ESCOLA DE CIÊNCIAS DA SAÚDE

PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA

EFEITO DO CANABIDIOL, UM COMPONENTE DA *CANNABIS SATIVA*, NA MUCOSITE ORAL INDUZIDA EM CAMUNDONGOS SOB QUIMIOTERAPIA COM 5-FLUOROURACIL: AVALIAÇÃO CLÍNICA, HISTOLÓGICA, HEMATOLÓGICA E BIOQUÍMICA

LETÍCIA DE FREITAS CUBA GUERRA

2018

Pontifícia Universidade Católica
do Rio Grande do Sul

Pontifícia Universidade Católica do Rio Grande do Sul

Escola de Ciências da Saúde

Programa de Pós-Graduação em Odontologia

Doutorado em Estomatologia Clínica

LETÍCIA DE FREITAS CUBA GUERRA

EFEITO DO CANABIDIOL, UM COMPONENTE DA *CANNABIS SATIVA*, NA MUCOSITE ORAL INDUZIDA EM CAMUNDONGOS SOB QUIMIOTERAPIA COM 5-FLUOROURACIL: AVALIAÇÃO CLÍNICA, HISTOLÓGICA, HEMATOLÓGICA E BIOQUÍMICA

Linha de Pesquisa: Enfermidades da Região Bucomaxilofacial: Estudos Clínicos, Imunológicos e Anatomopatológicos

Porto Alegre

2018

LETÍCIA DE FREITAS CUBA GUERRA

EFEITO DO CANABIDIOL, UM COMPONENTE DA *CANNABIS SATIVA*, NA MUCOSITE ORAL INDUZIDA EM CAMUNDONGOS SOB QUIMIOTERAPIA COM 5-FLUOROURACIL: AVALIAÇÃO CLÍNICA, HISTOLÓGICA, HEMATOLÓGICA E BIOQUÍMICA

Linha de Pesquisa: Enfermidades da Região Bucomaxilofacial: estudos clínicos, imunológicos e anatomopatológicos

Tese apresentada como parte dos requisitos obrigatórios para a obtenção do título de Doutor em Estomatologia Clínica pelo Programa de Pós-Graduação em Odontologia da Escola de Ciências da Saúde da Pontifícia Universidade Católica do Rio Grande do Sul.

Orientadora: Profa. Dra. Maria Antonia Zancanaro de Figueiredo

2018

LETÍCIA DE FREITAS CUBA GUERRA

EFEITO DO CANABIDIOL, UM COMPONENTE DA *CANNABIS SATIVA*, NA MUCOSITE ORAL INDUZIDA EM CAMUNDONGOS SOB QUIMIOTERAPIA COM 5-FLUOROURACIL: AVALIAÇÃO CLÍNICA, HISTOLÓGICA, HEMATOLÓGICA E BIOQUÍMICA

Linha de Pesquisa: Enfermidades da Região Bucomaxilofacial: estudos clínicos, imunológicos e anatomopatológicos

Tese apresentada como parte dos requisitos obrigatórios para a obtenção do título de Doutor em Estomatologia Clínica pelo Programa de Pós-Graduação em Odontologia da Escola de Ciências da Saúde da Pontifícia Universidade Católica do Rio Grande do Sul.

Aprovada em: _____ de _____ de _____.

Banca Examinadora:

_____
Prof. Dra. Karen Cherubini (PUCRS)

_____
Prof. Dra Maria Martha Campos (PUCRS)

_____
Prof. Dra Sabrina Pozatti Moure (ULBRA)

_____
Profa. Dra. Maria Antonia Zancanaro de Figueiredo - Orientadora (PUCRS)

_____
Prof. José Antonio Poli de Figueiredo (UFRGS) (Suplente)

Porto Alegre
2018

"Por vezes sentimos que aquilo que fazemos não é senão uma gota de água no oceano. Mas o oceano seria menor se lhe faltasse uma gota."

"A todos os que sofrem, dai sempre um sorriso de alegria. Não lhes proporciones apenas os vossos cuidados, mas também o vosso coração."

Madre Teresa de Calcutá

AGRADECIMENTOS

Aos meus pais Neila e Edgar, obrigada pelo apoio incondicional, pelo amor sem limites, pela maneira como me ensinaram a conduzir a vida, pela forma terna de valorizarem desde os mais simples dos meus "feitos". Orgulhá-los é meu grande objetivo de vida.

Ao meu avô Zé, que me inspira lá do céu.

À minha Vó Idália, minha eterna gratidão por ter proporcionado condições para que lá atrás eu estudasse e me apaixonasse pela odontologia.

Ao meu marido, Vinícius, por esses longos anos de parceria, por estar ao meu lado diante de cada novo desafio apoiando, incentivando, acreditando na minha capacidade, por vezes, mais do que eu mesma, por aceitar as mudanças repentinas de planos e de humor, por conhecer a fórmula exata de acalmar minha mente ansiosa e meu coração apressado e, não menos importante, por me ensinar mil vezes a calcular a diluição das drogas para este trabalho.

Aos meus irmãos e demais familiares, obrigada por compreenderem meus momentos de reclusão e por tantos momentos de descontração que me proporcionaram nesses 4 anos.

Aos amigos fiéis que foram incansáveis em me fazer rir, que vibraram a cada nova história sobre ratos e tornaram essa jornada mais suave e prazerosa.

A coordenação do Curso de Odontologia da UNIPAR Campus Francisco Beltrão pelo apoio e incentivo através da liberação das minhas atividades docentes para todas as necessidades vinculadas ao programa de doutorado.

Aos colegas da UNIPAR, que foram imprescindíveis quando precisei me ausentar e, além disso, se tornaram verdadeiros amigos.

Aos meus alunos que são a fonte de energia para os momentos difíceis, que são o motivo pelo qual deixo minha casa toda semana, para aprender mais do que ensinar.

LISTA DE ABREVIATURAS

5FU	5-fluorouracil
AOX	Antioxidantes/ Antioxidants
CAT	Catalase
CBD	Canabidiol/Cannabidiol
CT	Chemotherapy
EO	Estresse oxidativo
FR	Free radicals
GSH	Glutatina reduzida/Reduced glutathione
HE	Hematoxilina e Eosina
IP	Intraperitoneal
MO	Mucosite oral
OM	Oral mucositis
OS	Oxidative stress
QT	Quimioterapia
RL	Radicais livres
ROS	Espécies reativas de oxigênio/ Reactive oxygen species
RT	Radioterapia/ Radiotherapy

RESUMO

As neoplasias malignas representam um problema de saúde pública mundial. As estimativas mostram que, em 2012, ocorreram 14,1 milhões de casos novos de câncer e 8,2 milhões de óbitos. A epidemiologia do câncer denota a importância do avanço em pesquisas na área, desde estratégias de diagnóstico precoce à contenção dos danos associados a sua ocorrência e terapêutica empregada. Atualmente, as principais modalidades de tratamento contemplam a cirurgia, a radioterapia (RT) e a quimioterapia (QT). A QT é amplamente empregada no tratamento de neoplasias malignas sólidas e hematológicas, uma vez que age destruindo as células tumorais. No entanto, não é seletiva, atingindo também células saudáveis de rápida renovação como as da mucosa oral. Em consequência ao dano às células normais, esta terapia gera efeitos deletérios importantes como, por exemplo, a mucosite oral (MO). Esta condição caracteriza-se pela presença de ulcerações dolorosas que podem evoluir para quadros tão graves que comprometam o curso do tratamento oncológico. Associada a etiologia da MO está o estresse oxidativo (EO) gerado pela QT, que seria capaz de induzir a produção de espécies reativas de oxigênio (ROS) responsáveis pelo dano celular e iniciação das lesões. O canabidiol (CBD) é o principal componente não-psicotrópico da *Cannabis sativa* e desempenha potentes efeitos antiinflamatórios, antioxidantes e analgésicos. A presente tese 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 na prevenção e manejo da MO, sugerindo o caráter promissor desta droga. O segundo trata de um estudo experimental desenvolvido em modelo animal, com objetivo de avaliar a resposta clínica, histológica, hematológica e estresse oxidativo da administração intraperitoneal do CBD, nas doses de 3 mg/kg, 10 mg/kg e 30 mg/kg por 4 e 7 dias, no reparo da MO quimioinduzida no ventre lingual de 90 camundongos CF-1. O tratamento com CBD foi capaz de diminuir os escores clínicos da MO em ambos os tempos experimentais ($p < 0,005$) e reduziu a intensidade da resposta inflamatória, porém sem significância estatística. Na contagem de eritrócitos, leucócitos, plaquetas e atividade das enzimas antioxidantes CAT e GSH, observou-se melhores resultados nos grupos tratados com CBD. Dessa forma, concluiu-se que o CBD é capaz de regular o processo inflamatório da MO, podendo representar uma alternativa promissora no manejo dessa condição.

Palavras chave: estomatologia; quimioterapia; mucosite oral; antioxidantes; canabidiol.

ABSTRACT

ABSTRACT

Malignant neoplasms are a public health problem worldwide. The global estimate shows that in 2012, there were 14.1 million new cases of cancer and 8.2 million deaths. The epidemiology of cancer shows the importance of advancement in research in the area, from strategies of early diagnosis to containment of the damages associated with its occurrence and therapies used. Currently, the main treatment modalities include surgery, radiotherapy (RT) and chemotherapy (CT). CT is widely used in the treatment of solid and hematological malignances, where its aim is to destroy the tumor cells. However, it is not selective and also affects healthy cells of rapid renewal, such as those of the oral mucosa. As a consequence of damage to normal cells, this therapy accounts for substantial deleterious effects, such as oral mucositis (OM). This condition is characterized by the presence of painful ulcerations that can progress to such severe conditions, where the course of cancer treatment is compromised. Associated with the etiology of OM is oxidative stress (OS) generated by CT, which can induce the production of reactive oxygen species (ROS), responsible for cell damage and the initiation tissue lesions. Cannabidiol (CBD) is the main non-psychotropic component of *Cannabis sativa* and has potent antiinflammatory, antioxidant and analgesic effects. This thesis consists of 2 scientific papers. The first is a literature review, whose objective was to evaluate the different mechanisms of action of CBD, which may be involved in the prevention and management of OM, suggesting the promising property of this drug. The second one deals with an experimental study conducted in an animal model, with the objective of evaluating the clinical, histological, hematological and oxidative stress effects of the intraperitoneal administration of CBD, at doses of 3, 10 and 30 mg/kg for 4 and 7 days, in the repair of chemo-induced OM on the tongue ventrum of 90 CF-1 mice. CBD treatment decreased the OM clinic scores at both experimental times (p<0.005) and reduced the intensity of the inflammatory response, but not statistically significant. With regard to erythrocyte, leukocyte and platelet counts and antioxidant enzyme activity, CAT and GSH, the groups treated with CBD showed better results. Thus, we concluded that CBD is able to regulate the inflammatory process of OM, and may represent a promising alternative in the management of this condition.

Keywords: stomatology; chemotherapy; oral mucositis; antioxidants; cannabidiol.

SUMÁRIO

SUMÁRIO

1. INTRODUÇÃO

As doenças e agravos não transmissíveis são os principais responsáveis pelo adoecimento e morte da população no mundo. Estima-se que, em 2008, 36 milhões dos óbitos (63%) ocorreram em consequência dessas doenças, sendo o câncer responsável por 21% destes. As transições demográficas e epidemiológicas globais sinalizam um impacto cada vez maior da carga de câncer nas próximas décadas. Estima-se, para o Brasil, biênio 2018-2019, a ocorrência de 600 mil casos novos de câncer, para cada ano. Essas estimativas refletem o perfil de um país que possui os cânceres de próstata, pulmão, mama feminina, além de cólon e reto, entre os mais incidentes, entretanto ainda apresenta altas taxas para os cânceres do colo do útero, estômago e esôfago (INCA, 2018).

Dentre as modalidades terapêuticas usadas no manejo das neoplasias malignas destaca-se a QT, amplamente utilizada de forma exclusiva ou concomitante a cirurgia e RT. Trata-se da utilização de drogas, injetáveis ou por via oral, com o objetivo de destruir ou bloquear o crescimento das células neoplásicas malignas. Estão disponíveis centenas de tipos de medicamentos contra o câncer, cada um com ação específica e com toxicidade ou efeitos colaterais específicos. Alguns quimioterápicos são bem tolerados, enquanto outros podem causar efeitos adversos importantes (HUANG et al, 2018).

A QT age direta ou indiretamente na estrutura do DNA interferindo na sua duplicação. No entanto, não é seletiva às células tumorais, atingindo também os tecidos saudáveis da mucosa oral, sendo capaz de gerar efeitos deletérios que podem causar diversas complicações dentre as quais destaca-se a MO (THOMSEN, VITETTA, 2018).

O termo mucosite surgiu em 1980 para descrever reações inflamatórias na mucosa bucal de pacientes submetidos à radio ou quimioterapia (PETERSON, CARRIELLO, 2004). Hoje é considerada a mais severa complicação não hematológica da terapia do câncer, ocorrendo em cerca de 40 a 80% dos pacientes submetidos a esta modalidade terapêutica (REZAZADEH et al, 2018). Caracteriza-se por eritema, seguido de ulcerações dolorosas na mucosa bucal que interferem no estado nutricional e na qualidade de vida dos pacientes, podendo até mesmo limitar ou interromper a terapia oncológica. Sua evolução é complexa, uma vez que é influenciada por outras complicações, como xerostomia, disgeusia, odinofagia e infecções oportunistas como, por exemplo, a candidíase.

Além disso, representa um fator de risco para infecções sistêmicas que podem requerer antibioticoterapia de amplo espectro e até mesmo internação hospitalar, elevando significativamente os custos do tratamento (KUO *et al* 2018, THOMSEN, VITETTA 2018; TIAN *et al* 2018; SONIS, VILLA 2018).

Há evidências consideráveis de que os efeitos citotóxicos da QT são decorrentes das reações físico-químicas que levam à produção de radicais livres (RL). Estes compostos são espécies reativas com um ou mais elétrons não pareados em sua última camada eletrônica, o que o torna altamente instável. Estas moléculas buscam estabilidade em outros elementos, como proteínas, lipídeos e DNA, causando a desestruturação das mesmas, gerando reações em cadeia que culminarão no que chamamos de EO. A formação dos RL e o consequente EO estariam fortemente associados à iniciação da MO (SONIS, 2004; SONIS *et al*, 2004; LALLA, SONIS, PETERSON, 2008; SONIS, 2010, AL DASOOQUI *et al*, 2013; KUO *et al* 2018, THOMSEN, VITETTA 2018; TIAN *et al* 2018; SONIS, VILLA 2018).

A mucosite tem sido foco de diversos estudos, pois sua prevenção e/ou tratamento efetivo permitiria doses terapêuticas mais agressivas para a neoplasia maligna e provável aumento das taxas de sobrevida. Vários métodos são sugeridos na prevenção e manejo da MO, tais como manutenção da higiene bucal, uso de agentes antiinflamatórios, antimicrobianos, anestésicos tópicos, protetores de mucosa, laserterapia e fatores de crescimento, embora a maioria delas seja utilizada de forma paliativa (SAITO *et al*, 2014; KUO *et al*, 2018; SONIS, VILLA, 2018).

A *Cannabis sativa* (também conhecida como maconha) é uma planta psicoativa que contém mais de 500 componentes. Dois destes tem sido alvo de diversas investigações científicas tendo em vista as suas propriedades farmacológicas: o tetrahidrocanabinol (THC) e o canabidiol (CBD) (LAFEYE *et al*, 2017).

O THC é o principal componente psicoativo da *Cannabis*, funcionando principalmente como um agonista parcial nos receptores CB1 e CB2. Estudos demonstram efeitos positivos no controle da dor, apetite, digestão, emoções e processos de pensamento mediado pelo sistema endocanabinoide. No entanto, está associado a eventos adversos psicoativos dependendo da dose e tolerância prévia do paciente (CHEN *et al*, 2016; MACCALUM, RUSSO 2018).

O CBD, ao contrário, tem pouca afinidade com esses receptores, não exercendo atividade psicotrópica. Em vez disso, é um modulador alostérico negativo de CB1, com efeitos farmacológicos

em vários outros sistemas receptores, desempenhando ação analgésica, anti-inflamatória e antioxidante. Seu potencial terapêutico pode ser uma ferramenta útil no tratamento de doenças ou condições raras e de difícil manejo como síndromes centrais de sensibilidade (fibromialgia, síndrome da fadiga crônica, enxaquecas, intestino irritável) ou esclerose múltipla, dor neuropática e náusea refratária (CHEN *et al*, 2016; KOZELA *et al*, 2016; MACCALUM, RUSSO 2018).

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 pesquisa realizada em modelo animal avaliando cicatrização de lesões ulceradas, publicada recentemente por nosso grupo de pesquisa, sugere que o CBD possa ser uma alternativa terapêutica capaz de modular o processo inflamatório favorecendo a velocidade de cicatrização das lesões (KLEIN *et al*, 2018).

Embora a incidência da MO durante a terapia antineoplásica e as consequências correspondentes sejam extremamente graves, estratégias de prevenção e tratamento permanecem um desafio para a odontologia. Baseado na compreensão do mecanismo de desenvolvimento da MO bem como do potencial anti-inflamatório e antioxidante do CBD o objetivo deste estudo foi avaliar a resposta clínica, histológica, hematológica e bioquímica do uso do CBD sobre a MO induzida no ventre da língua de camundongos sob QT com 5FU.

A presente tese foi estruturada na forma de 2 artigos científicos. Apresenta-se, inicialmente, uma revisão de literatura, destacando o potencial uso do CBD como agente antiinflamatório e antioxidante para MO. No segundo artigo, foi realizado um estudo experimental, analisando-se sob vários parâmentros o processo de reparo da MO induzida na língua de camundongos tratados com distintas doses de CBD.

2. OBJETIVOS

2.1. Objetivo geral

• Avaliar o efeito da administração intraperitoneal de diferentes concentrações de canabidiol na mucosite oral induzida na língua de camundongos, sob quimioterapia com 5-fluorouracil.

2.2. Objetivos específicos

• Avaliar clinicamente o efeito da administração intraperitoneal de diferentes concentrações de canabidiol na mucosite oral induzida na língua de camundongos sob quimioterapia com 5-fluorouracil, comparando-os entre si;

• Avaliar histologicamente o efeito da administração intraperitoneal de diferentes concentrações de canabidiol na mucosite oral induzida na língua de camundongos sob quimioterapia com 5-fluorouracil, comparando-os entre si;

• Avaliar hematologicamente o efeito da administração intraperitoneal de diferentes concentrações de canabidiol em camundongos sob quimioterapia com 5-fluorouracil, considerando as variáveis leucócitos, eritrócitos e plaquetas, comparando-os entre si;

• Avaliar através da análise bioquímica do estresse oxidativo o efeito de diferentes concentrações de canabidiol em camundongos sob quimioterapia com 5-fluorouracil,considerando as variáveis catalase e glutationa redutase, comparando-os entre si.

3. ARTIGO I

O artigo a seguir intitula-se "**CANNABIDIOL: AN ALTERNATIVE THERAPEUTIC AGENT FOR ORAL MUCOSITIS?**" Foi aceito e publicado pelo periódico Journal of Clinical Pharmacy and Therapeutics (Anexo A), o qual apresenta Qualis B1 e Fator de Impacto 1.661.

Review article

Running title: **Cannabis and oral mucositis**

Cannabidiol: an alternative therapeutic agent for oral mucositis?

Letícia de Freitas Cuba, MSc[a]; Fernanda Gonçalves Salum, PhD[b]; Karen Cherubini, PhD[b]; Maria Antonia Zancanaro de Figueiredo PhD[b].

Division of Oral Medicine – PUCRS Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, RS, Brazil.

Conflicts of interest: The authors declare that they have no conflict of interest.

[a]PhD student of Oral Medicine, Pontifical Catholic University of Rio Grande do Sul(PUCRS), Porto Alegre, Brazil.

[a]Clinical Lecturer of Oral Medicine, Paranaense University (UNIPAR), Francisco Beltrão, Brazil.

[b]Professor of Oral Medicine, Pontifical Catholic University of Rio Grande do Sul (PUCRS), Porto Alegre, Brazil.

Corresponding author: Maria Antonia Z. de Figueiredo
Av. Ipiranga 6690 – Sala 231 CEP 90610-000 - Porto Alegre - RS- Brazil
antonia.figueiredo@pucrs.br   telephone   (+5551)   99930005   fax   number (+5551)33203254

Cannabidiol: an alternative therapeutic agent for oral mucositis?

Summary

What is known and objective: Chemo- and radiotherapy are therapeutic modalities often used in patients with malignant neoplasms. They kill tumor cells but act on healthy tissues as well, resulting in adverse effects. Oral mucositis is especially of concern, due to the morbidity that it causes. We reviewed the literature on the etiopathogenesis of oral mucositis and the activity of cannabidiol, to consider the possibility of its use for the prevention and treatment of oral mucositis.

Methods: We searched the PUBMED database and selected complete articles published in English that met the inclusion criteria for the period 1998 to 2016 . The search terms "cannabinoids", "cannabidiol", "oxidative stress", "antioxidants", "oral mucositis" were used.

Results and discussion: The control of oxidative stress may prevent and alleviate oral mucositis. Studies have demonstrated that cannabidiol is safe to use and possesses antioxidant, antiinflammatory and analgesic properties.

What is new and conclusions: The literature on the use of cannabidiol in dentistry is still scarce. Studies investigating the use of cannabidiol in oral mucositis and other oxidative stress-mediated side effects of chemotherapy and radiotherapy on the oral mucosa should be encouraged.

Key words: cannabinoids, cannabidiol, oxidative stress, antioxidants, oral mucositis

WHAT IS KNOWN AND OBJECTIVE

With an increase in life expectancy and an aging population, the frequency of chronic non-communicable diseases such as cancer has been increasing. Consequently, there is a greater demand for antineoplastic treatments, which are responsible for major adverse effects. Oral mucositis (OM) is a complication commonly observed, particularly with radiotherapy (RT) of the head and neck area and with chemotherapy (CT). It is a painful and debilitating condition of varying severity. It is characterized by the presence of ulcerated lesions in the oral cavity, which usually leads to difficulty in eating and consequential loss of weight and malnutrition, and susceptibility to opportunistic infections. OM affects the quality of life of patients and can become a dose-limiting factor in treatment. [1-6]

There is considerable evidence that the cytotoxic effects of CT and RT result from physico-chemical reactions that lead to excessive production of free radicals (FR), which favors an oxidant–antioxidant imbalance and oxidative stress (OS). This could mediate the development of oral lesions and OM. [4, 5, 7-11] However, despite our understanding of the pathogenesis of this condition, its prevention and treatment are still considered a challenge to the dentist.

The use of cannabinoids, components of *Cannabis sativa*, in the treatment of complex diseases has been extensively investigated in different medical areas. Among the cannabinoids, cannabidiol (CBD) is the non-psychotropic cannabinoid of greatest abundance in the plant.[12] According to reported studies, CBD has antioxidant, antiinflammatory and immunomodulatory properties and is devoid of psychoactive properties, and its minimal side effects make it safe for use in humans.[12-15] This compound has shown promising results in the control of serious diseases such as epilepsy, Alzheimer's disease, multiple sclerosis, chronic pain, cachexia, diabetes and sepsis and in the treatment of various types of cancer.[16-19]

The use of CBD in the area of dentistry has been studied little, and its effects in the treatment of oral diseases are not well known. Nonetheless, considering its properties, mechanisms of action and favorable results in treating other complex diseases, it is believed that it can exert a positive therapeutic effect on some pathologies that are still challenging for the oral surgeon, for example, OM.

The purpose of this review was to evaluate the therapeutic viability of CBD on the basis of scientific publications that discuss its mechanism of action, suggesting possibilities for future investigations regarding the use of this compound in dentistry.

METHODS

Accordingly, we carried out a review of the literature on the subject in the PubMed database. We used the **key words** "cannabinoids", "cannabidiol", "oxidative stress" and "oral mucositis" to search for full articles published in English from 1995 to 2016. Additional papers were obtained from the reference lists in the "hits". Classic and more recent studies covered mechanisms of action, indications of use, and possible future uses of CBD as well. Also included were publications regarding the etiology, clinical features and forms of treatment of OM.

RESULTS

Oral mucositis

OM is frequently observed in patients receiving antineoplastic therapies. It occurs in about 30 to 70% of patients treated with RT of the head and neck area and in 40 to 80% of patients undergoing CT. When the two modalities are combined, the incidence of these lesions tends to increase, ranging from 50 to 100%.[3, 6, 20, 21]

OM manifests as erythema of the oral mucosa or even extremely painful ulcerations. Its severity has been determined by various classifications, where the one recommended by the World Health of Organization (WHO) is the most widely used today. This takes into consideration clinical criteria, such as the presence and absence of injury, and subjective criteria, such as the presence of pain and ability to eat (Table 1). Its clinical features vary according to the type of cancer therapy used and the health of the patient.[3, 6, 21, 22]

Table 1. Classification of OM according to WHO criteria.

GRADE 0	No sign or symptom
GRADE 1	Erythema and slight pain
GRADE 2	Presence of ulcers and pain, still able to eat
GRADE 3	Presence of ulcers and pain and unable to eat solid food
GRADE 4	Presence of ulcers, unable to swallow, with need of parenteral or enteral support

Episodes of OM can greatly affect the quality of life of patients as well as the course of their cancer treatment. Oral pain is a common symptom, making it difficult or impossible to eat, leading in some cases to malnutrition. Moreover, resultant oral lesions are a port of entry for opportunistic pathogens, leading to infections that may require antibiotics, hospitalization and even discontinuation of cancer treatment. In addition to clinical consequences, OM has a significant economic impact, since the necessity of parenteral feeding, antibiotic therapy, control of pain and hospitalization significantly increase the costs of treatment.[2, 3, 6, 11, 21, 23]

Despite the clinical and economic implications of OM, no effective therapy has been developed to date. Some interventions are successful, although most of them are palliative. Patients should be encouraged to maintain good oral hygiene and use antiinflammatory agents, antibiotics, topical anesthetics and protective substances for the mucosa. Low-intensity laser therapy and the administration of epithelial growth factors are considered available resources in preventive interventions. However, they have high costs and require skilled staff. [2, 11, 21]

The understanding of the pathogenesis of OM is essential to the development of new preventive and therapeutic alternatives. Oxidative stress and proinflammatory cytokines (such as TNF-α) are directly involved in mucosal destruction secondary to cancer treatment. Specifically, an increase in reactive oxygen species (ROS) occurs after radiation treatment or the administration of chemotherapeutic drugs with a subsequent number of events that cause direct tissue damage. Several studies have investigated the genes responsible for the control of ROS metabolism; the presence of deletion-specific nucleotide polymorphisms in a group of cancer patients was associated with a greater mucositis risk. A new approach to predict

which patients will develop mucositis is the identification of certain single nucleotide polymorphisms (SNPs) using a genome-wide association and candidate gene approaches. [24]

Sonis *et al* in 2004 proposed the theory of five stages of OM (Table 2). Accordingly, its progression is seen not only as a result of direct cell damage but also as a series of complex biological events in the cells and tissues of the submucosa. [8-10, 25-26]

Table 2: Phases of development of OM. Sonis *et al* 2004.

Phase 1	**Initiation:** Formation of reactive oxygen species (ROS) due to CT or RT. oxidant–antioxidant imbalance.
Phase 2	**Response to primary damage:** Activation of transcription factors such as NF-kB.
Phase 3	**Signaling and amplification:** Regulation of proinflammatory cytokines: (TNF-α, interleukin 1-β and interleukin 6).
Phase 4	**Ulceration:** Ulceration of mucosa, inflammatory infiltrate rich in macrophages, neutrophils, mastocytes and bacterial colonization.
Phase 5	**Wound healing:** Differentiation of cells and tissues with restoration of integrity of mucosa.

According to this proposal, it is suggested that the formation of ROS arising from the action of CT and RT would be capable of generating an oxidant–antioxidant imbalance and thereby activate proinflammatory cytokines responsible for tissue damage. Therefore, different strategies of prevention and treatment of OM have been aimed at the control of OS. [1, 4, 5, 7, 27, 28]

Cannabidiol

Cannabis sativa, popularly known as marijuana, is the most used illegal drug in the world and can be associated with many health problems. This plant contains over 545 substances, which have different effects in the body. More than 100 of them are classified as cannabinoids, and since the end of the nineteenth century, marijuana has been widely studied for medicinal use. [15, 29, 30]

The term cannabinoid refers to a heterogeneous group of molecules that act on cannabinoid receptors present in the body. They can be produced endogenously, where anandamide

(AEA) and 2-arachidonoylglycerol (2AG) are the endocannabinoids best known and studied to date. There are also derivatives in the plant known as phytocannabinoids such as delta-9-tetrahydrocannabidiol (THC) and CBD, or synthetic cannabinoids that are developed in the laboratory. [31]

The two non-endogenous compounds most investigated are THC and CBD. It is known that limitations to the therapeutic use of THC stems from its capacity to cause psychoactive effects mediated through receptors in the central nervous system (CNS). CBD is totally devoid of psychoactive properties, due to the low affinity of these receptors for CBD, thus offering greater safety in clinical use. [12, 32]

Cannabinoid receptors were identified in the 1980s and named by order of discovery, i.e., CB1 and CB2.[14] They are involved in the modulation of neuronal functions and inflammatory processes and in the etiology of some diseases. Although CB1 and CB2 receptors share considerable structural similarities, they differ with regard to distribution and activity. CB1 receptors are located mainly in the CNS, peripheral nervous system (PNS) and some organs, mediating effects on cognition, memory, motor performance and perception of pain. On the other hand, CB2 receptors are mainly expressed in the immune system and play an important role in the establishment of inflammatory processes, since they are involved in the reduction of proinflammatory cytokines.[14, 29, 33, 34] Thus, it is known that the type of receptor that has greater affinity for each compound is crucial to the resulting pharmacological effects.

CBD was isolated in 1942 and its molecular structure determined in 1963, and interest in its pharmacodynamics was only aroused after many years. To date, the mechanisms of action of CBD have not been fully elucidated, and the cell signaling pathways involved are still little known. In the last decade, there has been a substantial growth in medical studies on the application of CBD, mainly motivated by the discovery of its antiinflammatory, antioxidant and neuroprotective activities.[14, 35, 36]

Studies show that this compound is capable of suppressing the production of proinflammatory mediators through the suppression of cellular immune response, which can be important in the treatment of various 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 antiinflammatory action of CBD. [18]

Many authors mention that the effect of CBD on OS can be more potent than that of classic antioxidants, such as α-tocopherol. A pioneer study conducted by Julius Axelrod and David Wink in 1998 showed that CBD was a potent neuroprotective antioxidant. The authors noted that there was a greater efficacy against glutamate-induced neurotoxicity than with ascorbate or α-tocopherol. This suggests that CBD has potential therapeutic application in neurodegenerative disorders associated with OS.[13, 17, 18, 33, 37-41]

Rajan *et al* evaluated the antiinflammatory, antioxidative, and antiapoptotic effects of low doses of CBD. The results showed that 5 μM CBD had a moderate antiinflammatory effect (TNF-α, IL-10) and oxidative markers (inducible nitric oxide synthase, nuclear factor erythroid 2-related factor 2, nitrotyrosine). Their in vitro results demonstrated the antiinflammatory, antioxidant, and antiapoptotic effects of CBD. [42]

DISCUSSION

An increase in FR, as evidenced by cell damage caused by CT and/or RT, is capable of causing an imbalance in OS. This, in turn, stimulates the production of proinflammatory mediators responsible for tissue damage in OM. Thus, CBD could act both in the control of OS and the suppression of the inflammatory response, having a protective role against the development of OM. However, this hypothesis remains to be tested in a clinical and molecular setting, since we did not find specific studies covering this topic.

In the area of dentistry, only one experimental study of CBD was found, which was carried out in a rat model with induced periodontitis. After 30 days of treatment with 5 mg/kg CBD daily, morphological analysis of alveolar bone loss demonstrated that the animals treated with the compound exhibited a regional decrease in bone loss and a lower expression of NF-kB. Moreover, in the gingival tissue of the CBD-treated group, there was a decrease in neutrophil migration, with reduced production of interleukin-1β and TNF-α.[43] These results suggest that CBD may be useful in the control of inflammation caused by induced periodontal disease in rats. The possibility of searching for similar results in study models of OM can evaluate research in this area, aimed at obtaining favorable clinical effects.

A literature review of the use of cannabinoids in the management of chronic pain in dentistry was also found. The authors addressed the possibility of using CBD in the control of symptoms of burning mouth syndrome, neuralgia of the trigeminal nerve and postherpetic

neuralgia. On the basis of the proven involvement of the endocannabinoid system in analgesia, promising results in studies of pain of various etiologies (Table 3) and of the mechanism of action of CBD, the authors suggested that this could be a potential therapeutic agent for the above disorders. The prospect of controlling pain with CBD can be another favorable way of managing OM. [30]

In the United Kingdom, Canada and other countries, an oral spray containing 50% CBD (Sativex) can be legally prescribed for the purpose of relieving pain and spasticity associated with multiple sclerosis, which denotes the safety of its use in humans.[31, 41-44] However, an observational study in a limited sample with 8 patients investigated the effects of oral use of this product. The most frequently reported findings were a burning sensation after application, dry mouth, halitosis and hyperkeratosis. However, the authors warned of the possibility of these results not being linked specifically to CBD but rather to the high concentration of alcohol in the product.[43]

Even though we evaluated studies on CBD conducted in the medical area, the literature that supports the therapeutic potential of its use is still limited and lacks well-designed clinical studies, regardless of the disorder in question. Most findings are from *in vitro* or *in vivo* preclinical research and clinical cases.[34] There are several reports of the protective action of CBD in other disorders associated with the toxicity of anticancer therapy mediated by OS, as well as systemic effects that may be of interest, such as its antiemetic activity and ability to improve renal and cardiac function (Table 3).

Table 3: Preclinical studies of CBD in animal models to control adverse effects of CT.

Study/Model	CT Agent	Dose CBD	Results	Reference
Antiemetic activity	LiCl	2.5 mg	Improvement in the control of vomiting with fewer side effects when combined with other compounds. [47].	Rock EM, Parker LA 2015
Antiemetic activity	Cisplatin	2.5 or 0.5 mg	Antiemetic and antinausea effects by indirect activation of 5-HT receptor[48]	Rock *et al* 2012
Prevention of nephrotoxicity	Cisplatin	2.5 or 10 mg	Decrease in ROS and improvement in renal function. [12]	Pan 2008
Prevention of cardiotoxicity	Doxorubicin	5 mg	Decrease in proinflammatory cytokines, attenuation of decline in antioxidants and improvement in cardiac function. [49]	Fouad *et al* 2013
Prevention of cardiotoxicity	Doxorubicin	10 mg	Decrease in cardiac dysfunction, improvement in cardiac mitochondrial function and decrease in oxidative stress. [32]	Hao *et al* 2015
Prevention of neuropathic pain	Paclitaxel	2.5 or 10 mg	Attenuation of pain without hampering treatment efficacy.[50]	Ward *et al* 2014

While Table 3 shows various events associated with the adverse effects of CT, the studies cited are mediated by OS as well as OM. On the basis of the favorable results of these studies, it can be proposed that CBD is capable of exerting some protective effect in oral tissues in the management of OM (Fig 1). However, there is still a lack of consensus in the dose to be administered, which confirms the need for new studies that can establish dose-response parameters.

Figure 1: Potential anti-mucositis actions of CBD
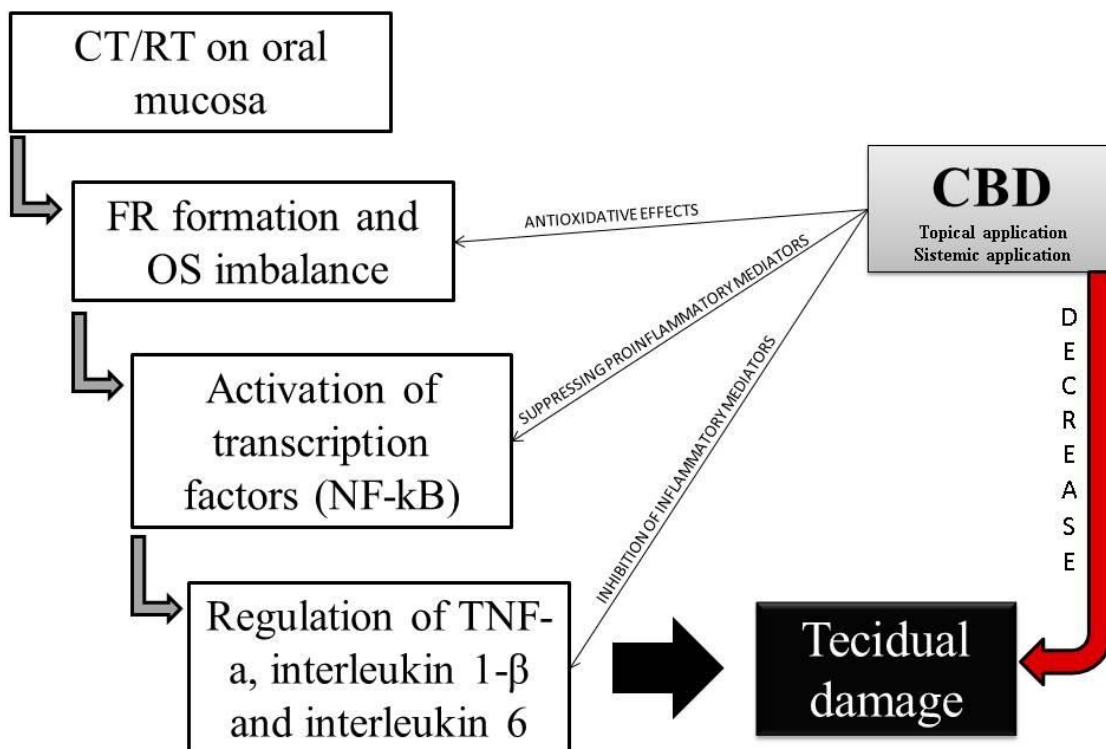
A recent review of the literature concluded that medical marijuana has potential therapeutic applications in oncology, but the available evidence and legal status pose a challenge for physicians and oncology providers. There is moderate evidence for the use of cannabis in pain management. Some patients subjectively report benefit from cannabis for nausea, appetite, sleep, and anxiety problems, but the level of published evidence remains low. Clinical trials are underway, but the legal status in some countries presents challenges to research. It is critical that oncology professionals are able to at least address the known risks and adverse effects of marijuana when questions arise from patients. Current available evidence is conflicting in terms of cancer risk. There is preliminary research on the anticancer effects of cannabis, which may balance out the risk to some extent. Overall, medical marijuana may have use in cancer care, but more research is needed to better inform physicians and patients.[51]

As an alternative to treat complications of antineoplasic therapies, added to eventual beneficial effects, it is important to consider the interaction of CBD with tumoral cells. Risks and benefits of antioxidant agents for antineoplasic have been debated among oncologists,

radiotherapists and other professionals. It is speculated that drugs able to protect healthy tissues could also protect tumor cells, jeopardizing regular treatment.

Recent studies point to CBD as a promising alternative to cancer treatment due to its ability to inhibit 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 neoplasm is to be treated and respective drug. [52, 53, 54]

It is compulsory to further investigate CBD in the management of OM to unveil its actual potenciality.

WHAT IS NEW AND CONCLUSIONS

The lack of effective strategies for the prevention of OM have prompted researchers to investigate new therapeutic agents, in view of the serious consequences caused by this disease. Studies on the action of various types of antioxidant agents in the prevention of OM represent a significant part of current research regarding this disease, which could contribute to the better understanding of the biopathology of OM and of the role of FR in its development.

The antioxidant, antiinflammatory and analgesic activity of CBD, along with its safety, oare decisive factors in stirring interest in its use in dentistry. In view of the foregoing, we believe that studies should be encouraged in testing CBD in the treatment of difficult-to-manage oral diseases, such as OM. Its mechanism of action seems to involve functions that could play a positive role in the prevention and treatment of OM.

References:

1. Lalla RV, Bowen J, Barasch A, Elting L, Epstein J, Keefe DM, McGuire DB, Migliorati C, Nicolatou-Galitis O, Peterson DE, Raber-Durlacher JE, Sonis ST, Elad S. Mucositis Guidelines Leadership Group of the Multinational Association of Supportive Care in Cancer and International Society of Oral Oncology (MASCC/ISOO) MASCC/ISOO clinical practice guidelines for the management of mucositis secondary to cancer therapy. *Cancer*, 2014; 10:1453-1461. doi:10.1002/cncr.28592, Review. Erratum in: Cancer. 2015 Apr 15; 121:1339

2. de Araújo AA, Varela H, de Medeiros CA, de Castro Brito GA, de Lima KC, de Moura LM, et al. Azilsartan reduced TNF-α and IL-1β levels, increased IL-10 levels and upregulated VEGF, FGF, KGF, and TGF-α in an oral mucositis model. *PLoS One*, 2015; 10:e0116799. doi: 10.1371/journal.pone.0116799. eCollection 2015.

3. Barkokebas A, Silva IH, de Andrade SC, Carvalho AA, Gueiros LA, Paiva SM, et al. Impact of oral mucositis on oral-health-related quality of life of patients diagnosed with cancer. *J Oral Pathol Med*, 2015; 44:746-751. doi: 10.1111/jop.12282.

4. de Freitas Cuba L, Salum FG, Cherubini K, de Figueiredo MA. Antioxidant agents: a future alternative approach in the prevention and treatment of radiation-induced oral mucositis? *Altern Ther Health Med*, 2015; 21:36-41. Review. PMID: 25830279

5. de Freitas Cuba L, Braga Filho A, Cherubini K, Salum FG, Figueiredo MA. Topical application of Aloe vera and vitamin E on induced ulcers on the tongue of rats subjected to radiation: clinical and histological evaluation. *Support Care Cancer*, 2015. doi: 10.1007/s00520-015-3048-3

6. Miyamoto H, Kanayama T, Horii K, Kawai T, Tsuchimochi T, Shigetomi T, et al. The relationship between the severity of radiation-induced oral mucositis and the myeloperoxidase levels in rats. *Oral Surg Oral Med Oral Pathol Oral Radiol*, 2015; 120:329-336. doi: 10.1016/j.oooo.2015.04.012.

7. Urbain P, Raynor A, Bertz H, Lambert C, Biesalski HK. Role of antioxidants in buccal mucosa cells and plasma on the incidence and severity of oral mucositis after allogeneic hematopoietic cell transplantation. *Support Care Cancer*, 2012; 20:1831-1838. doi:10.1007/s00520-011-1284-8

8. Sonis ST. Pathobiology of mucositis. *Semin Oncol Nurs*, 2004; 20:11-15. doi:10.1053/j.soncn.2003.10.003

9. Sonis ST. The pathobiology of mucositis. *Nat Rev Cancer*, 2004; 4:277-284. doi:10.1038/nrc1318

10. Sonis ST, Elting LS, Keefe D, Peterson D, Schubert M, HauerJensen M et al. Perspective on cancer therapy-induced mucosal injury - pathogenesis, measurement,

epidemiology, and consequences for patients. *Cancer*, 2004; 100:1995-2024. doi:10.1002/cncr.2016

11. Al-Dasooqui N, Sonis ST, Bowen JM, Bateman E, Blijlevens N, Gibson RJ et al. Emerging evidence on the pathobiology of mucositis. *Support Care Cancer*, 2013; 21:2075-2083. doi:10.1007/s00520-013-1810-y

12. Pan H, Mukhopadhyay P, Rajesh M, Patel V, Mukhopadhyay B, Gao B, et al. Cannabidiol attenuates cisplatin-induced nephrotoxicity by decreasing oxidative/nitrosative stress, inflammation, and cell death. *J Pharmacol Exp Ther*, 2009; 328:708-714. doi: 10.1124/jpet.108.147181

13. Cassol-Jr OJ, Comim CM, Silva BR, Hermani FV, Constantino LS, Felisberto F, et al. Treatment with cannabidiol reverses oxidative stress parameters, cognitive impairment and mortality in rats submitted to sepsis by cecal ligation and puncture. *Brain Res*, 2010; 1348:128-138. doi: 10.1016/j.brainres.2010.06.023

14. Burstein S. Cannabidiol (CBD) and its analogs: a review of their effects on inflammation. *Bioorg Med Chem*, 2015; 23:1377-1385. doi: 10.1016/j.bmc.2015.01.059.

15. Friedman D, Devinsky O. Cannabinoids in the Treatment of Epilepsy. *N Engl J Med*, 2015; 373:1048-1058. doi: 10.1056/NEJMra1407304.

16. Elbaz M, Nasser MW, Ravi J, Wani NA, Ahirwar DK, Zhao H, et al. Modulation of the tumor microenvironment and inhibition of EGF/EGFR pathway: novel anti-tumor mechanisms of Cannabidiol in breast cancer. *Mol Oncol*, 2015; 9:906-919. doi: 10.1016/j.molonc.2014.12.010.

17. Yang L, Rozenfeld R, Wu D, Devi LA, Zhang Z, Cederbaum A. Cannabidiol protects liver from binge alcohol-induced steatosis by mechanisms including inhibition of oxidative stress and increase in autophagy. *Free Radic Biol Med*, 2014; 68:260-267. doi: 10.1016/j.freeradbiomed.2013.12.026.

18. Booz GW. Cannabidiol as an emergent therapeutic strategy for lessening the impact of inflammation on oxidative stress. *Free Radic Biol Med*, 2011; 51:1054-1061. doi: 10.1016/j.freeradbiomed.2011.01.007.

19. Davis MP. Cannabinoids for Symptom Management and Cancer Therapy: The Evidence. *J Natl Compr Canc Netw*, 2016; 14:915-922.

20. Miranzadeh S, Adib-Hajbaghery M, Soleymanpoor L, Ehsani M. Effect of adding the herb Achillea millefolium on mouthwash on chemotherapy induced oral mucositis in cancer patients: A double-blind randomized controlled trial. *Eur J Oncol Nurs*, 2015; 19:207-213. doi: 10.1016/j.ejon.2014.10.019.

21. Bonomi M, Batt K. Supportive Management of Mucositis and Metabolic Derangements in Head and Neck Cancer Patients. *Cancers (Basel)*, 2015; 7:1743-1757. doi: 10.3390/cancers7030862.

22. Epstein JB, Thariat J, Bensadoun RJ, Barasch A, Murphy B, Kolnick L et al. Oral complications of cancer and cancer therapy. *CA Cancer J Clin*, 2012; 62: 400-422. doi: 10.3322/caac.21157.

23. Morimata J, Otomaru T, Murase M, Haraguchi M, Sumita Y, Taniguchi H. Investigation of factor affecting health-related quality of life in head and neck cancer patients. *Gerodontology*, 2012; 30:194-200. doi: 10.1111/j.1741-2358.2012.00662.x.

24. Alessandro Villa & Stephen T. Sonis. Pharmacotherapy for the management of cancer regimen-related oral mucositis, *Expert Opinion on Pharmacotherapy*, 2016. DOI: 10.1080/14656566.2016.1217993

25. Sonis ST. Oral mucositis. *Anticancer Drugs*, 2011; 22:607-612. doi: 10.1097/CAD.0b013e3283462086.

26. Sonis ST. Oral mucositis in head and neck cancer: risk, biology, and management. *Am Soc Clin Oncol Educ Book*, 2013. doi: 10.1200/EdBook_AM.2013.33.e236.

27. Yarom N, Ariyawardana A, Hovan A, Barasch A, Jarvis V, Jensen SB et al. Systematic review of natural agents for the management of oral mucositis in cancer patients. *Support Care Cancer*, 2013; 21:3209-3221. doi:10.1007/s00520-013-1869-5.

28. Aghamohamamdi A, Hosseinimehr SJ. Natural Products for Management of Oral Mucositis Induced by Radiotherapy and Chemotherapy. *Integr Cancer Ther*, 2015; 15:60-68. doi: 10.1177/1534735415596570.

29. Atakan Z. Cannabis, a complex plant: different compounds and different effects on individuals. *Ther Adv Psychopharmacol*, 2012; 2:241-254. doi: 10.1177/2045125312457586.

30. Gould J. The cannabis crop. *Nature*, 2015; 525:S2-3. doi: 10.1038/525S2a.

31. McDonough P, McKenna JP, McCreary C, Downer EJ. Neuropathic orofacial pain: cannabinoids as a therapeutic avenue. *Int J Biochem Cell Biol,* 2014; 55:72-78. doi: 10.1016/j.biocel.2014.08.007.

32. Hao E, Mukhopadhyay P, Cao Z, Erdélyi K, Holovac E, Liaudet L et al. Cannabidiol Protects against Doxorubicin-Induced Cardiomyopathy by Modulating Mitochondrial Function and Biogenesis. *Mol Med*, 2015; 21:38-45. doi: 10.2119/molmed.2014.00261.

33. Fine PG, Rosenfeld MJ. The endocannabinoid system, cannabinoids, and pain. *Rambam Maimonides Med J*, 2013; 4:e0022. doi: 10.5041/RMMJ.10129.

34. van Amsterdam J, Brunt T, van den Brink W. The adverse health effects of synthetic cannabinoids with emphasis on psychosis-like effects. *J Psychopharmacol*, 2015; 29:254-263. doi: 10.1177/0269881114565142.

35. Baron EP. Comprehensive Review of Medicinal Marijuana, Cannabinoids, and Therapeutic Implications in Medicine and Headache: What a Long Strange Trip It's Been. *Headache*, 2015; 55:885-916. doi: 10.1111/head.12570.

36. Ibeas Bih C, Chen T, Nunn AV, Bazelot M, Dallas M, Whalley BJ. Molecular Targets of Cannabidiol in Neurological Disorders. *Neurotherapeutics,* 2015; 12:699-730. doi: 10.1007/s13311-015-0377-3.

37. Giacoppo S, Mandolino G, Galuppo M, Bramanti P, Mazzon E. Cannabinoids: New Promising Agents in the Treatment of Neurological Diseases. *Molecules*, 2014; 19:18781-18816. doi:10.3390/molecules191118781.

38. Hampson AJ, Grimaldi M, Axelrod J, Wink D. Cannabidiol and (-)Delta9-tetrahydrocannabinol are neuroprotective antioxidants. *Proc Natl Acad Sci USA*, 1998; 95:8268-8273.

39. Borges RS, Batista J Jr, Viana RB, Baetas AC, Orestes E, Andrade MA, et al. Understanding the molecular aspects of tetrahydrocannabinol and cannabidiol as antioxidants. *Molecules*, 2013; 18:12663-12674. doi: 10.3390/molecules181012663.

40. Rajesh M, Mukhopadhyay P, Bátkai S, Patel V, Saito K, Matsumoto S. Cannabidiol attenuates cardiac dysfunction, oxidative stress, fibrosis, inflammatory and cell death signaling pathways in diabetic cardiomyopathy. *J Am Coll Cardiol*, 2010; 56:2115-2125. doi:10.1016/j.jacc.2010.07.033.

41. Aviello G, Romano B, Borrelli F, Capasso R, Gallo L, Piscitell F et al. Chemopreventive effect of the non-psychotropic phytocannabinoid cannabidiol on experimental colon cancer. *J Mol Med*, 2012; 90:925-934. doi 10.1007/s00109-011-0856-x.

42. Rajan TS, Giacoppo S, Iori R, De Nicola GR, Grassi G, Pollastro F, Bramanti P, Mazzon E. Anti-inflammatory and antioxidant effects of a combination of cannabidiol and moringin in LPS-stimulated macrophages. *Fitoterapia*, 2016; 112:104-115. doi: 10.1016/j.fitote.2016.05.008. Epub 2016 May 20.

43. Napimoga MH, Benatti BB, Lima FO, Alves PM, Campos AC, Pena-Dos-Santos DR et al. Cannabidiol decreases bone resorption by inhibiting RANK/RANKL expression and pro-inflammatory cytokines during experimental periodontitis in rats. *Int Immunopharmacol*, 2009;9:216-222. doi: 10.1016/j.intimp.2008.11.010.

44. Welty TE, Luebke A, Gidal BE. Cannabidiol: promise and pitfalls. *Epilepsy Curr*, 2014; 14:250-252. doi: 10.5698/1535-7597-14.5.250.

45. Russo M, Naro A, Leo A, Sessa E, D'Aleo G, Bramanti P, et al. Evaluating Sativex® in Neuropathic Pain Management: A Clinical and Neurophysiological Assessment in Multiple Sclerosis. *Pain Med*, 2016. doi: http://dx.doi.org/10.1093/pm/pnv080

46. Scully C. Cannabis; adverse effects from an oromucosal spray. *Br Dent J*, 2007; 203:336-337. doi: 10.1038/bdj.2007.749.

47. Rock EM, Parker LA. Synergy between cannabidiol, cannabidiolic acid, and Δ^9-tetrahydrocannabinol in the regulation of emesis in the Suncus murinus (house musk shrew). *Behav Neurosci*, 2015; 129:368-370. doi: 10.1037/bne0000057.

48. Rock EM, Bolognini D, Limebeer CL, Cascio MG, Anavi-Goffer S, Fletcher PJ et al. Cannabidiol, a non-psychotropic component of cannabis, attenuates vomiting and nausea-like behaviour via indirect agonism of 5-HT(1A) somatodendritic autoreceptors in the dorsal raphe nucleus. *Br J Pharmacol*, 2012; 16:2620-2634. doi: 10.1111/j.1476-5381.2011.01621.x.

49. Fouad AA, Albuali WH, Al-Mulhim AS, Jresat I. Cardioprotective effect of cannabidiol in rats exposed to doxorubicin toxicity. *Environ Toxicol Pharmacol*, 2013; 36:347-357. doi: 10.1016/j.etap.2013.04.018.

50. Ward SJ, McAllister SD, Kawamura R, Murase R, Neelakantan H, Walker EA. Cannabidiol inhibits paclitaxel-induced neuropathic pain through 5-HT(1A) receptors without diminishing nervous system function or chemotherapy efficacy. *Br J Pharmacol*, 2014; 171:636-645. doi: 10.1111/bph.12439.

51. Birdsall SM, Birdsall TC, Tims LA. The Use of Medical Marijuana in Cancer. *Curr Oncol Rep*, 2016; 18:40. doi: 10.1007/s11912-016-0530-0.

52. Fowler CJ. Δ9-tetrahydrocannabinol and cannabidiol as potential curative agents for cancer. A critical examination of the preclinical literature. *Clin Pharmacol Ther.* 2015; 6:587-596. doi: 10.1002/cpt.84.

53. Fraguas-Sánchez AI, Fernández-Carballido A, Torres-Suárez AI. Phyto-, endo- and synthetic cannabinoids: promising chemotherapeutic agents in the treatment of breast and prostate carcinomas. *Expert Opinion on Investigational Drugs*, 2016; 11:1311-1323. doi: 10.1080/13543784.2016.1236913.

54. Davis MP.Cannabinoids for Symptom Management and Cancer Therapy: The Evidence. *J Natl Compr Canc Netw*. 2016;14:915-922. PMID: 27407130.

4. ARTIGO II

O artigo a seguir intitula-se "**EFFECT OF CANNABIDIOL, A COMPONENT OF *CANNABIS SATIVA*, ON 5-FLUOROURACIL-INDUCED ORAL MUCOSITIS IN MICE: CLINICAL, HISTOLOGICAL, HEMATOLOGICAL AND BIOCHEMICAL EVALUATION**" e será submetido ao periódico Oral Oncology, o qual possui Qualis A1 e Fator de Impacto 4.636.

Effect of cannabidiol, a component of *Cannabis sativa*, on 5-fluorouracil-induced oral mucositis in mice: clinical, histological, hematological and biochemical evaluation

Letícia de Freitas Cuba, MSc[a] ; Fernanda Gonçalves Salum, PhD[b] ; Francisco Silveira Guimarães, PhD [c] ; Karen Cherubini, PhD [d]; Ruchielli Loureiro Borghetti, PhD[e]; Maria Antonia Zancanaro de Figueiredo PhD[d].
Division of Oral Medicine – PUCRS Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, RS, Brazil.

[a]PhD student of Oral Medicine, Pontifical Catholic University of Rio Grande do Sul (PUCRS), Porto Alegre, Brazil.
[a]Clinical Lecturer of Oral Medicine, Paranaense University (UNIPAR), Francisco Beltrão, Brazil.
[b]Senior Lecturer of Oral Medicine, Pontifical Catholic University of Rio Grande do Sul (PUCRS), Porto Alegre, Brazil.
[c] Department of Pharmacology, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Brazil.
[d]Professor of Oral Medicine, Pontifical Catholic University of Rio Grande do Sul (PUCRS), Porto Alegre, Brazil.
[e]Department of Oral Medicine, São Lucas Hospital, Porto Alegre, Brazil.

Corresponding author: Maria Antonia Z. de Figueiredo
Av. Ipiranga 6690 – Sala 231 CEP 90610-000 – Porto Alegre – RS- Brazil
antonia.figueiredo@pucrs.br, telephone (+5551) 999930005 fax number (+5551)33203254

Word count: 4139

Abstract

Objectives: 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.

Materials and Methods: We used 90 mice in which oral mucositis was induced using a protocol with 5-fluorouracil 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 and negative control), 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 antioxidant enzyme activity, the groups treated

with CBD showed better results, but only some of these variables showed statistically significant differences.

Conclusions: Altogether, these findings suggest that CBD exerted antiinflammatory and antioxidant activity favoring a faster resolution of oral mucositis in this animal model.

Key words: Oral Mucositis, Chemotherapy, Cannabidiol; *Cannabis sativa*; Mice

Introduction

Oral mucositis (OM) is a common complication in patients undergoing chemotherapy (CT). Its occurrence depends on the therapeutic protocol used, and can affect 60 to 80% of patients under this antineoplastic therapy[1–3]. It is a debilitating condition that sets in around 5 to 7 days after administration of CT and persists usually for days to weeks. It presents clinically as an inflammatory response, with areas of ulceration of the mucosa, in varying degrees of severity. It is accompanied by painful symptomatology and dysphagia, and may result in weight loss, malnutrition, and susceptibility to opportunistic infections as well. It affects patients' quality of life and may become a dose-limiting factor in treatment [1,3,4].

5-Fluorouracil (5-FU) belongs to the class of antimetabolite anticancer drugs capable of interfering with essential biochemical processes. It is widely used in the treatment of various solid malignancies such as those that affect the gastrointestinal tract, gynecological system and breast, head and neck, and other anatomical sites. 5-FU therapy is associated with a wide range of adverse effects, including gastrointestinal mucositis, which is sometimes accompanied by abdominal pain, nausea, vomiting, diarrhea and dehydration, and is one of the chemotherapeutic agents with the greatest association with the incidence of the OM [5–10].

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 (ROS) and oxidative stress (OS). The consistent reports linked to the formation of free radicals after exposure to CT, added to the results of various studies, demonstrate that mucosal lesions can be attenuated and even prevented by agents able to limit OS caused by free radicals. These agents are called antioxidants (AOX), 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 [3,6,11–16].

Canabidiol (CBD) is a component of *Cannabis sativa* (marijuana), a plant that exerts potent antiinflammatory, immunomodulatory, antiemetic and analgesic effects through the activation of cannabinoid receptors (CB1 and CB2) located in the central nervous system and immune cells [17,18]. Studies have reported that CBD does not have psychotropic activity and exerts beneficial effects in neuropsychiatric diseases and inflammatory disorders, through its antioxidant, antiinflammatory and immunomodulatory action [19–26]. On the basis of

these premises, it is believed that CBD can be an alternative in the treatment of CT-induced OM.

In view of the biological complexity of the pathogenesis of OM and considering the 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 the tongue ventral induced by 5-FU.

Materials and Methods

Animal model

The sample consisted of 90 male mice of the CF-1 strain, 10 weeks old, weighing between 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 accommodated and acclimatized in the CeMBE, 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, placed in micro isolators with constant temperature of 23 ± 1º C, relative humidity of 50 ± 5% and 12-h light-dark cycle (lights on at 07 h, lights off at 19 h).

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).

Study groups and experimental times

The animals were identified by their tail as to which group they belonged according to the following scheme (Fig. 1).

technique, with some modifications, has been extensively used to induce OM similar to that occurring in humans [27–29]. Immediately after the mechanical trauma on the 4th day, the animals began to receive treatment determined for each group, IP every 24 h. 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, at 4 and 7 days of treatment.

Hematological analysis

Prior to euthanasia, the animals were anesthetized by inhalation of isoflurane and then blood samples collected by the cardiac puncture method (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 collected 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 determined.

Clinical and histological evaluation

Immediately after euthanasia, region was clinically evaluated in the tongue ventrum subjected to the trauma by 2 blinded examiners, 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 [28–30]:

0 - Normal
1 - Erythema
2 - Epithelial desquamation
3 - Ulcer on up to 25% of surface
4 - Ulcer on more than 25% up to 50% of surface
5 - Ulcer on more than 50% up to 75% of surface
6 - 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 h, where the longitudinal section of the ulcer area was in 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. Intra-examiner calibration was done by reanalysis of 20 slides with a 7-day interval between observations (Kappa=0.889 ± 0.061, p<0.001). Next, the field with the highest intensity of inflammatory response was chosen to determine the score, according to the criteria below [31–34]:

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

Biochemical analysis of oxidative stress

For OS evaluation, the liver was surgically removed from each animal. 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 antioxidant enzyme catalase (CAT) and antioxidant reduced glutathione (GSH) were measured using a spectrophotometer Cary 100.

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 was used in the analysis of inflammatory response and comparative OM between the study times.

The significance level was set at 5% for all analyses.

Results

During the experiment, 11 animals died. Therefore, 79 mice were included in the study with following distribution: 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) and 5B (n=9). All animals lost weight during the experiment, as shown in Fig. 2.
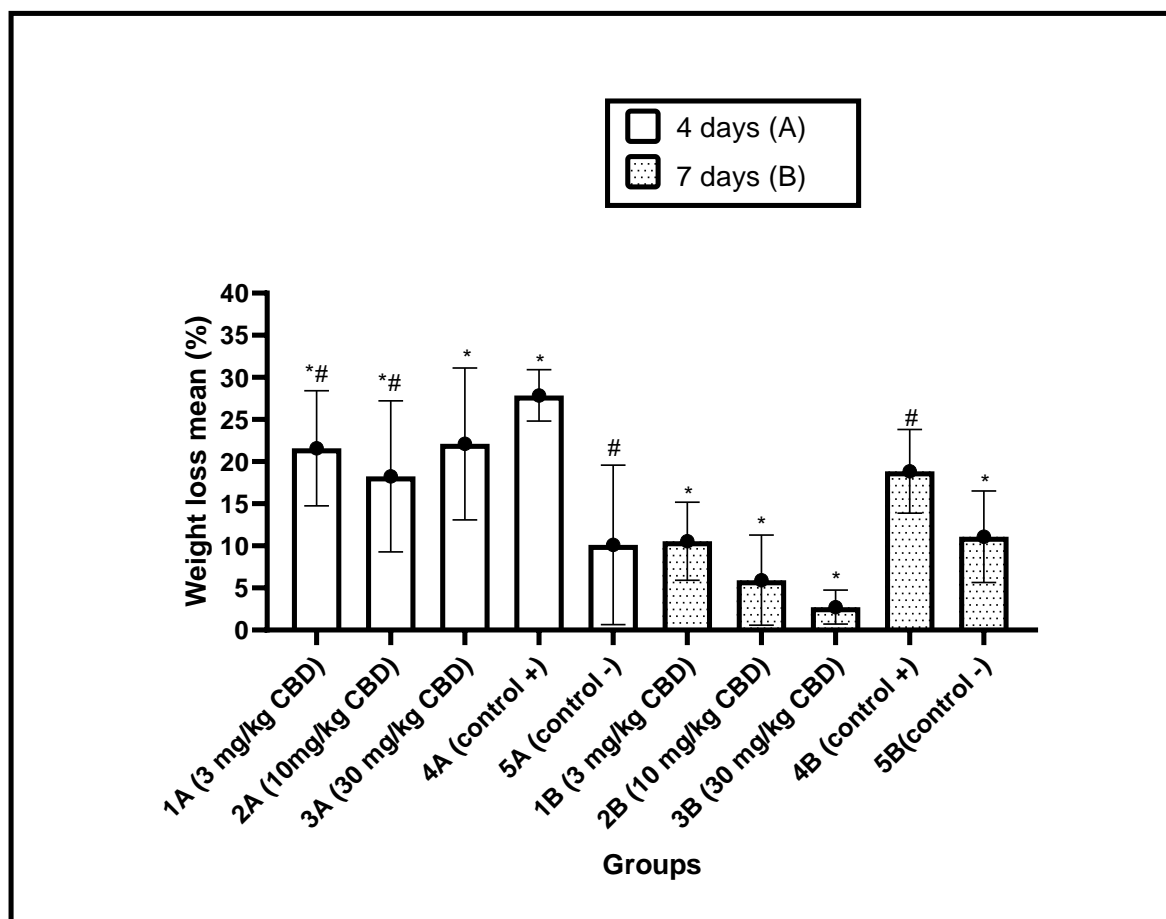
Fig. 2. Mean ± standard deviation weight loss of study groups at 4 and 7 days.
*#: Different symbols indicate statistically significant difference between the groups

Among the groups that received CT there was no statistical difference in the experimental time of 4 days ($p=0.135$). However, the greatest weight loss was observed in group 4A animals, which differed significantly from group 5A ($p=0.001$). Among the groups treated with CBD, 2A showed less weight loss, statistically similar to group 5A ($p=0.326$).

In the 7-day evaluation, all groups treated with CBD showed lower weight loss than the control groups, although there was a statistically significant difference only in relation to group 4B ($p=0.000$), showing no significant difference compared with group 5B ($p=0.371$). The least weight loss was observed in group 3B.

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 did not show a statistically significant difference (p=0.655).

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 was found in all animals at 4 days and was completely healed in the 7-day analysis (Fig. 3). The classification of the OM lesions is described in Table 1.
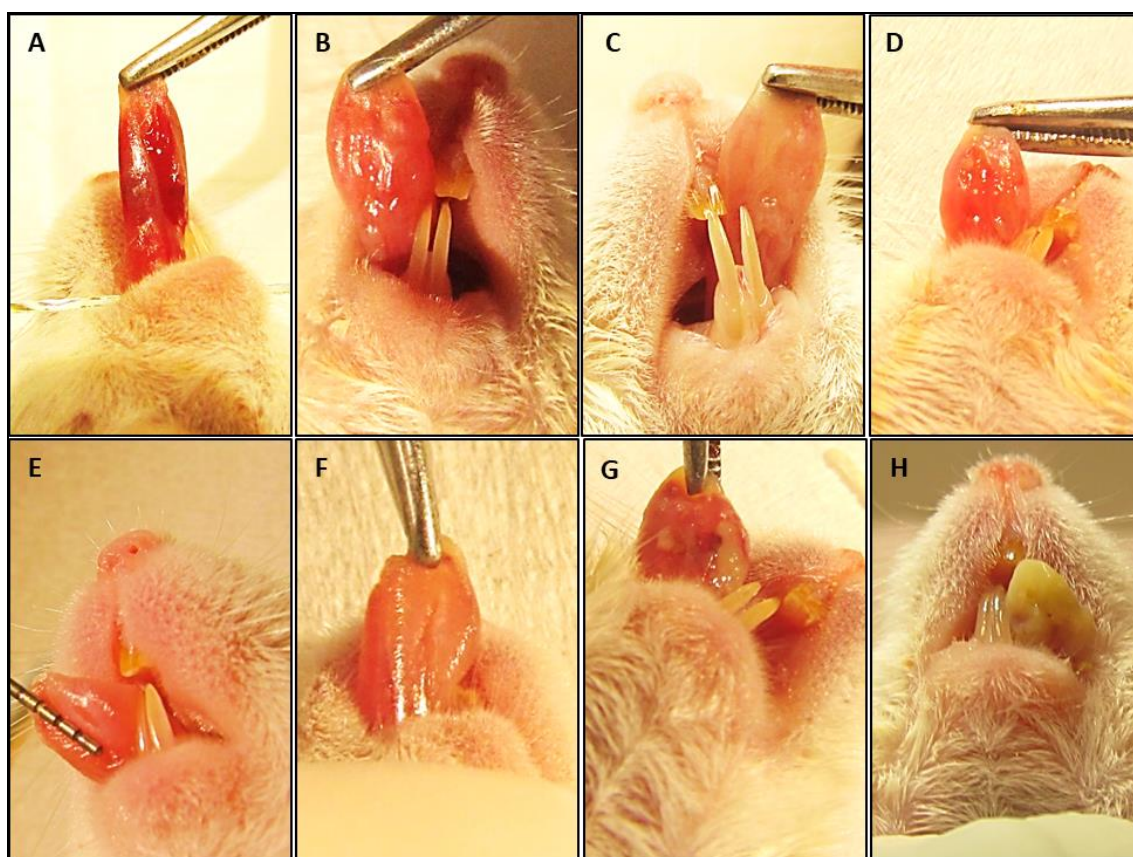
Fig. 3. Clinical evaluation of OM. (A) Immediately after mechanical trauma. (B) Animal in group 2A showing grade 3 OM. (C) Animal in group 3A showing grade 4 OM. (D) Animal in group 4A showing grade 5 OM. (E) Animal in group 1B showing a discrete ulcerated lesion. (F) Animal in group 2B showing grade 3 OM. (G) Animal in group 4B showing grade 5 OM. (H) Animal in group 4B showing grade 6 OM.

In the 4-day clinical analysis, all animals displayed some degree of OM ranging from grade 3 to 5. However, the severity of the lesions was greater in group 4A, differing

statistically from the others. When comparing the groups treated with CBD, there was no statistical difference between them (p=0.580; p=0.756; p=0.788). In the 7-day experimental period, there was a decrease in the severity of OM in all groups that received CBD treatment, ranging from grade 0 to 3, but no statistically significant difference was found between these groups (p=0.419; p=0.659; p=0.792). In the animals of groups 4A and 4B (positive control), the severity of the lesions remained unchanged or worsened, varying from grade 3 to 6 (p=0.001) (Fig. 3). 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 (p=0.002) and 3A and 3B (p=0.029).

Table 1

Comparison of classification of induced OM in relation to study groups that received chemotherapy (Kruskal-Wallis and Student-Newman-Keuls test).

Classification of OM	TIME							
	4 days				7 days			
	1A	2A	3A	4A	1B	2B	3B	4B
	(*n*=8)	(*n*=7)	(*n*=9)	(*n*=9)	(*n*=9)	(*n*=7)	(*n*=5)	(*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*	0.006				0.001			
*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]

*comparison between treatment and control
** rank means followed by different letters are significantly different
1= 3 mg/Kg CBD; 2= 10 mg/Kg CBD; 3= 30 mg/Kg CBD; 4= placebo

Histological analysis of inflammatory response

The intensity of the inflammatory response varied from mild to severe (Fig. 4) in all groups that received 5-FU in the 4-day analysis, with no statistically significant difference

between these groups (p=0.763). However, at the same experimental time, the negative control group (5A) displayed mild to moderate inflammation but not significantly different compared with the other study groups (p=0.643).

In the 7-day analysis, the inflammatory response scores remained between mild and intense in groups 1B, 2B, 3B and 4B with no significant difference (p=0.095), while most of group 5B animals showed scores reduced to mild (p=0.003).

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 (p=0.004) and 4A and 4B (p=0.05). The classification of the inflammatory response is described in Table 2.
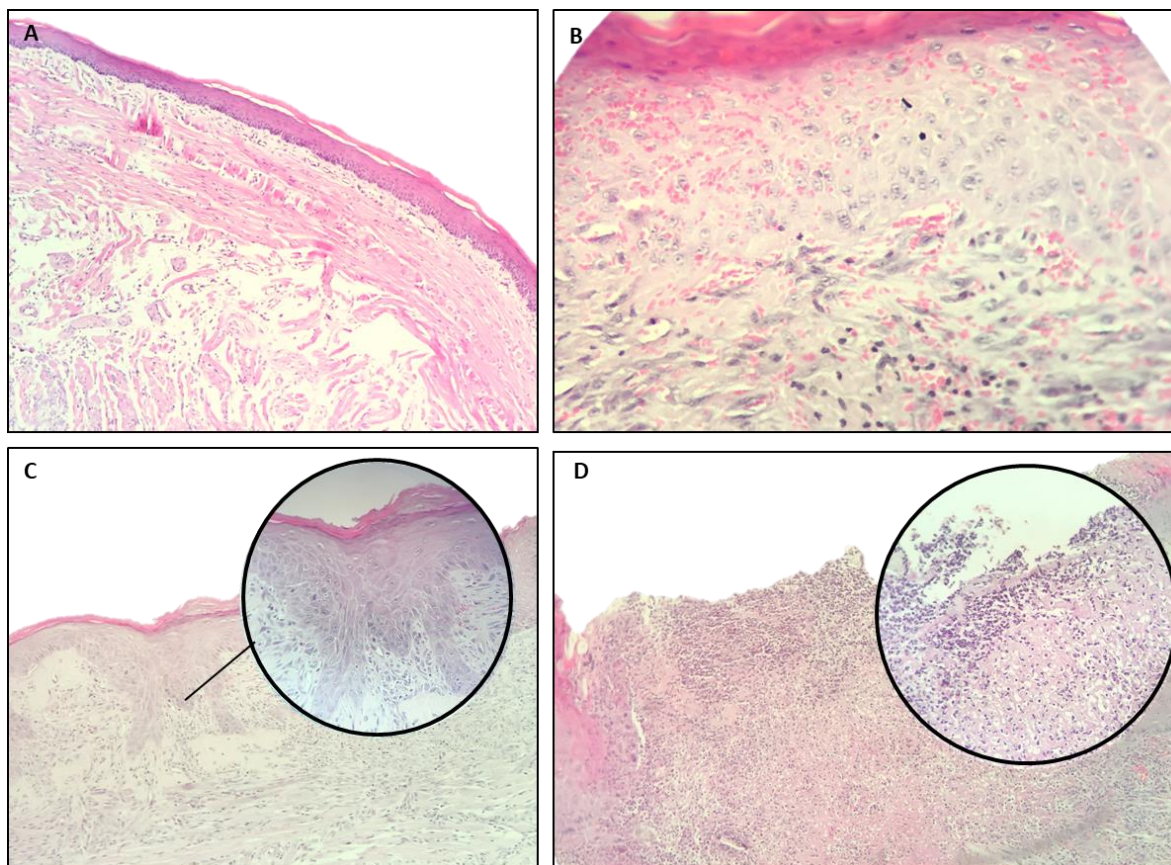
Fig. 4. A: Photomicrograph showing sparse mononuclear cells, characterizing mild inflammation, animal in group 1A (HE, 100x). B: Photomicrograph showing the presence of inflammatory infiltrate of mononuclear cells with neutrophils and sparse eosinophils, characterizing moderate inflammation, animal in group 1B (HE, 400x). C: Photomicrograph showing remodeling of the epithelium in the ulcer area, animal in group 2B (HE, 100x). D: Photomicrograph showing the presence of inflammatory infiltrate of polymorphonuclear

cells, characterizing intense inflammation, edema and ulceration, animal in group 4B (HE, 100x).

Table 2

Comparison of inflammatory response in relation to study groups (Kruskal-Wallis and Student-Newman-Keuls test).

Inflammatory response	4 days					7 days				
	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*	0.643					0.003				
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]

* comparison between groups
**rank means followed by different letters are significantly different
1= 3 mg/Kg CBD; 2= 10 mg/Kg CBD; 3= 30 mg/Kg CBD; 4= placebo; 5= without CT

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 (p=0.001), in leukocyte count in relation to groups 2A (p=0.001) and 5A (p=0.000), and in platelet count only in relation to group 5A (p=0.018). For the OS variables, there were no statistically significant differences between groups in relation to CAT, but group 4A differed from all groups treated with CBD (1A: p=0.03; 2A: p=0.004; 3A: p=0.016).

In the 7-day experimental period, the analysis of hematological variables showed statistical difference only in relation to erythrocytes in the comparison between controls 4B and 5B (p=0.008). In the OS evaluation, significant differences were found between the positive controls and groups 1B and 2B for both variables (CAT: p=0.000; p=0.000 and GSH: p=0.000; p=0.000).

Table 3

Comparison of hematological and oxidative stress response in relation to study groups and times (ANOVA and Tukey's test).

Groups/Times	1 (Mean ± standard deviation)**	2 (Mean ± standard deviation)**	3 (Mean ± standard deviation)**	4 (Mean ± standard deviation)**	5 (Mean ± standard deviation)**
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**	*0.004*	*0.002*	*0.06*	*0.000*	*0.072*
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**	*0.011*	*0.075*	*0.017*	*0.001*	*0.174*
Platelets					
A (*n*=9)	994.38 ± 213.7^{ABa}	1068.71 ± 524.7^{ABa}	1055.11 ± 236.9^{ABa}	830.11 ± 99.0^{Aa}	1283.43 ± 139.1^{Ba}
B (*n*=5)	1614.11 ± 306.8^{Ab}	1593.86 ± 328.1^{Ab}	1684.8 ± 373.6^{Ab}	1643.11 ± 428.5^{Ab}	1460.25 ± 105.8^{Aa}
*p value**	*0.000*	*0.044*	*0.002*	*0.000*	*0.096*
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**	*0.001*	*0.003*	*0.005*	*0.206*	*0.028*
GSH					
A (*n*=7)	12.03 ± 2.40^{Aa}	12.91 ± 1.39^{Aa}	12.19 ± 1.83^{Aa}	9.31 ± 2.09^{Ba}	$11.46 \pm .79^{ABa}$
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**	*0.000*	*0.000*	*0.008*	*0.124*	*0.235*

* Comparison between times

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

1= 3 mg/Kg CBD; 2= 10 mg/Kg CBD; 3= 30 mg/Kg CBD; 4= placebo; 5= without CT

Discussion

OM is a frequent and highly debilitating side effect in patients undergoing antineoplastic CT, capable of compromising the prognosis and affecting the patients' quality of life. However, to date, there are no effective treatments for OM, only palliative strategies [2,4]. This is the first study conducted to evaluate the preventive and therapeutic effect of CBD on 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 consensus on the ideal dose for antioxidant and antiinflammatory 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 [19,20,23,25,34–38].

The induction of mucositis in an animal model is technically difficult since the protocols already described in the international 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, which met similar standards to those desired for this experimental model, and previous experiences of the researchers, a protocol with 5-FU and mechanical trauma in the tongue ventrum was chosen [28,29]. The experimental times were also determined from the existing literature, in which there are reports that, with this induction protocol, there is peak severity of OM lesions at 4 days, followed by the beginning of the healing process at 7 days [27–30].

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, it is justified the need to adapt 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 [28–30].

Regarding weight loss during the experiment, it was observed that at 4 days. This was more evident at 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 antineoplastic treatments [39,40].

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 [41]. The

antiemetic action of this compound has been reported by several authors and related to the indirect activation of receptors associated with these stimuli [42,43]. In a recent study published by Callejas et al., where the antiinflammatory 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 [38].

These data also corroborate the clinical findings that showed the greater severity of the lesions in the oral cavity in the first experimental time making it difficult to maintain feeding and for the improvement of the lesions during the subsequent days, recovering the ability to feed.

In the macroscopic analysis of induced OM, the groups treated with CBD showed degrees of severity significantly lower than those of the control group in 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 present the positive evolution of the 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.

In corroboration with our findings, 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 proinflammatory cytokines such as TNF-alpha, Nf-Kb and interleukins, and migration of neutrophils to the periphery of the damaged tissues, which would reduce the tissue inflammatory reaction [21,34,35,44].

On the other hand, it is possible to elucidate that the leukopenia observed in the groups that received 5-FU in the 4-day period justifies the lower tissue inflammatory response 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 conducted by MacCallum and Russo revealed that CBD is able to reduce the cytotoxic effects of CT. [18].

CAT and GSH are endogenous AOX capable of eliminating free radicals. This enzyme activity and metabolite show high levels when the body is under OS [22,45]. The results of the analysis of these biochemical parameters indicate that in the evaluation of 4 days both AOX were increased compared with the group control. In the 7-day 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 the 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 proinflammatory 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 antiinflammatory action of CBD [20,21,25,37,44,46,47].

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. The authors suggested that CBD exerted an antiinflammatory action in the early stages of the healing process [34].

Many investigators have noted that the action of CBD on OS may be more potent than with classic AOX, such as α-tocopherol [4,26,34]. 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 proinflammatory mediators responsible for the tissue damage in OM the results obtained 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.

It should also be noted that the administration of CBD seems to be safe at the doses used. A recent review of 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 [48].

The serious consequences of OM in oral tissues stimulate 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 AOX 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 induce in a variety of health conditions/

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 oxidative stress, favoring tissue repair of induced OM. Further studies are

encouraged using standardized approaches to obtain solid scientific evidence that could support the inclusion of this substance in the guidelines for OM.

Acknowledgements

Conflict of interest statement

The authors declare no conflicts of interest.

References

[1]   Reyes-Gibby CC, Melkonian SC, Wang J, Yu RK, Shelburne SA, Lu C, et al. Identifying novel genes and biological processes relevant to the development of cancer therapy-induced mucositis: An informative gene network analysis. PLoS One 2017;12:1–21. doi:10.1371/journal.pone.0180396.

[2]   Peng H, Chen B Bin, Chen L, Chen YP, Liu X, Tang LL, et al. A network meta-analysis in comparing prophylactic treatments of radiotherapy-induced oral mucositis for patients with head and neck cancers receiving radiotherapy. Oral Oncol 2017;75:89–94. doi:10.1016/j.oraloncology.2017.11.001.

[3]   Sonis ST, Villa A. Phase II investigational oral drugs for the treatment of radio/chemotherapy induced oral mucositis. Expert Opin Investig Drugs 2018;27:147–54. doi:10.1080/13543784.2018.1427732.

[4]   Koohi-Hosseinabadi O, Ranjbar Z, Sepehrimanesh M, AndisheTadbir A, Poorbaghi SL, Bahranifard H, et al. Biochemical, hematological, and pathological related healing effects of Elaeagnus angustifolia hydroalcoholic extract in 5-fluorouracil-induced oral mucositis in male golden hamster. Environ Sci Pollut Res 2017;24:24447–53. doi:10.1007/s11356-017-0137-5.

[5]   Lalla RV, Sonis ST, Peterson DE. Management of Oral Mucositis in Patients Who Have Cancer. Dent Clin North Am 2008;52:61–77. doi:10.1016/j.cden.2007.10.002.

[6]   Al-Dasooqi N, Sonis ST, Bowen JM, Bateman E, Blijlevens N, Gibson RJ, et al. Emerging evidence on the pathobiology of mucositis. Support Care Cancer 2013;21:3233–41. doi:10.1007/s00520-013-1900-x.

[7]   Lalla R V., Bowen J, Barasch A, Elting L, Epstein J, Keefe DM, et al. MASCC/ISOO clinical practice guidelines for the management of mucositis secondary to cancer therapy. Cancer 2014;120:1453–61. doi:10.1002/cncr.28592.

[8]   Focaccetti C, Bruno A, Magnani E, Bartolini D, Principi E, Dallaglio K, et al. Effects of 5-fluorouracil on morphology, cell cycle, proliferation, apoptosis, autophagy and ros production in endothelial cells and cardiomyocytes. PLoS One 2015;10:1–25. doi:10.1371/journal.pone.0115686.

[9]   Kalpadakis C, Pangalis G a, Dimopoulou MN, Vassilakopoulos TP, Kyrtsonis M-C, Korkolopoulou P, et al. Rituximab monotherapy is highly effective in splenic marginal zone lymphoma. Hematol Oncol 2007;25:127–31. doi:10.1002/hon.

[10]   Rtibi K, Selmi S, Grami D, Amri M, Sebai H, Marzouki L. Contribution of oxidative stress in acute intestinal mucositis induced by 5 fluorouracil (5-FU) and its pro-drug capecitabine in rats. Toxicol Mech Methods 2018;28:262–7. doi:10.1080/15376516.2017.1402976.

[11]   Sonis ST. Pathobiology of mucositis. Semin Oncol Nurs 2004;20:11–5. doi:10.1053/j.soncn.2003.10.003.

[12]   Sonis ST. Mucositis: The impact, biology and therapeutic opportunities of oral mucositis. Oral Oncol 2009;45:1015–20. doi:10.1016/j.oraloncology.2009.08.006.

[13]   Sonis ST. New thoughts on the initiation of mucositis. Oral Dis 2010;16:597–600. doi:10.1111/j.1601-0825.2010.01681.x.

[14]   Sonis ST. Oral mucositis. Anticancer Drugs 2011;22:607–12. doi:10.1097/CAD.0b013e3283462086.

[15]   Yuan A, Sonis S. Emerging therapies for the prevention and treatment of oral mucositis. Expert Opin Emerg Drugs 2014;19:343–51. doi:10.1517/14728214.2014.946403.

[16]   Villa A, Sonis ST. Pharmacotherapy for the management of cancer regimen-related oral mucositis. Expert Opin Pharmacother 2016;17:1801–7. doi:10.1080/14656566.2016.1217993.

[17]   Lafaye G, Karila L, Blecha L, Benyamina A. Cannabis, cannabinoids, and health. Dialogues Clin Neurosci 2017;19:309–16.

[18]   MacCallum CA, Russo EB. Practical considerations in medical cannabis administration and dosing. Eur J Intern Med 2018;49:12–9. doi:10.1016/j.ejim.2018.01.004.

[19] Pan H, Mukhopadhyay P, Rajesh M, Patel V, Mukhopadhyay B, Gao B. Cannabidiol Attenuates Cisplatin-Induced Nephrotoxicity by Decreasing Oxidative / Nitrosative Stress , Inflammation , and Cell Death. Alcohol 2009;328:708–14. doi:10.1124/jpet.108.147181.cells.

[20] Cassol-Jr OJ, Comim CM, Silva BR, Hermani F V., Constantino LS, Felisberto F, et al. Treatment with cannabidiol reverses oxidative stress parameters, cognitive impairment and mortality in rats submitted to sepsis by cecal ligation and puncture. Brain Res 2010;1348:128–38. doi:10.1016/j.brainres.2010.06.023.

[21] Rajesh M, Mukhopadhyay B, Bátkai S, Patel V, Saito K, Matsumoto S, et al. NIH Public Access. J Am Coll Cardiol 2010;56:2115–25. doi:10.1016/j.jacc.2010.07.033.Cannabidiol.

[22] Booz GW. Cannabidiol as an emergent therapeutic strategy for lessening the impact of inflammation on oxidative stress. Free Radic Biol Med 2011;51:1054–61. doi:10.1016/j.freeradbiomed.2011.01.007.

[23] Yang L, Rozenfeld R, Wu D, Devi LA, Zhang Z, Cederbaum A. Cannabidiol protects liver from binge alcohol-induced steatosis by mechanisms including inhibition of oxidative stress and increase in autophagy. Free Radic Biol Med 2014;68:260–7. doi:10.1016/j.freeradbiomed.2013.12.026.

[24] Burstein S. Cannabidiol (CBD) and its analogs: A review of their effects on inflammation. Bioorganic Med Chem 2015;23:1377–85. doi:10.1016/j.bmc.2015.01.059.

[25] Elbaz M, Nasser MW, Ravi J, Wani NA, Ahirwar DK, Zhao H, et al. Modulation of the tumor microenvironment and inhibition of EGF/EGFR pathway: Novel anti-tumor mechanisms of Cannabidiol in breast cancer. Mol Oncol 2015;9:906–19. doi:10.1016/j.molonc.2014.12.010.

[26] Cuba LF, Salum FG, Cherubini K, Figueiredo MAZ. Cannabidiol: an alternative therapeutic agent for oral mucositis? J Clin Pharm Ther 2017;42:245–50. doi:10.1111/jcpt.12504.

[27] Aras MH, Sezer U, Erkilic S, Demir T, Dagli SN. Effect of dietary boron on 5-fluorouracil induced oral mucositis in rats. Eur J Dent 2013;7:310–4. doi:10.4103/1305-7456.115415.

[28] Ottaviani G, Gobbo M, Sturnega M, Martinelli V, Mano M, Zanconati F, et al. Effect of class IV laser therapy on chemotherapy-induced oral mucositis: A clinical and experimental study. Am J Pathol 2013;183:1747–57. doi:10.1016/j.ajpath.2013.09.003.

[29] Skeff MA, Brito GAC, De Oliveira MG, Braga CM, Cavalcante MM, Baldim V, et al.

S-nitrosoglutathione accelerates recovery from 5-fluorouracil-induced oral mucositis. PLoS One 2014;9:1–25. doi:10.1371/journal.pone.0113378.

[30]   Patel A, Biswas S, Shoja MH, Ramalingayya G V, Nandakumar K. Protective effects of aqueous extract of Solanum nigrum Linn. leaves in rat models of oral mucositis. ScientificWorldJournal 2014;2014:345939. doi:10.1155/2014/345939 [doi].

[31]   Figueiredo JAP, Pesce HF, Gioso MA, Figueiredo MAZ. The histological effects of four endodontic sealers implanted in the oral mucosa: Submucous injection versus implant in polyethylene tubes. Int Endod J 2001;34:377–85. doi:10.1046/j.1365-2591.2001.00407.x.

[32]   Ramos IF de AS, Biz MT, Paulino N, Scremin A, Della Bona Á, Barletta FB, et al. Histopathological analysis of corticosteroid-antibiotic preparation and propolis paste formulation as intracanal medication after pulpectomy: an in vivo study. J Appl Oral Sci 2012;20:50–6. doi:10.1590/S1678-77572012000100010.

[33]   de Freitas Cuba L, Braga Filho A, Cherubini K, Salum FG, Figueiredo MAZ de. Topical application of Aloe vera and vitamin E on induced ulcers on the tongue of rats subjected to radiation: clinical and histological evaluation. Support Care Cancer 2016;24:2557–64. doi:10.1007/s00520-015-3048-3.

[34]   Klein M, de Quadros De Bortolli J, Guimarães FS, Salum FG, Cherubini K, de Figueiredo MAZ. Effects of cannabidiol, a *Cannabis sativa* constituent, on oral wound healing process in rats: Clinical and histological evaluation. Phytother Res 2018:1–7. doi:10.1002/ptr.6165.

[35]   Napimoga MH, Benatti BB, Lima FO, Alves PM, Campos AC, Pena-dos-Santos DR, et al. Cannabidiol decreases bone resorption by inhibiting RANK/RANKL expression and pro-inflammatory cytokines during experimental periodontitis in rats. Int Immunopharmacol 2009;9:216–22. doi:10.1016/j.intimp.2008.11.010.

[36]   Aviello G, Romano B, Borrelli F, Capasso R, Gallo L, Piscitelli F, et al. Chemopreventive effect of the non-psychotropic phytocannabinoid cannabidiol on experimental colon cancer. J Mol Med 2012;90:925–34. doi:10.1007/s00109-011-0856-x.

[37]   Chen J, Hou C, Chen X, Wang D, Yang P, He X, et al. Protective effect of cannabidiol on hydrogen peroxide-induced apoptosis, inflammation and oxidative stress in nucleus pulposus cells. Mol Med Rep 2016;14:2321–7. doi:10.3892/mmr.2016.5513.

[38]   Callejas GH, Figueira RL, Gonçalves FLL, Volpe FAP, Zuardi AW, Crippa JA, et al. Maternal administration of cannabidiol promotes an anti-inflammatory effect on the intestinal wall in a gastroschisis rat model. Brazilian J Med Biol Res 2018;51:1–9. doi:10.1590/1414-431x20177132.

[39]   Arribas L, Hurtós L, Taberna M, Peiró I, Vilajosana E, Lozano A, et al. Nutritional changes in patients with locally advanced head and neck cancer during treatment. Oral Oncol 2017;71:67–74. doi:10.1016/j.oraloncology.2017.06.003.

[40]   Lin MC, Shueng PW, Chang WK, Mu-Hsin Chang P, Feng HC, Yang MH, et al. Consensus and clinical recommendations for nutritional intervention for head and neck cancer patients undergoing chemoradiotherapy in Taiwan. Oral Oncol 2018;81:16–21. doi:10.1016/j.oraloncology.2018.03.016.

[41]   Gorter RW. Cancer cachexia and cannabinoids. Forsch Komplementarmed 1999;6:21–2. doi:10.1159/000057152.

[42]   Rock EM, Bolognini D, Limebeer CL, Cascio MG, Anavi-Goffer S, Fletcher PJ, et al. Cannabidiol, a nonpsychotropic component of cannabis, attenuates vomiting and nausea-like behaviour via indirect agonism of 5-HT 1A somatodendritic autoreceptors in the dorsal raphe nucleus. Br J Pharmacol 2012;165:2620–34. doi:10.1111/j.1476-5381.2011.01621.x.

[43]   Rock EM, Parker LA. Synergy Between Cannabidiol, Cannabidiolic Acid, and Δ^9-Tetrahydrocannabinol in the Regulation of Emesis in the Suncus Murinus (House Musk Shrew). Behav Neurosci 2015;129:368–70. doi:10.1037/bne0000057.

[44]   Rajan TS, Giacoppo S, Iori R, De Nicola GR, Grassi G, Pollastro F, et al. Anti-inflammatory and antioxidant effects of a combination of cannabidiol and moringin in LPS-stimulated macrophages. Fitoterapia 2016;112:104–15. doi:10.1016/j.fitote.2016.05.008.

[45]   Cruz É de P da, Campos L, Pereira F da S, Magliano GC, Benites BM, Arana-Chavez VE, et al. Clinical, biochemical and histological study of the effect of antimicrobial photodynamic therapy on oral mucositis induced by 5-fluorouracil in hamsters. Photodiagnosis Photodyn Ther 2015;12:298–309. doi:10.1016/j.pdpdt.2014.12.007.

[46]   Borges RS, Batista J, Viana RB, Baetas AC, Orestes E, Andrade MA, et al. Understanding the molecular aspects of tetrahydrocannabinol and cannabidiol as antioxidants. Molecules 2013;18:12663–74. doi:10.3390/molecules181012663.

[47]   Kozela E, Juknat A, Gao F, Kaushansky N, Coppola G, Vogel Z. Pathways and gene networks mediating the regulatory effects of cannabidiol, a nonpsychoactive cannabinoid, in autoimmune T cells. J Neuroinflammation 2016;13:1–19. doi:10.1186/s12974-016-0603-x.

[48]   Iffland K, Grotenhermen F. An Update on Safety and Side Effects of Cannabidiol: A Review of Clinical Data and Relevant Animal Studies. Cannabis Cannabinoid Res 2017;2:139–54. doi:10.1089/can.2016.0034.

5. DISCUSSÃO COMPLEMENTAR

O câncer é responsável por quase uma em cada 6 mortes no mundo. Mais de 14 milhões de pessoas desenvolvem neoplasias malignas todos os anos, e estima-se que esse número suba para mais de 21 milhões até 2030. Dados recentes da Organização Mundial da Saúde (OMS) apontam que 8,8 milhões de pessoas morrem em função desta grave patologia por ano, principalmente em países de baixa e média renda (OMS, 2017).

A crescente incidência de câncer na população mundial está associada às expressivas mudanças no perfil demográfico de muitos países em desenvolvimento, especialmente o envelhecimento da população, sendo consequência, entre outros fatores, do processo de urbanização, industrialização e dos avanços da ciência e da tecnologia. Além disso, unem-se os novos estilos de vida e a exposição, ainda mais intensa, a fatores de risco próprios do mundo contemporâneo. Esse processo de mudança demográfica, associado à transformação nas relações entre as pessoas e seu ambiente, trouxe uma alteração importante no perfil de morbimortalidade, diminuindo a ocorrência das doenças infectocontagiosas e colocando as doenças crônico-degenerativas, como o câncer, no novo centro de atenção dos problemas de doença e morte da população (INCA, 2018).

A OMS preconiza o diagnóstico precoce como a principal forma de redução de danos associados ao câncer, uma vez que, ao dectectar a doença em estágio inicial seu tratamento será mais rápido e eficaz gerando menor sofrimento ao paciente e menos custos aos sistemas de saúde. Atualmente o custo econômico anual do câncer através de gastos com saúde do paciente e perda de produtividade foi estimado em US $ 1,16 trilhão (OMS, 2017).

Dentre as principais estratégias de tratamento das neoplasias malignas destaca-se a QT. Esta modalidade consiste no emprego de drogas que objetivam destruir as células tumorais, no entanto, não atuam exclusivamente sobre essas, atingindo também células normais de rápida renovação como as da medula óssea, mucosas e couro cabeludo. Os efeitos terapêuticos e tóxicos dos quimioterápicos dependem do tempo de exposição e da concentração plasmática da droga. A toxicidade é variável para os diversos tecidos e depende da droga utilizada (INCA, 2018)

Nos últimos anos, houve um progresso notável na terapia do câncer, visando a redução da toxicidade, da necessidade de interrupção da QT e buscando a intensificação dos quimioterápicos,

embora reações adversas e complicações induzidas pela QT ainda não sejam incomuns. Sabe-se que cerca de 40% a 80% dos pacientes submetidos a esta modalidade terapêutica desenvolvem reações adversas na cavidade oral, sendo a MO uma das mais debilitantes e capaz de comprometer o curso da oncoterapia (SAITO *et al*, 2014; REZAZADEH *et al,* 2018).

Em resposta às diretrizes recentemente publicadas sobre a MO, está se tornando prática comum a inclusão de intervenções para prevenir esta complicação como um componente essencial da terapia do câncer (SAITO *et al*, 2014). Muitas tentativas vem sendo direcionadas para o desenvolvimento de formulações visando evitar ou minimizar a MO. A administração tópica ou sistêmica de substâncias como sucralfato, vitamina E, clorexidina, agentes anti-inflamatórios, fatores de crescimento, citocinas, além de uma série de fitoterápicos apresentam resultados promissores. No entanto, até o momento, eles não reduziram significativamente a incidência e a gravidade da mucosite nos pacientes (CUBA *et al*, 2015; REZAZADEH *et al*, 2018; THONSEM, VITETTA, 2018).

Sonis *et al*, descreveram o desenvolvimento da MO em 5 fases, nas quais o EO induzido pelos quimioterápicos é descrito como o principal responsável por desencadear uma cascata de reações de culminam no rompimento do tecido epitelial. A partir dessa hipótese, passou-se a investigar substâncias capazes de impedir ou modular o EO, atuando como protetores da mucosa (SONIS *et al*, 2004; SONIS, 2010; REZAZADEH *et al*, 2018; SONIS, VILLA, 2018).

O CBD foi isolado em 1942, no entanto, o interesse por sua farmacodinâmica só foi despertado muitos anos após. Até o momento, os mecanismos de ação do CBD não foram totalmente elucidados e as vias de sinalização celular envolvidas, são ainda pouco conhecidas. Na última década houve um expressivo crescimento nos estudos visando a aplicação médica do CBD, motivado principalmente pela descoberta da sua capacidade anti-inflamatória, antioxidante que, segundo estudos prévios, pode ser mais potente do que antioxidantes clássicos como o alpha tocoferol (IFFLAND, GOTENHERMEN, 2017; PISANTI *et al*, 2017; BARON, 2018).

Diante do crescente interesse nas propriedades curativas do CBD, considera-se indispensável a discussão a respeito das polêmicas envolvendo o uso medicinal da sua planta de origem, a *Cannabis sativa*, popularmente conhecida como maconha. 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, 2018).

Em 2016, a Agência Nacional de Vigilancia Sanitária (ANVISA) autorizou e a prescrição médica e importação por pessoa física de medicamentos e produtos derivados do CBD e THC desde que exclusivamente para tratamento de saúde, sendo estes, até então, parte da lista de compostos proibidos. Além disso, em 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, 2018). É utilizado para o tratamento sintomático da espasticidade relacionada à esclerose múltipla, sendo indicado para pacientes que não respondem às terapias convencionais (ANVISA, 2018).

Ainda em 2017, a ANVISA publicou nota de esclarecimento comunicando que não apresentava oposição à investigações científicas com a maconha para uso medicinal. Porém, especificamente no que diz respeito ao cultivo da *Cannabis* destinado a fins científicos ou médicos, incluindo a obtenção de insumo para a fabricação de medicamentos registrados ou para o eventual tratamento de pacientes autorizados pelas autoridades governamentais, entendeu-se que o tema merece regulamentação (ANVISA, 2018).

Este estudo visa agregar à inserção da odontologia neste debate, uma vez que a compreensão das propriedades terapêuticas do CBD e seus mecanismos de ação revelam um potencial benefício para muitas patologias orais que são desafiadoras para os cirurgiões dentistas assim como a MO. Estimulados pela expressiva participação de pesquisadores brasileiros nos estudos científicos com os derivados da *Cannabis*, a partir da criação de um Centro de Pesquisa em Canabinoides vinculado à Faculdade de Medicina de Ribeirão Preto, nosso grupo de pesquisa, vem desenvolvendo experiementos testando distintas aplicabilidades do CBD na área da estomatologia (CUBA *et al*, 2017; KLEIN *et al*, 2018).

Embora os principais conceitos e achados deste estudo estejam amplamente discutidos ao longo dos artigos que o compõe, alguns aspectos metodológicos merecem maior detalhamento. A

escolha do modelo animal, apesar de classificada como baixo nível de evidência pela Oxford Centre for Evidence-based Medicine, permite uma rígida padronização da amostra e dos critérios de análise, além de baixo custo e menores entraves burocráticos quando comparada aos ensaios clínicos. Seus resultados permitem ou desencorajam elucubrações e planejamento de estudos futuros em humanos. Os desfechos desta pesquisa provocam pretensões de novos projetos em modelos experimentais de maior nível de evidência.

Com relação à metodologia de indução da MO, encontra-se na literatura uma gama de variações do clássico método desenvolvido por Sonis e colaboradores em 1990, o qual consiste em duas aplicações de 5FU nas doses de 100 mg/Kg e 65 mg/Kg seguidos de 2 momentos de trauma mecânico (SONIS *et al*, 1990). No experimento de Tanideh e colaboradores, as aplicações de 5FU foram modificadas para 3 doses de 60 mg/kg em uma linhagem de *hamsters* dourados machos (TANIDEH *et al*, 2014). Lima e colaboradores empregaram, em ratos *Wistar*, 3 doses de 30 mg do mesmo quimioterápico, porém, realizaram o trauma mecânico através de indução química (LIMA *et al*, 2015). Araújo *et al,* realizaram seus experimentos em *hamsters sírios* utilizando o protocolo de duas doses de 60 mg/kg e trauma mecânico único. Já Ottaviani *et al,* empregaram em camundongos C57BL/6 a mesma dose porém com trauma por indução química (OTTAVIANI *et al*, 2013; ARAÚJO *et al*, 2015).

Neste estudo optou-se pela utilização de camundongos em função do menor peso corporal requerendo menor volume de CBD. A linhagem CF-1, disponível na instituição, porém não descrita nos protocolos estudados, demandou a realização de um projeto piloto no qual algumas variações das diferentes metodologias citadas acima foram testadas em 1 animal cada uma. A partir de então optou-se pela que apresentou resultados clínicos mais semelhantes a MO em humanos, ou seja, duas aplicações de 60 mg/kg de 5FU e 2 momentos de indução mecânica.

Além das distintas doses e métodos utilizados para traumatizar a mucosa, os estudos diferem ainda na localização anatômica a ser analisada. A mucosa jugal é o sítio mais frequentemente usado, porém o ventre lingual e fornix também são citados (ARAS *et al,* 2013; OTTAVIANI *et al*, 2013; TANIDEH *et al*, 2014; ARAÚJO *et al*, 2015; CRUZ *et al*, 2015; LIMA *et al*, 2015). Neste estudo optou-se pelo ventre lingual uma vez que a mucosa não-ceratinizada é mais costumeiramente acometida pela MO. Outra vantagem do sítio utilizado é o melhor 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.

A inclusão de um grupo controle negativo (5A e 5B) objetivou a avaliação da efetividade da quimioterapia tanto nas variáveis clínicas quanto laboratoriais. Além disso buscou-se estabelecer um parâmetro de comparação dos dados hematológicos e bioquímicos, uma vez que estes podem variar de acordo com a linhagem dos animais utilizados (CASTELO BRANCO *et al*, 2011). Alguns achados secundários deste estudo também corroboram com a ação agressiva do agente quimioterápico utilizado. Nos animais que receberam 5FU, foi observada perda e umedecimento de pelos bem como atrofia parcial ou completa das papilas do dorso lingual (APÊNDICES 7 e 11). A perda de pelos é um efeito psicologicamente devastador que frequentemente afeta humanos sob tratamento quimioterápico (PAUS *et al*, 2013). A atrofia das papilas está fortemente associada a dificuldade de alimentação e consequente perda de peso, evidenciada nos animais do grupo controle positivo, uma vez que as funções de proteção e sensoriais das papilas estavam afetadas. Em humanos, este é um efeito adverso da QT que afeta consideravelmente sua qualidade de vida (PONTICELLI *et al*, 2017). Considerando ainda os 11 óbitos registrados, observou-se que 4 destes ocorreram logo após o procedimento anestésico para o trauma mecânico. Os demais se deram entre 6 e 9 dias após a QT. Portanto, acredita-se que possam estar relacionados a toxicidade da droga utilizada e não ao CBD, uma vez que em recente revisão sistemática da literatura constatou-se que o mesmo não é citotóxico para células normais, sendo considerado seguro para uso em animais e humanos(IFFLAND, GROTENHERMEN, 2017).

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. Sendo assim, um veículo de diluição deve ser utilizado previamente a incorporação à solução salina. As amostras foram pesadas em doses diárias, protegidas da luz, acondicionadas sob refrigeração e diluídas somente minutos antes de cada aplicação (APÊNDICE 6). Baseado na metodologia de estudos prévios optou-se pelo uso do *Tween* 80 (NAPIMOGA *et al*, 2009; CASSOL-JR *et al,* 2010).

A via intraperitoneal de administração do CBD é a mais frequentemente utilizada em estudos com animais. Apesar de sua farmacodinâmica ainda ser pouco clara em muitos aspectos, a farmacocinética já é muito bem definida. Sabe-se que quando administrado por via oral o CBD apresenta significativamente menor biodisponibilidade do que por via IP (PISANTI *et al*, 2017).

Estudos relatam que o nível plasmático de CBD, quando aplicado por via IP, pode ser 7 vezes maior do que por via oral (IFFLAND, GROTENHERMEN, 2017). Todavia, a reprodutibilidade deste tipo de tratamento em humanos é relativamente inviável.

Estudos em diferentes modelos de doença sugerem que, mesmo quando administrado topicamente, o CBD é capaz de apresentar propriedades terapêuticas significativas. Em um modelo de monoartrite induzida em ratos tratados com gel de CBD por via de aplicação transdérmica foi constatado que esta terapia foi efetiva na melhora da sintomatologia, exerceu ação antinflamatória, não apresentou efeitos psicoativos, além de demonstrar excelente concentração plasmática (HAMMEL *et al*, 2016). Giacoppo e colaboradores (2015) testaram o uso tópico de um creme na concentração de 98% de CBD em camundongos para o tratamento de encefalomielite autoimune induzida. Os autores sugerem que esta é uma via segura de administração e de baixo custo. Além disso, os resultados demonstraram que houve menor expressão de citocinas pró-inflamatórias e estresse oxidativo. Chelliah e colaboradores publicaram uma série de 3 casos clínicos em que pacientes acometidos de epidermólise bolhosa fizeram uso tópico de CBD. Os resultados demonstraram redução da dor e rápida cicatrização das feridas. 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.

No tocante aos resultados obtidos neste estudo, de maneira geral considera-se coerente com os achados da literatura, sendo promissores para o manejo da MO. Por outro lado, os dados da análise histológica, nos quais a intensidade da resposta inflamatória foi inversa aos achados clínicos na comparação entre os tempos experimentais, deixa ainda uma questão a ser elucidada. Algumas investigações adicionais como, por exemplo, imunoistoquímica para marcadores inflamatórios (TNF-α, IL1-β, IL6), análise histomorfométrica da epitelização, coloração picrosirius poderiam ser interessantes, visando enriquecer a análise dos efeitos do CBD no reparo tecidual.

De acordo com o que foi exposto nesta tese, acredita-se que os achados obtidos possam subsidiar estudos que envolvam e favoreçam o uso do CBD em diferentes campos da área médica e odontológica.

REFERÊNCIAS

AGHAMOHAMAMDI A, HOSSEINIMEHR SJ. Natural Products for Management of Oral Mucositis Induced by Radiotherapy and Chemotherapy. **Integr Cancer Ther**, v. 15, n. 1, p.60-68, 2015.

AL-DASOOQUI N, et al. Emerging evidence on the pathobiology of mucositis. **Support Care Cancer**, v. 21, n. 11, p. 2075-2083, 2013.

ANVISA. **Atualizada lista de produtos com canabidiol importados.** Disponível em: portal.anvisa.gov.br/.../atualizada-lista...produtos-com-canabidiol-importados/219201.

ANVISA. **Maconha: Anvisa não é contra uso para fins medicinais.** Disponível em: portal.anvisa.gov.br/rss/-/asset_publisher/Zk4q6UQCj9Pn/content/id/3470896.

ANVISA. **Lista oficial de fármacos inclui Cannabis.** Disponível em: portal.anvisa.gov.br/rss/-/asset.../lista-oficial-de-farmacos-inclui-cannabis-/219201.

ARAS MH, et al. Effect of dietary boron on 5-fluorouracil induced oral mucositis in rats. **Eur J Dent**, v. 7, n. 3, p. 310-314, 2013.

ARRIBAS L, et al. Nutritional changes in patients with locally advanced head and neck cancer during treatment. **Oral Oncol,** v. 71, p.67-74, 2017.

ATAKAN Z. Cannabis, a complex plant: different compounds and different effects on individuals. **Ther Adv Psychopharmacol**, v. 2, n. 6, p. 241-254, 2012.

AVIELLO G, et al. Chemopreventive effect of the non-psychotropic phytocannabinoid cannabidiol on experimental colon cancer. **J Mol Med**, v, 90, n. 8, p. 925-934, 2012.

BARKOKEBAS A, et al. Impact of oral mucositis on oral-health-related quality of life of patients diagnosed with cancer. **J Oral Pathol Med**, v. 44, n. 9, p. 746-751, 2015.

BARON EP. Comprehensive Review of Medicinal Marijuana, Cannabinoids, and Therapeutic Implications in Medicine and Headache: What a Long Strange Trip It's Been. **Headache**, v. 55, n. 6, p. 885-916, 2015.

BARON EP, Medicinal properties of cannabinoids, terpenes and flavonoids in cannabis, and potential roles in migraine, headache, and pain: An update on current evidence and cannabis science, **Headache**, v. In Press., 2018.

BIRDSALL SM, BIRDSALL TC, TIMS LA. The Use of Medical Marijuana in Cancer. **Curr Oncol Rep**, v. 18, n. 7, p. 40, 2016.

BONOMI M; BATT K. Supportive Management of Mucositis and Metabolic Derangements in Head and Neck Cancer Patients. **Cancers (Basel)**, v. 7, n. 3, p. 1743-1757, 2015.

BOOZ GW. Cannabidiol as an emergent therapeutic strategy for lessening the impact of inflammation on oxidative stress. **Free Radic Biol Med**, v. 51, n. 5, p. 1054-1061, 2011.

BORGES RS, et al. Understanding the molecular aspects of tetrahydrocannabinol and cannabidiol as antioxidants. **Molecules**, v. 18, n. 10, p. 12663-12674, 2013.

BURSTEIN S. Cannabidiol (CBD) and its analogs: a review of their effects on inflammation. **Bioorg Med Chem**, v. 23, n. 7, p. 1377-1385, 2015.

CALLEJAS GH, et al. Maternal administration of cannabidiol promotes an anti-inflammatory effect on the intestinal wall in a gastroschisis rat model. **Brazilian J Med Biol Res**, v. 51, n. 5, p. 1-9, 2018.

CASSOL-JR OJ, et al. Treatment with cannabidiol reverses oxidative stress parameters, cognitive impairment and mortality in rats submitted to sepsis by cecal ligation and puncture. **Brain Res**, v. 1348, p. 128-138, 2010.

CASTELLO BRANCO ACS, et al. Parâmetros bioquímicos e hematológicos de ratos Wistar e camundongos Swiss do Biotério Professor Thomas George. **Rev Bras Ciênc Saúde**, v. 15, n. 2, p. 209-214, 2011.

CHELLIAH M, et al. Self-initiated use of topical cannabidiol oil for epidermolysis bullosa. **Pediatr Dermatol**, v. 35, n. 4, p. e224-e227, 2018.

CHEN J, et al. Protective effect of cannabidiol on hydrogen peroxide-induced apoptosis, inflammation and oxidative stress in nucleus pulposus cells. **Mol Med Rep**, v. 14, n. 3, p. 2321-2327, 2016.

CRUZ É DE P DA, et al. Clinical, biochemical and histological study of the effect of antimicrobial photodynamic therapy on oral mucositis induced by 5-fluorouracil in hamsters. **Photodiagnosis Photodyn Ther**, v. 12, n. 2, p. 298-309, 2015.

CUBA LF, et al. Cannabidiol: an alternative therapeutic agent for oral mucositis? **J Clin Pharm Ther**, v. 42, n. 3, p. 245-250, 2017.

DAVIS MP.Cannabinoids for Symptom Management and Cancer Therapy: The Evidence. **J Natl Compr Canc Netw**, v. 14, n. 7, p 915-922, 2016.

DE ARAÚJO AA, et al. Azilsartan reduced TNF-α and IL-1β levels, increased IL-10 levels and upregulated VEGF, FGF, KGF, and TGF-α in an oral mucositis model. **PLoS One**, v. 10, n. 2, p. 1-16, 2015.

DE FREITAS CUBA L, et al. Antioxidant agents: a future alternative approach in the prevention and treatment of radiation-induced oral mucositis? **Altern Ther Health Med**, v. 21, n. 2, p 36-41, 2015.

DE FREITAS CUBA L, et al. Topical application of Aloe vera and vitamin E on induced ulcers on the tongue of rats subjected to radiation: clinical and histological evaluation. **Support Care Cancer**, v. 24, n. 6, p. 2557-2564, 2016.

ELBAZ M, et al. Modulation of the tumor microenvironment and inhibition of EGF/EGFR pathway: Novel anti-tumor mechanisms of Cannabidiol in breast cancer. **Mol Oncol**, v. 9, n. 4, p. 906-919, 2015.

EPSTEIN JB, et al. Oral complications of cancer and cancer therapy. **CA Cancer J Clin**, v. 62, p. 400-422, 2012.

FIGUEIREDO JAP, et al. The histological effects of four endodontic sealers implanted in the oral mucosa: Submucous injection versus implant in polyethylene tubes. **Int Endod J**, v. 34, n. 5, p 377-385, 2001.

FINE PG, ROSENFELD MJ. The endocannabinoid system, cannabinoids, and pain. **Rambam Maimonides Med J**, v. 4, n. 4, p. 1-15, 2013.

FOCACCETTI C, et al. Effects of 5-fluorouracil on morphology, cell cycle, proliferation, apoptosis, autophagy and ros production in endothelial cells and cardiomyocytes. **PLoS One**, v. 10, n. 2, p. 1-25, 2015.

FOWLER CJ. Δ9-tetrahydrocannabinol and cannabidiol as potential curative agents for cancer. A critical examination of the preclinical literature. **Clin Pharmacol Ther**, v. 97, n. 6, p. 587-596, 2015.

FOUAD AA, et al. Cardioprotective effect of cannabidiol in rats exposed to doxorubicin toxicity. **Environ Toxicol Pharmacol**, v. 36, n. 2, p. 347-357, 2013.

FRAGUAS-SÁNCHEZ AI, FERNÁNDEZ-CARBALLIDO A, TORRES-SUÁREZ AI. Phyto-, endo- and synthetic cannabinoids: promising chemotherapeutic agents in the treatment of breast and prostate carcinomas. **Expert Opin Investig Drugs**, v. 25, n. 11, p. 1311-1323, 2016.

FRIEDMAN D, DEVINSKY O. Cannabinoids in the Treatment of Epilepsy. **N Engl J Med**, v. 374, n. 1, p.1048-1058, 2015.

GIACOPPO S, et al. Cannabinoids: New Promising Agents in the Treatment of Neurological Diseases. **Molecules**, v. 19, n. 11, p. 18781-18816, 2014.

GIACOPPO S, et al. A new formulation of cannabidiol in cream shows therapeutic effects in a mouse model of experimental autoimmune encephalomyelitis. **DARU**, v. 23, n. 1, p. 1-17, 2015.

GOULD J. The cannabis crop. **Nature**, v. 525, n. 7570, s. 2-3, 2015.

GORTER RW. Cancer cachexia and cannabinoids. **Forsch Komplementarmed**, v. 6, n. 3, p. 21-22, 1999.

HAO E, et al. Cannabidiol Protects against Doxorubicin-Induced Cardiomyopathy by Modulating Mitochondrial Function and Biogenesis. **Mol Med**, v. 21, n. 6, p 38-45, 2015.

HAMPSON AJ, et al. Cannabidiol and (-)Delta9-tetrahydrocannabinol are neuroprotective antioxidants. **Proc Natl Acad Sci USA**, v. 95, n. 14, p. 8268-8273, 1998.

HAMMELL DC, et al. Transdermal cannabidiol reduces inflammation and pain-related behaviours in a rat model of arthritis. **Eur J Pain**, v. 20, n. 6, p. 936–948, 2016.

HUANG L, et al. SCID/NOD mice model for 5-FU induced intestinal mucositis: Safety and effects of probiotics as therapy. **Pediatric Neonat**, p. 1-9, 2018. Disponível em: https://linkinghub.elsevier.com/retrieve/pii/S1875957217306824

IBEAS BIH C, et al. Molecular Targets of Cannabidiol in Neurological Disorders. **Neurotherapeutics**, v. 12, n. 4, p. 699-730, 2015.

IFFLAND, K.; GROTENHERMEN, F. An update on safety and side effects of Cannabidiol: A review of clinical data and relevant animal studies. **Cannabis Cannabinoid Res**, v. 2, n. 1, p. 139-154, 2017.

INSTITUTO NACIONAL DO CÂNCER JOSÉ DE ALENCAR GOMES DA SILVA. Estimativa 2018: Incidência de Câncer no Brasil - Ministério da Saúde. Rio de Janeiro: INCA, 2018.

KALPADAKIS C, et al. Rituximab monotherapy is highly effective in splenic marginal zone lymphoma. **Hematol Oncol**, v. 25, n. 3, p.1271-31, 2007.

KLEIN M, et al. Effects of cannabidiol, a Cannabis sativa constituent, on oral wound healing process in rats: Clinical and histological evaluation. **Phyther Res**, n. april, p. 1-7, 2018.

KOOHI-HOSSEINABADI O, et al. Biochemical, hematological, and pathological related healing effects of Elaeagnus angustifolia hydroalcoholic extract in 5-fluorouracil-induced oral mucositis in male golden hamster. **Environ Sci Pollut Res**, v. 24, n. 31, p. 24447–24453, 2017.

KOZELA E, et al. Pathways and gene networks mediating the regulatory effects of cannabidiol, a nonpsychoactive cannabinoid, in autoimmune T cells. **J Neuroinflammation**, v. 13, n. 1, p 1-19, 2016.

KUO CC, et al. Meta-analysis of randomized controlled trials of the efficacy of propolis mouthwash in cancer therapy-induced oral mucositis. **Support Care Cancer**, v. 2018.

LAFAYE G, et al. Cannabis, cannabinoids, and health. **Dialogues Clin Neurosci**, v. 19, n. 3, p. 309-316, 2017.

LALLA RV, SONIS ST, PETERSON DE. Management of Oral Mucositis in Patients Who Have Cancer. **Dent Clin North Am**, v. 52, n. 1, p. 61-77, 2008.

LALLA RV, et al. MASCC/ISOO clinical practice guidelines for the management of mucositis secondary to cancer therapy. **Cancer**, v. 120, n. 10, p. 1453-1461, 2014.

LALLA RV, et al. Mucositis Guidelines Leadership Group of the Multinational Association of Supportive Care in Cancer and International Society of Oral Oncology (MASCC/ISOO) MASCC/ISOO clinical practice guidelines for the management of mucositis secondary to cancer therapy. **Cancer**, v. 120, n. 10, p. 1453-1461, 2014. Review. Erratum in: Cancer, v. 121, p.1339, 2015.

LIMA, G, et al, Amniotic membrane as a biological dressing for 5-fluoruracil-induced oral mucositis in rats, **Int J Oral Maxillofac Surg**, v. 44, n. 7, p. 845-851, 2015.

LIN MC, et al. Consensus and clinical recommendations for nutritional intervention for head and neck cancer patients undergoing chemoradiotherapy in Taiwan. **Oral Oncol**, v. 81, n. feb, p. 16-21, 2018.

MARTINS, A; PEREIRA, C; JUNIOR, E et al. À Descriminalização da Maconha a Luz da Política de Drogas do Brasil À Descriminalização da Maconha a Luz da Política de Drogas do Brasil. **Revista Científica Multidisciplinar Núcleo do Conhecimento**, v. 11, p. 281-05, 2016.

MACCALLUM CA, RUSSO EB. Practical considerations in medical cannabis administration and dosing. **Eur J Intern Med**, v. 49, n. jan, p. 12-19, 2018.

MCDONOUGH P, et al. Neuropathic orofacial pain: cannabinoids as a therapeutic avenue. **Int J Biochem Cell Biol**, v. 55, p. 72-78, 2014.

MIRANZADEH S, et al. Effect of adding the herb Achillea millefolium on mouthwash on chemotherapy induced oral mucositis in cancer patients: A double-blind randomized controlled trial. **Eur J Oncol Nurs**, v. 19, n. 3, p. 207-213, 2015.

MIYAMOTO H, et al. The relationship between the severity of radiation-induced oral mucositis and the myeloperoxidase levels in rats. **Oral Surg Oral Med Oral Pathol Oral Radiol**, v. 120, n. 3, p. 329-336, 2015.

MORIMATA J, et al. Investigation of factor affecting health-related quality of life in head and neck cancer patients. **Gerodontology**, v. 30, n. 3, p. 194-200, 2012.

NAPIMOGA, M. H. et al. Cannabidiol decreases bone resorption by inhibiting RANK/RANKL expression and pro-inflammatory cytokines during experimental periodontitis in rats. **Int Immunopharmacol**, v. 9, n. 2, p. 216-222, 2009.

ORGANIZAÇÃO MUNDIAL DA SAÚDE. **OMS: câncer mata 8,8 milhões de pessoas anualmente no mundo.** Disponível em: https://nacoesunidas.org/oms-cancer-mata-88-milhoes-de-pessoas-anualmente-no-mundo/

OTTAVIANI G, et al. Effect of class IV laser therapy on chemotherapy-induced oral mucositis: A clinical and experimental study. **Am J Pathol**, v.183, n. 6, p. 1747-1757, 2013.

OXFORD CENTRE FOR EVIDENCE-BASED MEDICINE. **Nível de Evidência Científica por Tipo de Estudo.** Disponível em: http://portalarquivos2.saude.gov.br/images/pdf/2014/janeiro/28/tabela-nivel-evidencia.pdf

PAN H, et al. Cannabidiol attenuates cisplatin-induced nephrotoxicity by decreasing oxidative/nitrosative stress, inflammation, and cell death. **J Pharmacol Exp Ther**, v. 328, n. 3, p. 708-714, 2009.

PATEL A, et al. Protective effects of aqueous extract of Solanum nigrum Linn. leaves in rat models of oral mucositis. **ScientificWorldJournal**, v. 2014, p. 345939, 2014.

PAUS R, et al. Pathobiology of chemotherapy-induced hair loss. **Lancet Oncol**, v. 14, n. 2, p. e50-e59, 2013.

PENG H, et al. A network meta-analysis in comparing prophylactic treatments of radiotherapy-induced oral mucositis for patients with head and neck cancers receiving radiotherapy. **Oral Oncol**, v. 75, n. october, p. 89-94, 2017.

PETERSON, D.E.; CARIELLO, A. Mucosal damage: a major risk factor for severe complications after cytotoxic therapy. **Semin Oncol**, v. 31, n. 3, p. 35-44. 2004.

PISANTI S, et al. Cannabidiol: State of the art and new challenges for therapeutic applications. **Pharmacol Ther**, v. 175, p. 133-150, 2017.

PONTICELLI E, et al. Dysgeusia and health-related quality of life of cancer patients receiving chemotherapy: A cross-sectional study. **Eur J Cancer Care**, v. 26, n. 2, p. 1-7, 2017.

RAJAN TS, et al. Anti-inflammatory and antioxidant effects of a combination of cannabidiol and moringin in LPS-stimulated macrophages. **Fitoterapia**, v. 112, p. 104-115, 2016.

RAJESH M, et al. Cannabidiol attenuates cardiac dysfunction, oxidative stress, fibrosis, inflammatory and cell death signaling pathways in diabetic cardiomyopathy. **J Am Coll Cardiol**, v. 56, n. 25, p. 2115-2125, 2010.

RAMOS IF, et al. Histopathological analysis of corticosteroid-antibiotic preparation and propolis paste formulation as intracanal medication after pulpectomy: an in vivo study. **J Appl Oral Sci**, v. 20, n. 1, p. 50-56, 2012.

REYES-GIBBY C, et al. Identifying novel genes and biological processes relevant to the development of cancer therapy-induced mucositis: An informative gene network analysis. **PLoS ONE**, v. 12, n. 7, p. 1-21, 2017.

REZAZADEH M, et al. A mucoadhesive thermosensitive hydrogel containing erythropoietin as a potential treatment in oral mucositis : in vitro and in vivo studies. **Drug Deliv Transl Res**, v.8, n. 5, p. 1226-1237, 2018.

ROCK E M, et al. Cannabidiol, a nonpsychotropic component of cannabis, attenuates vomiting and nausea-like behaviour via indirect agonism of 5-HT 1 A somatodendritic autoreceptors in the dorsal raphe nucleus. **Br J Pharmacol**, v. 165, n. 8, p. 2620-2634, 2012.

ROCK M; PARKER A. Synergy Between Cannabidiol, Cannabidiolic Acid, and Δ9-Tetrahydrocannabinol in the Regulation of Emesis in the Suncus Murinus (House Musk Shrew). **Behav Neuroscie**, v. 129, n. 3, p. 368-370, 2015.

RTIBI K, et al. Contribution of oxidative stress in acute intestinal mucositis induced by 5 fluorouracil (5-FU) and its pro-drug capecitabine in rats. **Toxicol Mech Methods**, v. 28, n. 4, p. 262-267, 2018.

RUSSO M, et al. Evaluating Sativex® in Neuropathic Pain Management: A Clinical and Neurophysiological Assessment in Multiple Sclerosis. **Pain Med**, v. 17, n. 6, p. 1145-1154, 2016.

SAITO H, et al, Effects of professional oral health care on reducing the risk of chemotherapy-induced oral mucositis. **Support Care Cancer**, v. 22, n. 11, p. 2935-2940, 2014.

SCULLY C. Cannabis; Adverse effects from an oromucosal spray. **Br Dent J**, v. 203, n. 6, p. 50-53, 2007.

SKEFF MA, et al. S-nitrosoglutathione accelerates recovery from 5-fluorouracil-induced oral mucositis. **PLoS ONE**, v. 9, n. 12, p. 1-25, 2014.

SONIS ST. Pathobiology of mucositis. **Semin Oncol Nurs**, v. 20, n. 1, p. 11-15, 2004.

SONIS ST. The pathobiology of mucositis. **Nat Rev Cancer**, v. 4, n. 1, p 277-284, 2004.

SONIS ST, et al. Perspective on cancer therapy-induced mucosal injury - pathogenesis, measurement, epidemiology, and consequences for patients. **Cancer**, v. 100, n. S9, p. 1995-2025, 2004.

SONIS ST. Mucositis: The impact, biology and therapeutic opportunities of oral mucositis. **Oral Oncol**, v. 45, n. 12, p. 1015-1020, 2009.

SONIS ST. New thoughts on the initiation of mucositis. **Oral Dis**, v. 16, n. 7, p. 597-600, 2010.

SONIS ST. Oral mucositis. **Anti-Cancer Drugs**, v. 22, n. 7, p. 607-612, 2011.

SONIS ST. Oral mucositis in head and neck cancer: risk, biology, and management. **Am Soc Clin Oncol Educ Book**, 2013.

SONIS ST; VILLA A. Phase II investigational oral drugs for the treatment of radio/chemotherapy induced oral mucositis. **Expert Opin Investig Drugs**, v. 27, n. 2, p. 147-154, 2018.

TANIDEH N, et al. Comparative assessment of the therapeutic effects of the topical and systemic forms of Hypericum perforatum extract on induced oral mucositis in golden hamsters. **Int J Oral Maxillofac Surg**, v. 43, n. 10, p. 1286-1292, 2014.

THOMSEN M; VITETTA L. Adjunctive Treatments for the Prevention of Chemotherapy- and Radiotherapy-Induced Mucositis. **Integr Cancer Ther**, p. 153473541879488, 2018.

TIAN X, et al. Efficacy and safety of oral zinc sulfate in the prevention of chemotherapy-induced oral mucositis. **Medicine**, v. 97, n. 21, p. e10839, 2018.

URBAIN P, et al. Role of antioxidants in buccal mucosa cells and plasma on the incidence and severity of oral mucositis after allogeneic haematopoietic cell transplantation. **Support Care Cancer**, v. 20, n. 8, p. 1831-1838, 2012.

VAN AMSTERDAM J; BRUNT T; VAN DEN BRINK W. The adverse health effects of synthetic cannabinoids with emphasis on psychosis-like effects. **J Psychopharmacol**, v. 29, n. 3, p. 254-263, 2015.

VILLA A; SONIS ST. Pharmacotherapy for the management of cancer regimen-related oral mucositis. **Expert Opin Pharmacother**, v. 17, n. 13, p. 1801-1807, 2016.

WARD SJ, et al. Cannabidiol inhibits paclitaxel-induced neuropathic pain through 5-HT 1A receptors without diminishing nervous system function or chemotherapy efficacy. **Br J Pharmacol**, v. 171, n. 3, p. 636-645, 2014.

WELTY TE; LUEBKE A; GIDAL BE. Cannabidiol: Promise and Pitfalls. **Epilepsy Curr**, v. 14, n. 5, p. 250-252, 2014.

YANG L, et al. Cannabidiol protects liver from binge alcohol-induced steatosis by mechanisms including inhibition of oxidative stress and increase in autophagy. **Free Radic Biol Med**, v. 68, p. 260-267, 2014.

YAROM N, et al. Systematic review of natural agents for the management of oral mucositis in cancer patients. **Support Care Cancer**, v. 21, n. 11, p. 3209-322, 20131.

YUAN A; SONIS ST. Emerging therapies for the prevention and treatment of oral mucositis. **Expert Opin Emerg Drugs**, v. 19, n. 3, p. 343-351, 2014.

ANEXOS

ANEXO A

IMPORTANT: Your article accepted in Journal of Clinical Pharmacy and Therapeutics   Entrada x   🖨 🖼

cs-author@wiley.com   05/01/2017 ☆   ↩   ▾
para mim ▾

文A inglês ▾   >   português ▾   Traduzir mensagem   Desativar para: inglês ✕

Dear Letícia Cuba,

Article ID: JCPT12504
Article: Cannabidiol: an alternative therapeutic agent for oral mucositis?
Journal: Journal of Clinical Pharmacy and Therapeutics

Congratulations on the acceptance of your article for publication in Journal of Clinical Pharmacy and Therapeutics.

Your article has been received by production. You may wish to access Wiley Author Services to view your article record. Please click here or paste this link into your browser to register for Wiley Author Services.

http://authorservices.wiley.com/index.html#register-invite/IaHJhLKiAKxt-hPuBYLBKs2S4ElbKstvd6SzR48nl9g=

Sincerely,
Wiley Author Services

ANEXO B

SIPESQ
Sistema de Pesquisas da PUCRS

PROPESQ

Código SIPESQ: 6926   Porto Alegre, 11 de novembro de 2015.

Prezado(a) Pesquisador(a),

        A Comissão Científica da FACULDADE DE ODONTOLOGIA da PUCRS apreciou e aprovou o Projeto de Pesquisa "EFEITO DO CANABIDIOL NA MUCOSITE ORAL INDUZIDA EM CAMUNDONGOS SOB QUIMIOTERAPIA COM 5-FLUOROURACI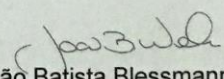L: AVALIAÇÃO CLÍNICA, HISTOLÓGICA, HEMATOLÓGICA E BIOQUÍMICA" coordenado por MARIA ANTONIA Z DE FIGUEIREDO. Caso este projeto necessite apreciação do Comitê de Ética em Pesquisa (CEP) e/ou da Comissão de Ética no Uso de Animais (CEUA), toda a documentação anexa deve ser idêntica à documentação enviada ao CEP/CEUA, juntamente com o Documento Unificado gerado pelo SIPESQ.

Atenciosamente,

Comissão Científica da FACULDADE DE ODONTOLOGIA

ANEXO C
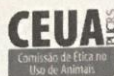
Pontifícia Universidade Católica do Rio Grande do Sul
PRÓ-REITORIA DE PESQUISA, INOVAÇÃO E DESENVOLVIMENTO
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

Ofício 01/2016 - CEUA   Porto Alegre, 07 de janeiro de 2016.

Prezado Sr(a). Pesquisador(a),

A Comissão de Ética no Uso de Animais da PUCRS apreciou e aprovou seu Protocolo de Pesquisa, registro CEUA 15/00488 intitulado **"Efeito do canabidiol na mucosite oral induzida em camundongos sob quimioterapia com 5-fluorouracil: Avaliação clínica, Histológica, Hematológica e Bioquímica"**.

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.

Nº de Animais	Espécie	Duração do Projeto
100	Mus musculus	03/2016 – 07/2016

ANIBIO – 2016 – 0002   Atenciosamente,

Prof. Dr. João Batista Blessmann Weber
Coordenador da CEUA/PUCRS

Ilma. Sra.
Profa. Dra. Maria Antonia Z. de Figueiredo
FO
Nesta Universidade

PUCRS

APÊNDICE 1

PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA - ÁREA DE CONCENTRAÇÃO EM ESTOMATOLOGIA CLÍNICA/PUCRS

EFEITO DO CANABIDIOL, UM COMPONENTE DA *CANNABIS SATIVA*, NA MUCOSITE ORAL INDUZIDA EM CAMUNDONGOS SOB QUIMIOTERAPIA COM 5-FLUOROURACIL: AVALIAÇÃO CLÍNICA, HISTOLÓGICA, HEMATOLÓGICA E BIOQUÍMICA

<u>FICHA DE AVALIAÇÃO CLÍNICA</u>

IDENTIFICAÇÃO

Rato nº: __   **Peso inicial: _____Kg**   **Peso final: _____Kg**

Tratamento: **Tempo:**

□ Grupo 1 (3mg)   □ Subgrupo A (4 dias)

□ Grupo 2 (10mg)   □ Subgrupo B (7 dias)

□ Grupo 3 (30mg)

□ Grupo 4 (placebo)

□ Grupo 5 (sem QT e placebo)

AVALIAÇÃO CLÍNICA LOCAL

□ Presença de úlcera: ( ) Sim ( ) Não   Tamanho: _____

□ Outras áreas de ulceração: □ Sim   □ Não

Localização: _____

CLASSIFICAÇÃO

( ) 0 - Normal

( ) 1 - Eritema

( ) 2 - Descamação epitelial

( ) 3 - Úlcera em até 25% da área da superfície

( ) 4 - Úlcera em mais de 25% até 50% da superfície

( ) 5 - Úlcera em mais de 50% até 75% da superfície

( ) 6 - Úlcera em mais de 75% da superfície

Fotos: _____

Data da avaliação: __/__/____.

APÊNDICE 2

PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA - ÁREA DE CONCENTRAÇÃO EM ESTOMATOLOGIA CLÍNICA/PUCRS

EFEITO DO CANABIDIOL, UM COMPONENTE DA CANNABIS SATIVA, NA MUCOSITE ORAL INDUZIDA EM CAMUNDONGOS SOB QUIMIOTERAPIA COM 5-FLUOROURACIL: AVALIAÇÃO CLÍNICA, HISTOLÓGICA, HEMATOLÓGICA E BIOQUÍMICA

<u>FICHA DE AVALIAÇÃO HISTOLÓGICA</u>

IDENTIFICAÇÃO

Rato nº: ___   **Lâmina nº:**_____

Tratamento	**Tempo**
□ Grupo 1 (3mg)	□ Subgrupo A (4 dias)
□ Grupo 2 (10mg)	□ Subgrupo B (7 dias)
□ Grupo 3 (30mg)	
□ Grupo 4 (placebo)	
□ Grupo 5 (sem QT e placebo)	

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

PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA - ÁREA DE CONCENTRAÇÃO EM ESTOMATOLOGIA CLÍNICA/PUCRS

EFEITO DO CANABIDIOL, UM COMPONENTE DA *CANNABIS SATIVA*, NA MUCOSITE ORAL INDUZIDA EM CAMUNDONGOS SOB QUIMIOTERAPIA COM 5-FLUOROURACIL: AVALIAÇÃO CLÍNICA, HISTOLÓGICA, HEMATOLÓGICA E BIOQUÍMICA

FICHA DE AVALIAÇÃO HEMATOLÓGICA E BIOQUÍMICA

IDENTIFICAÇÃO

Rato nº: __   **Peso inicial:** _____Kg   **Peso final:** _____Kg

Tratamento:

□ Grupo 1 (3mg)

□ Grupo 2 (10mg)

□ Grupo 3 (30mg)

□ Grupo 4 (placebo)

□ Grupo 5 (sem QT e placebo)

Tempo:

□ Subgrupo A (4 dias)

□ Subgrupo B (7 dias)

AVALIAÇÃO HEMATOLÓGICA E BIOQUÍMICA

Eritrócitos		GSH	
Hemoglobina		CAT	
Hematócrito			
VCM			
CHCM			
RDW			
Leucócitos			
Neutrófilos			
Linfócitos			
Monócitos			
Eosinófilos			
Basófilos			
Plaquetas			

Observações:_____

Data da coleta:__/__/__

APÊNDICE 4
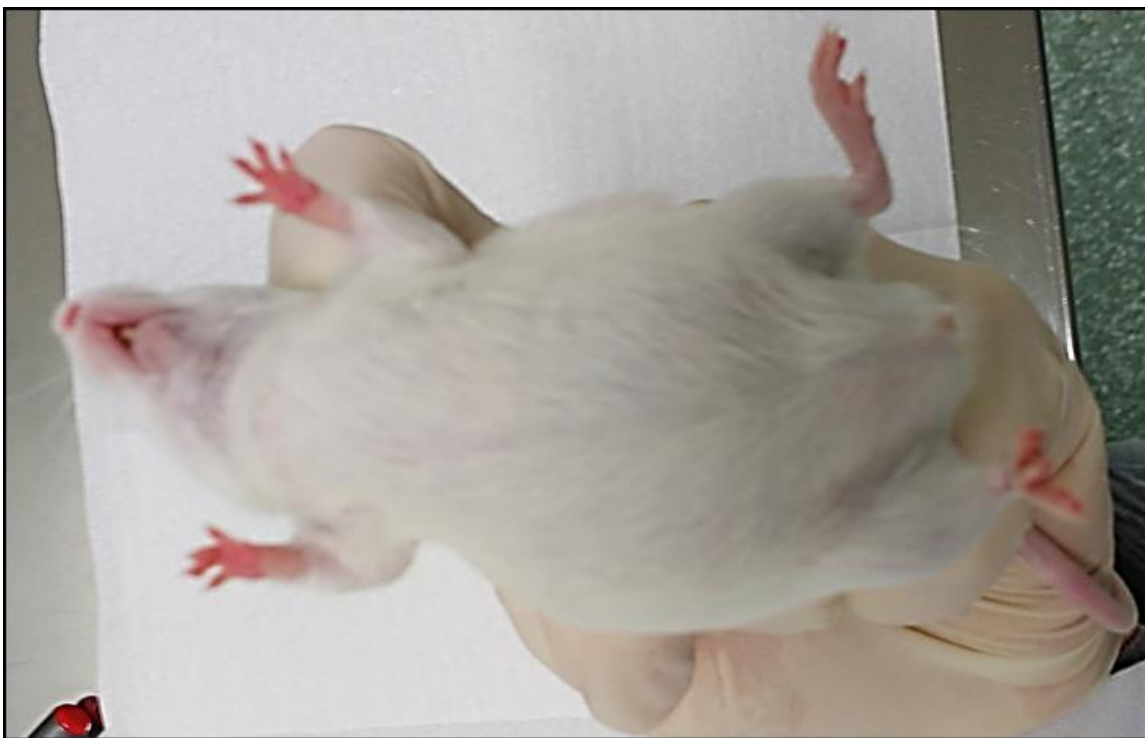
Contenção e posicionamento do animal para a injeção intraperitoneal das substâncias empregadas dispensando sedação.

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

APÊNDICE 5

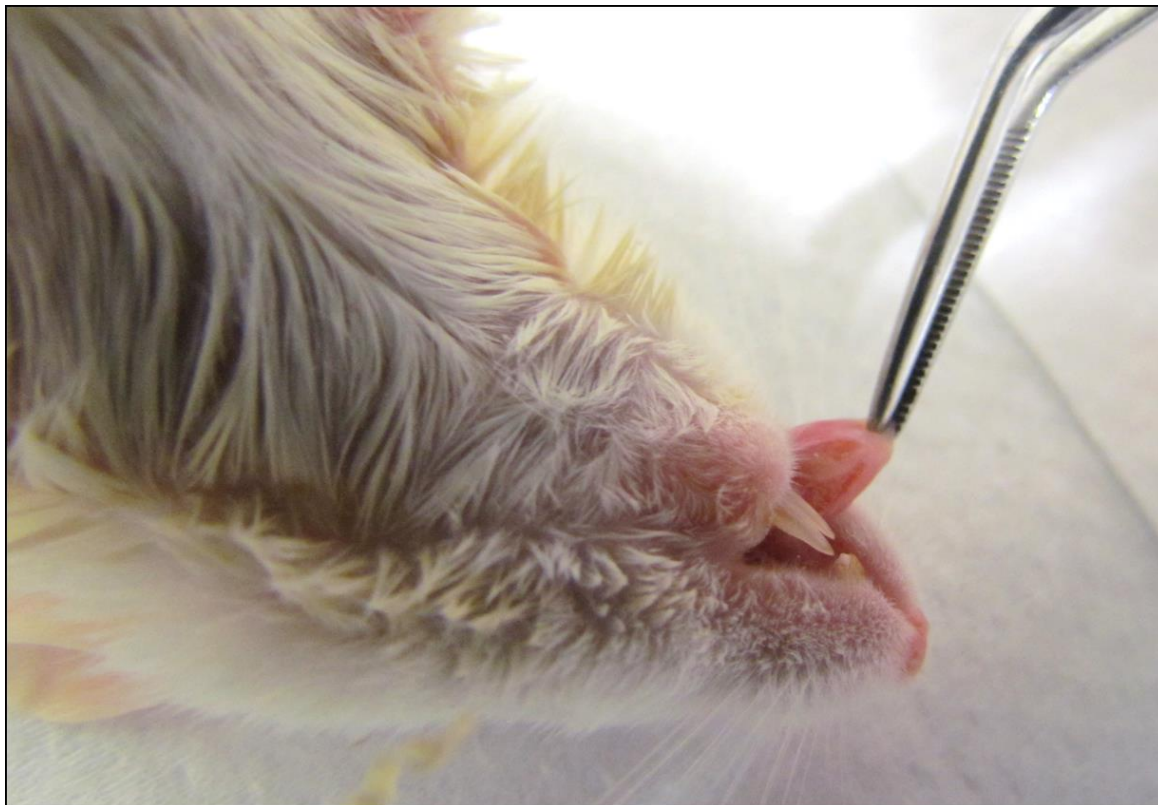Coleta das amostras de sangue através de punção cardíaca.

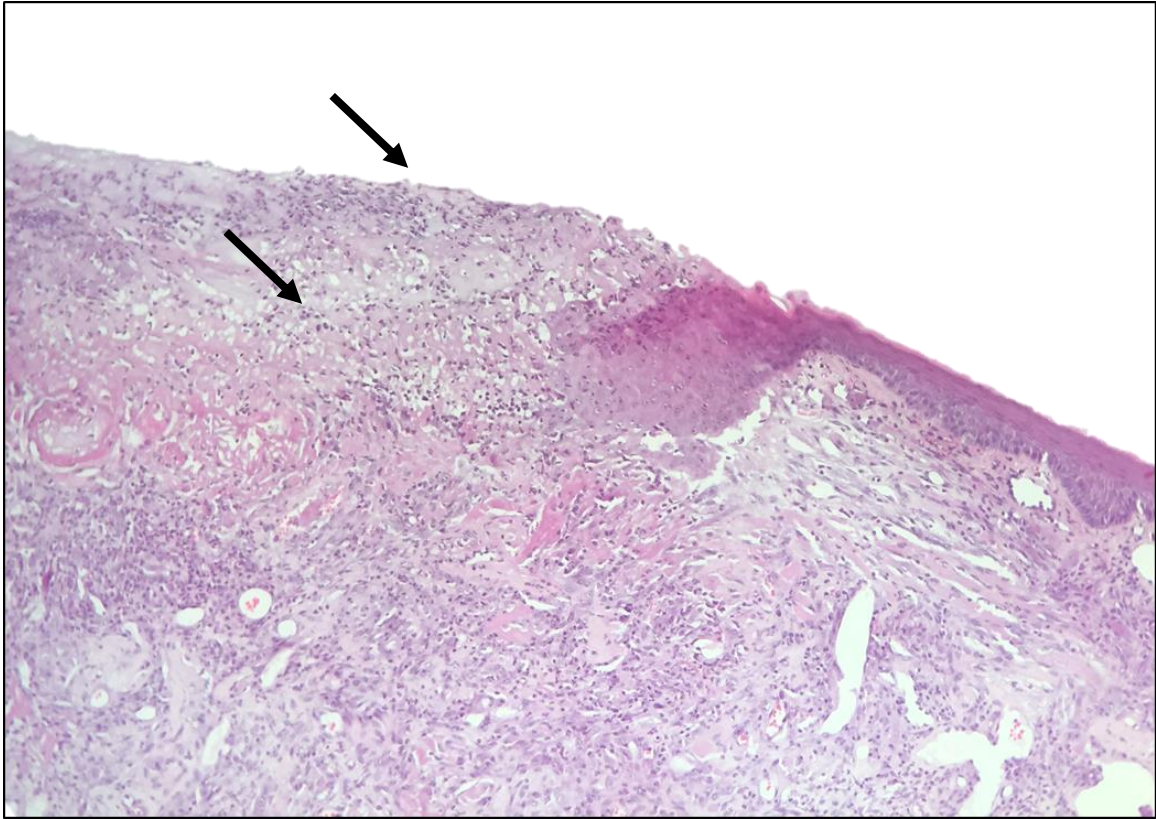
Coleta do fígado para análise de CAT e GSH.

APÊNDICE 6

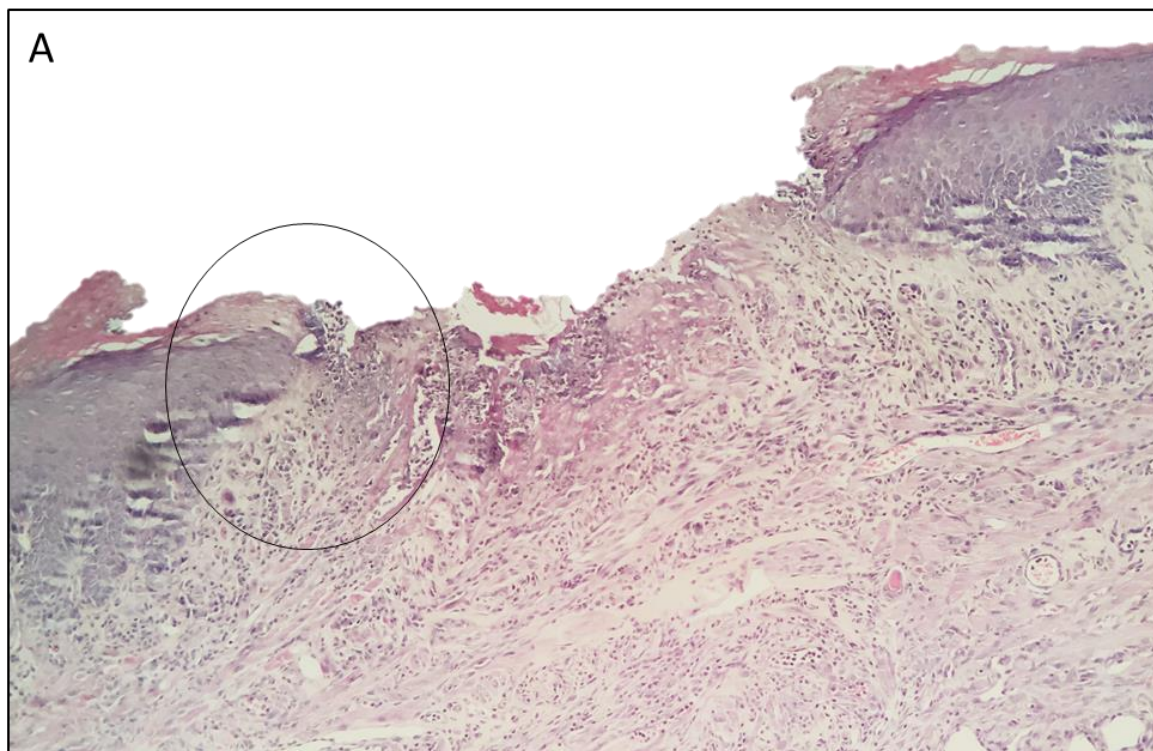Soluções preparadas imediatamente antes da aplicação nos animais.

APÊNDICE 7

Animal do grupo controle na avaliação de 7 dias (4B) apresentando extensa lesão ulcerada (MO grau 5), umedecimento e perda de pelos.
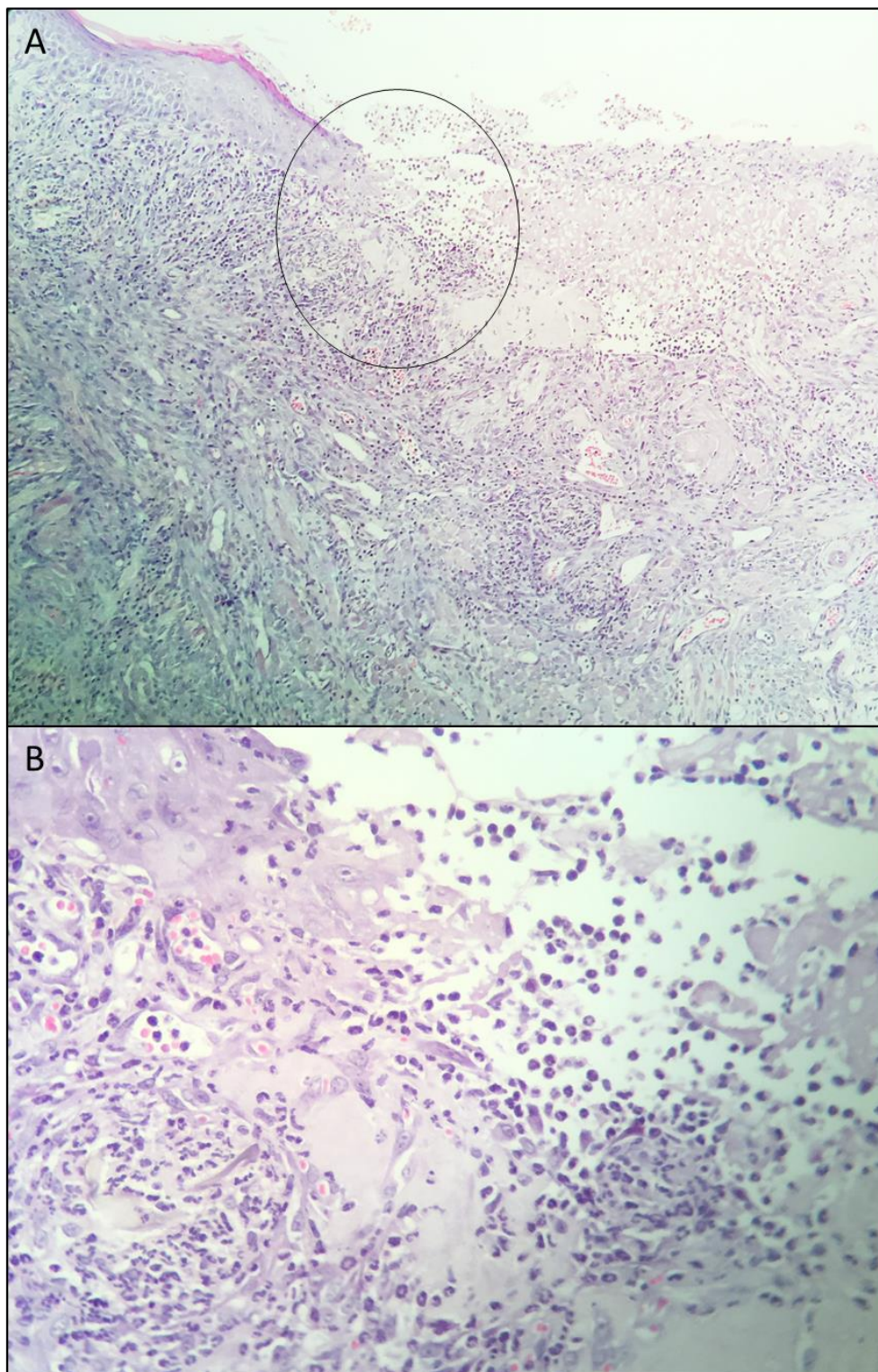
APÊNDICE 8

Fotomicrografia evidenciando área de tecido conjuntivo exposto, edema (HE – Aumento aproximado 100x).

APÊNDICE 9
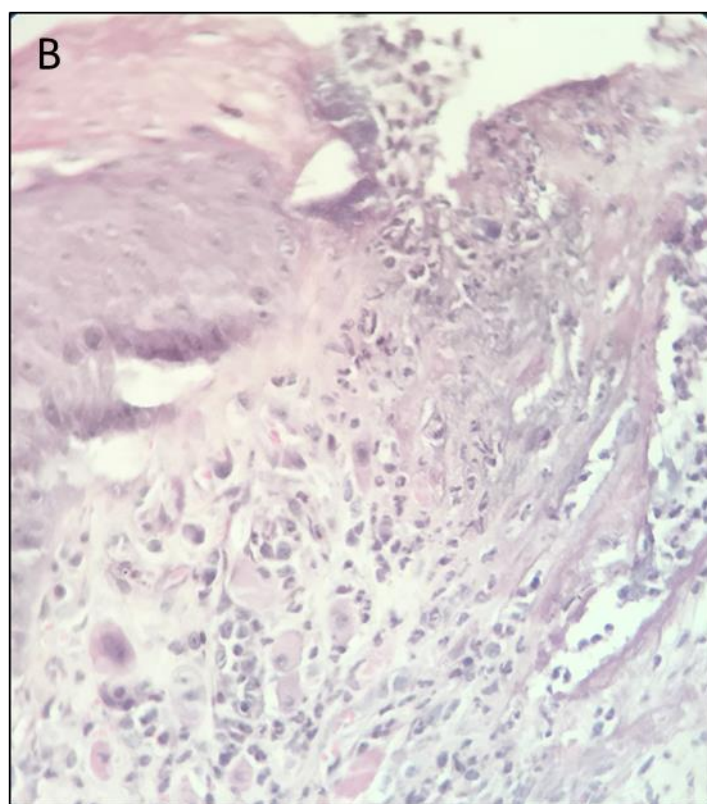
A - Fotomicrografia evidenciando a perda da continuidade do tecido epitelial, espessamento do mesmo, rica ceratinização nos bordos da úlcera e infiltrado inflamatório moderado (HE – aumento aproximado 100x) **B** - Infiltrado de células mononucleares, caracterizando processo inflamatório moderado (HE – aumento aproximado 400x)

APÊNDICE 10

A - Fotomicrografia evidenciando a perda da continuidade do epitélio, rica vascularização e intenso infiltrado inflamatório (HE – aumento aproximado 100x).
B - Infiltrado de células polimorfonucleares, caracterizando processo inflamatório intenso (HE – aumento aproximado 400x).

APÊNDICE 11
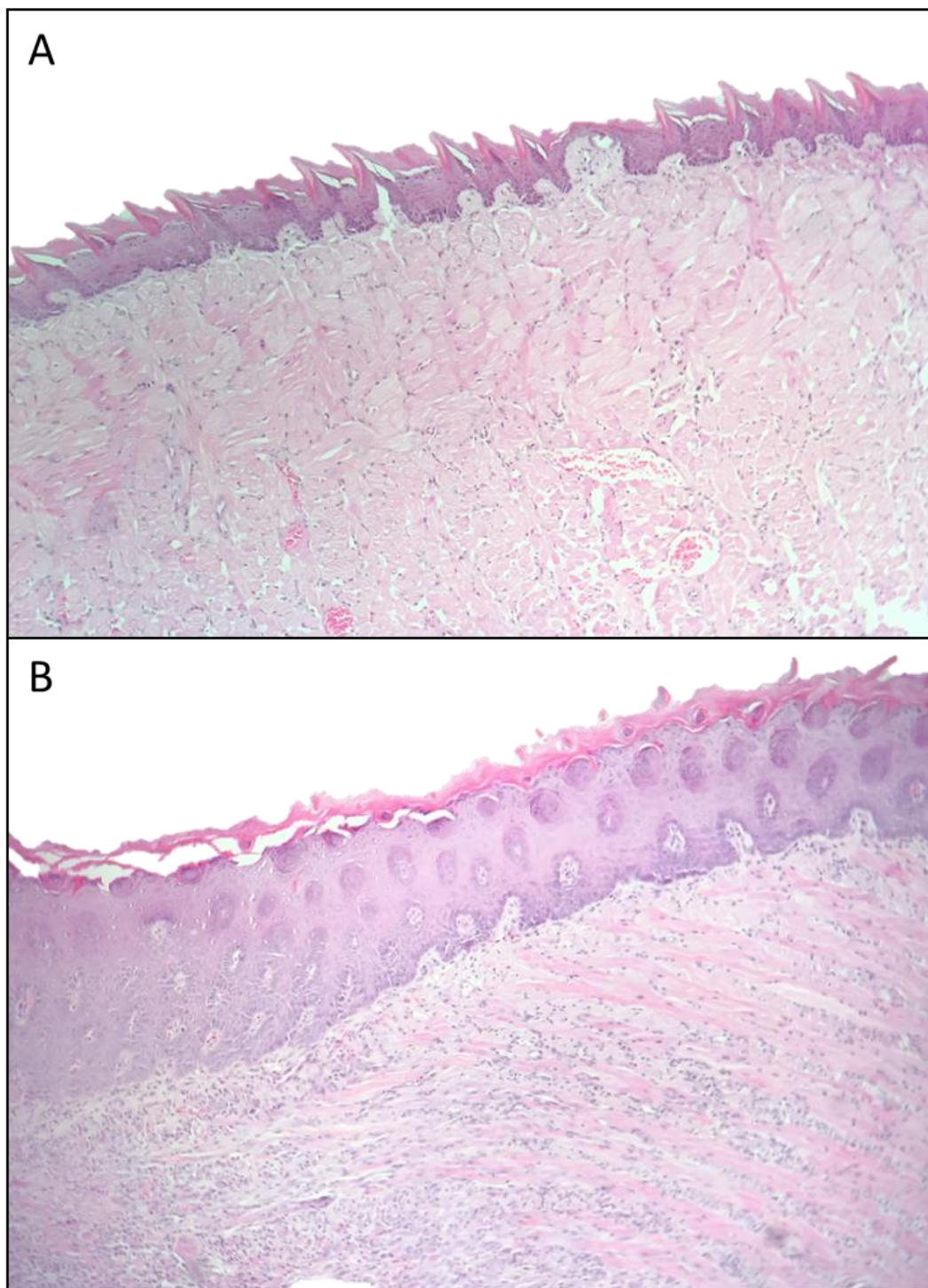
A - Fotomicrografia evidenciando a integridade das papilas linguais em animal do grupo controle negativo (HE – aumento aproximado 100x)
B - Perda de papilas e espessamento do epitélio em animal do grupo 1B o qual recebeu QT+ 3mg/Kg de CBD (HE – aumento aproximado 100x).