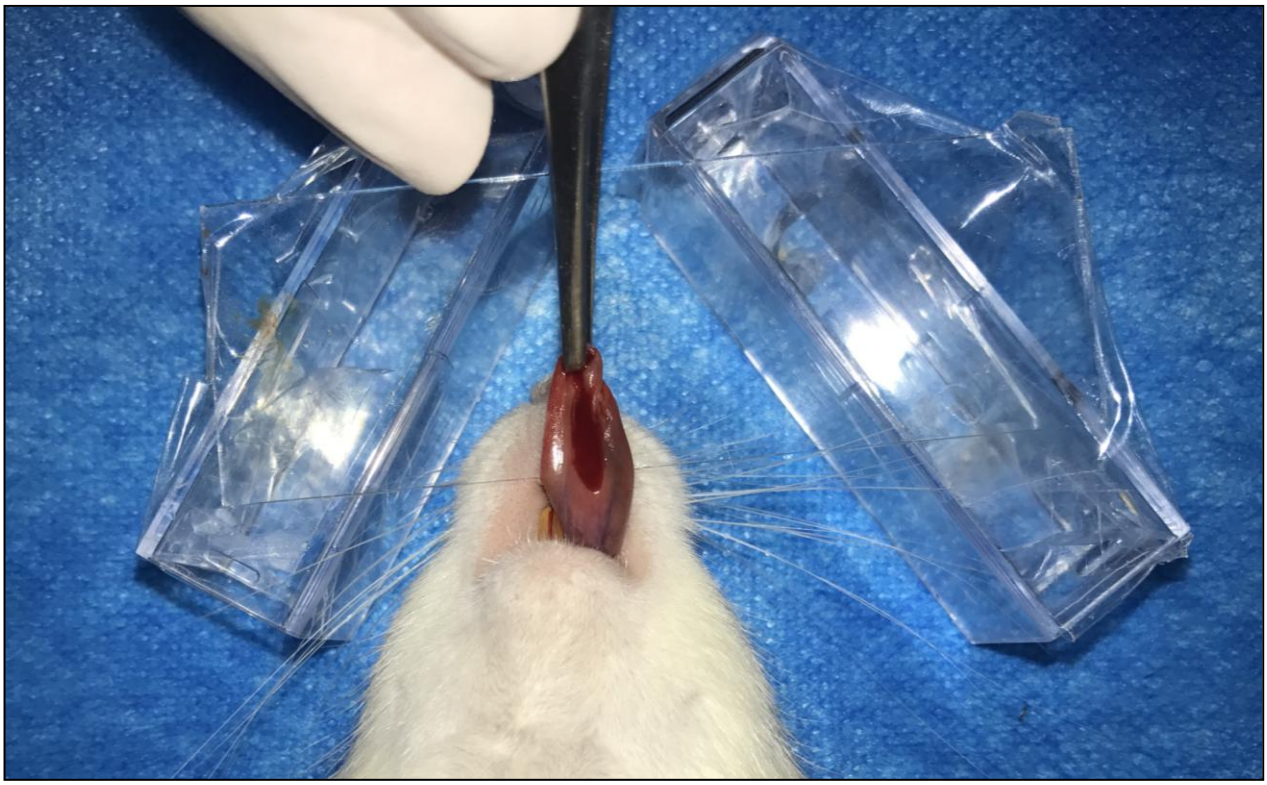
Variável/Resposta	SIM	NÃO
EDEMA		
PRESENÇA DE CÉLULAS INFLAMATÓRIAS:		
• Linfócitos		
• Plasmócitos		
• Macrófagos		
• Neutrófilos		
• Eosinófilos		
• Células gigantes		
FIBROPLASIA		

Escore:
[ ] 0 - Ausente: Ausência de inflamação
[ ] 1 - Leve: Células mononucleares esparsas
[ ] 2 - Moderada: Infiltrado mononuclear e/ou neutrófilos e eosinófilos esparsos
[ ] 3 - Intensa: Infiltrado polimorfonuclear de neutrófilos e eosinófilos

Observações:_____

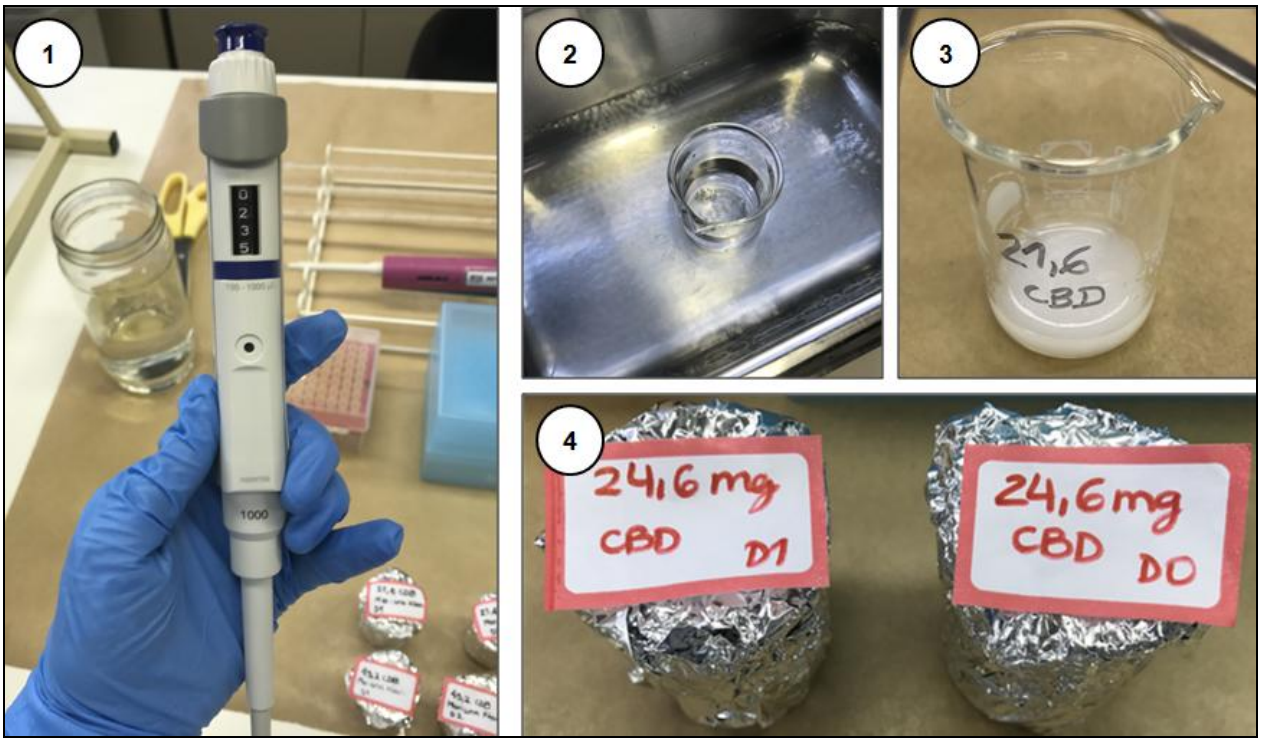
Fotos:_____ **Data da avaliação:**_____/____/_____

APÊNDICE 3 – Dispositivo confeccionado para o procedimento cirúrgico.



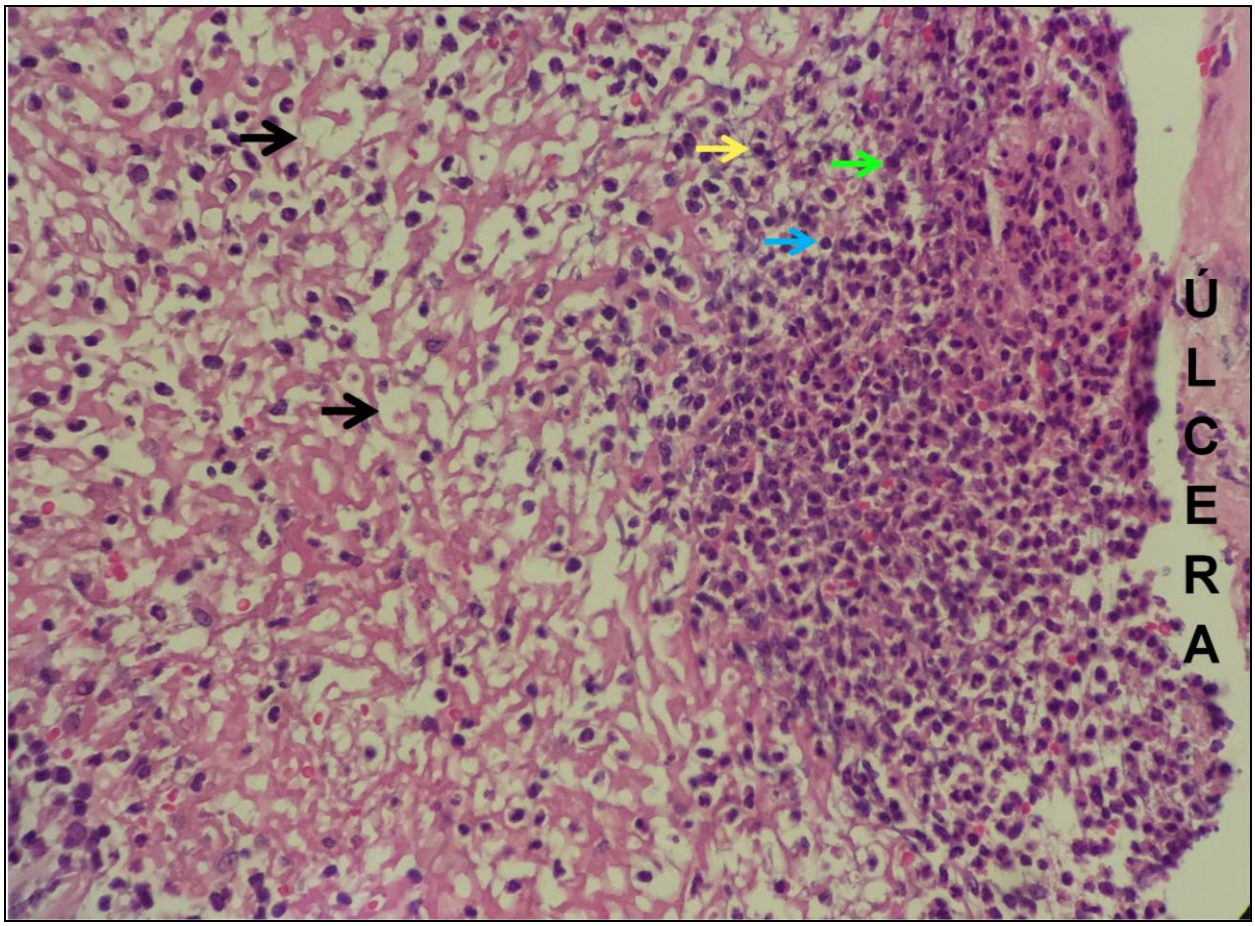
Dispositivo confeccionado com lâmina de vidro usado como anteparo da língua, auxiliando no controle da profundidade de penetração do *punch* e na padronização da lesão.

APÊNDICE 4 – Manipulação das soluções administradas.



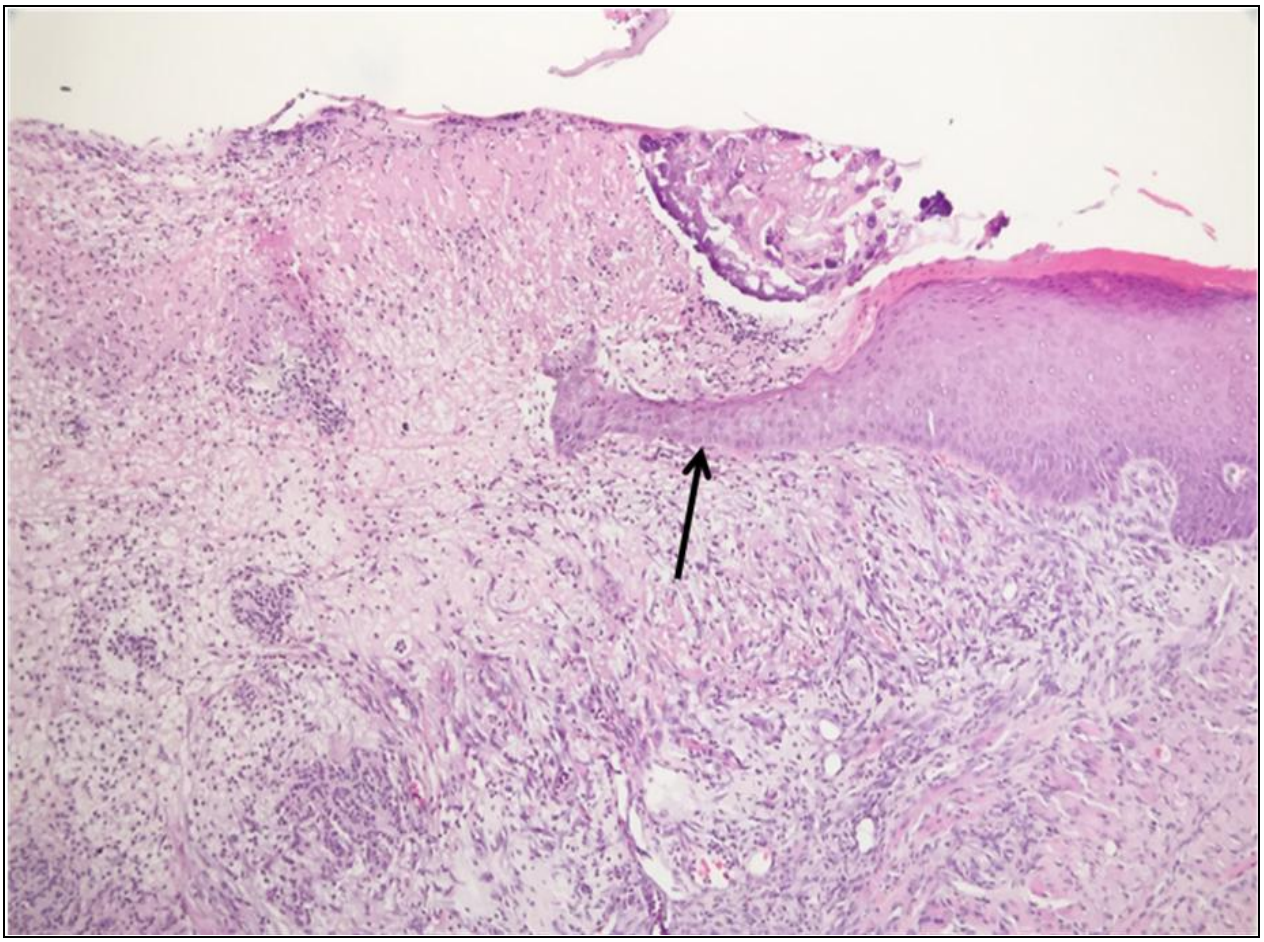
1. As soluções foram preparadas imediatamente antes da administração nos animais, em um volume de 1 mg/kg. **2.** Após a incorporação manual, a solução foi agitada em cuba ultrassônica para evitar formação de grânulos insolúveis. **3.** Aspecto final da solução de CBD após seu preparo. **4.** As soluções foram protegidas da luz até sua administração.

APÊNDICE 5 – Fotomicrografia do grupo controle com 3 dias de evolução.



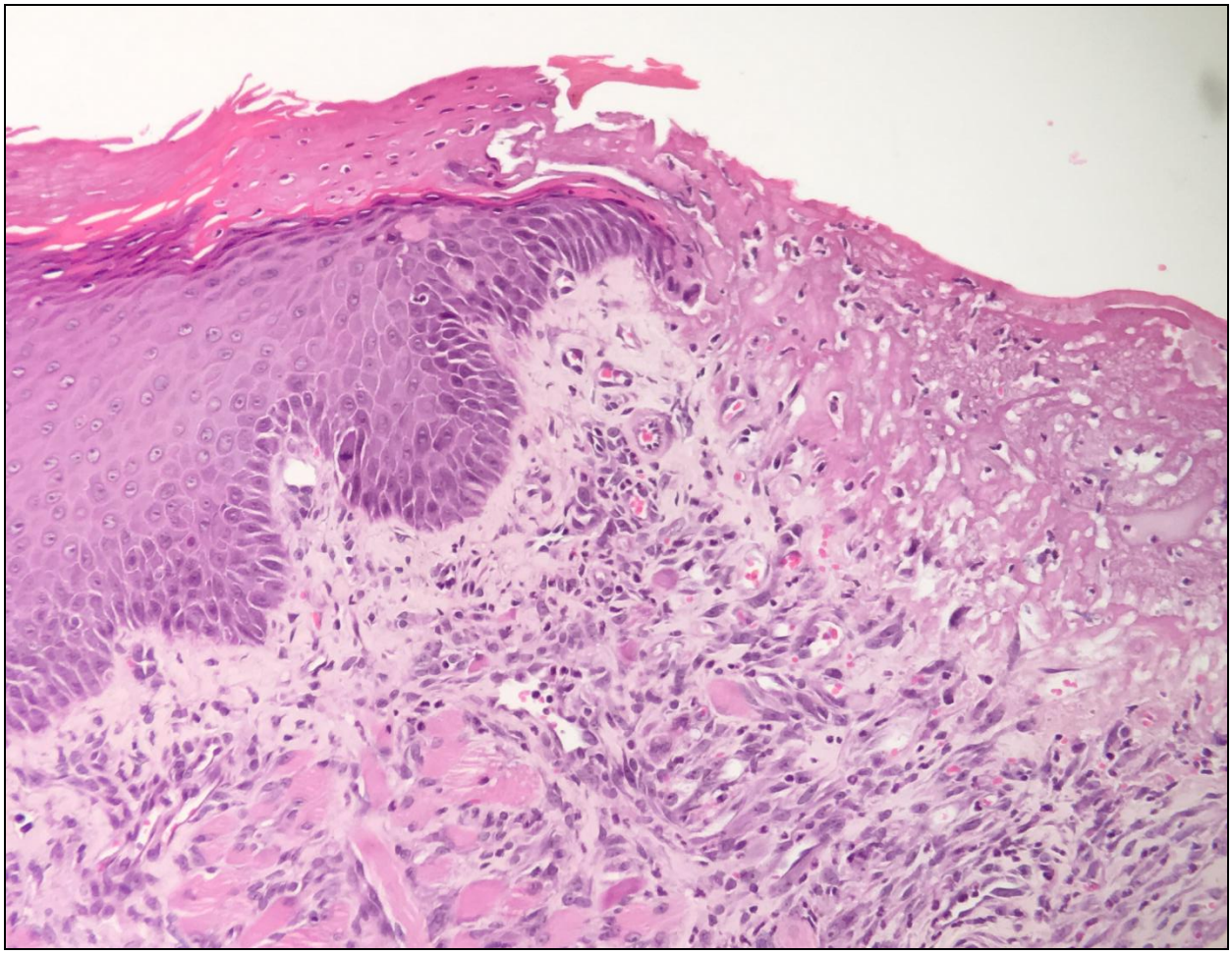
Lesão ulcerada com intenso infiltrado inflamatório na sua superfície - neutrófilos (seta amarela), plasmócitos (seta verde) e linfócitos (seta azul). As setas pretas indicam áreas de edema (HE, aumento aproximado de 400 x).

APÊNDICE 6 – Fotomicrografia do grupo CBD 5 mg/kg com 3 dias de evolução.



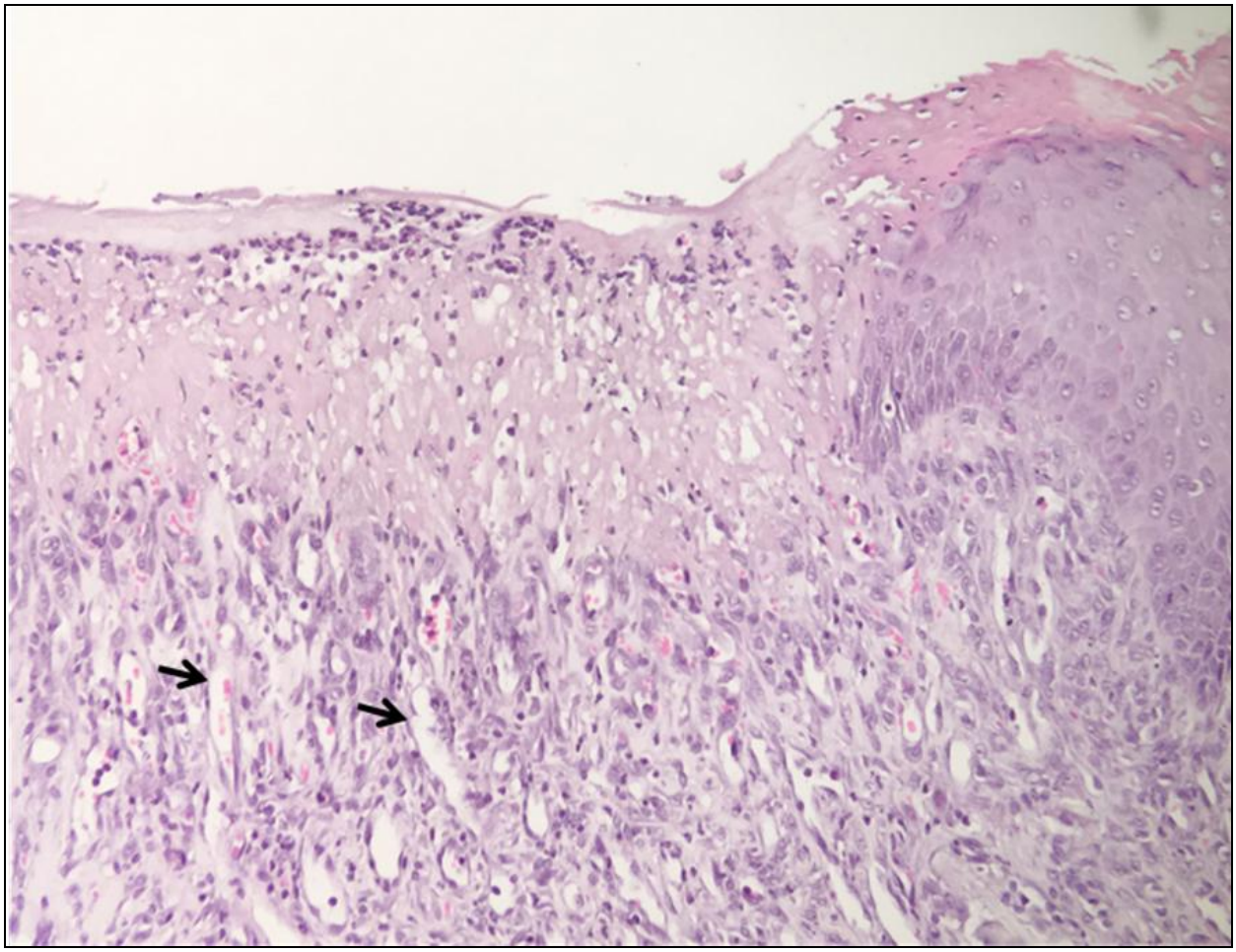
Áreas de tecido conjuntivo exposto, recoberto por membrana fibrinopurulenta e com infiltrado inflamatório crônico e difuso adjacente. Na seta, observa-se projeção epitelial para o tecido conjuntivo (HE, aumento aproximado de 100 x).

APÊNDICE 7 – Fotomicrografia do grupo CBD 10 mg/kg com 3 dias de evolução.



Observa-se área de tecido conjuntivo exposto, recoberto por membrana fibrinopurulenta e com infiltrado inflamatório esparso (linfócitos, neutrófilos e macrófagos). O tecido conjuntivo apresenta-se ricamente celularizado e o epitélio perilesional hiperplásico e hiperceratinizado (HE, aumento aproximado de 200 x).

APÊNDICE 8 – Fotomicrografia do grupo CBD 10 mg/kg com 7 dias de evolução.



Membrana de fibrina recobrindo a área de tecido conjuntivo exposto, que se apresenta ricamente vascularizado (setas). Tecido epitelial perilesional exibe hiperplasia e hiperceratose (HE, aumento aproximado de 200 x).

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