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Review Article

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Use of *Cannabis sativa* derivatives for the treatment of lymphomas: what is known so far?

Uso dos derivados da *Cannabis sativa* para o tratamento de linfomas: o que se conhece até o presente momento?

Taynara Maris da Silva[1]
Orcid: https://orcid.org/0000-0003-0304-5389

Lara Luiza Freitas de Oliveira[2]
Orcid: https://orcid.org/0000-0002-2115-0943

Anna Carolina de Moura Costa[3]
Orcid: https://orcid.org/0000-0001-6813-9007

Joaquim Maurício Duarte Almeida[4]
Orcid: https://orcid.org/0000-0001-5737-6963

Farah Maria Drumond Chequer[5]
Orcid: https://orcid.org/0000-0003-3514-2132

Abstract

Introduction: *Cannabis sativa* is a plant of great medicinal interest due to its various constituents with pharmacological properties, the main ones being cannabidiol (CBD) and Δ^9-tetrahydrocannabidiol (Δ^9-THC). A number of studies report the potential of phytocannabinoids for various diseases, including cancer, with lymphomas being cancers that mainly affect the body's defense cells. Objectives: To analyze what scientific evidence there is regarding the antitumor potential of phytocannabinoids against lymphomas. Materials and Methods: This is an integrative literature review using articles indexed in the following databases: PubMed, BVS, SciELO, CAPES Periodical and Science Direct, using the English descriptors: "*Cannabis*", "cannabidiol", "lymphoma" and "apoptosis", and Portuguese: "*Cannabis*", "canabidiol", "linfoma" and "apoptose", combined with the Boolean operators AND and OR, published until August 2021. Results: Six studies were found, these being carried out *in vivo*, *in vitro*, *ex vivo*, or clinical trial. The most studied phytocannabinoid was CBD and all selected studies addressed only non-Hodgkin's lymphomas. All trials demonstrated the effectiveness of the use of phytocannabinoids in the treatment of non-Hodgkin's lymphomas, and the antitumor effect was demonstrated through the induction of apoptosis, cytotoxicity, antiproliferation, and in the clinical trial by redirecting malignant cells to secondary organs. Conclusions: Most of the results obtained come from *in vitro* or animal research, with a lack of clinical studies being detected; in addition, more studies are needed in relation to the mechanisms involved in the action of phytocannabinoids against lymphomas and other cancers, since they are still little known.

Palavras-chave: anticancer agente; cannabidiol; hematologic neoplasms.

Resumo

Introdução: A *Cannabis sativa* é uma planta de grande interesse medicinal devido seus diversos constituintes com propriedades farmacológicas, sendo os principais o canabidiol (CBD) e o Δ^9-tetrahidrocanabidiol (Δ^9-THC). Uma série de estudos relatam o potencial dos fitocanabinoides para diversas doenças, incluindo o câncer, sendo os linfomas cânceres que afetam, principalmente, as células de defesa do organismo. Objetivos: Analisar o que há de evidências

[1] Universidade Federal de São João del-Rei. São João del-Rei/Minas Gerais, Brasil. E-mail: taynaramaris.silva@gmail.com
[2] Universidade Federal de São João del-Rei. São João del-Rei/Minas Gerais, Brasil. E-mail: luizalara422@gmail.com
[3] Universidade Federal de São João del-Rei. São João del-Rei/Minas Gerais, Brasil. E-mail: annacmourac@hotmail.com
[4] Universidade Federal de São João del-Rei. São João del-Rei/Minas Gerais, Brasil. E-mail: maudall@ufsj.edu.br
[5] Universidade Federal de São João del-Rei. São João del-Rei/Minas Gerais, Brasil. E-mail: farahchequer@ufsj.edu.br

científicas a respeito do potencial antitumoral dos fitocanabinoides contra os linfomas. Materiais e Métodos: Trata-se de uma revisão integrativa da literatura, utilizando artigos indexados nas bases de dados: PubMed, BVS, SciELO, Periódicos CAPES e *Science Direct*, através dos descritores em inglês: "*Cannabis*", "*cannabidiol*", "*lymphoma*" e "*apoptosis*", e português: "*Cannabis*", "canabidiol", "linfoma" e "apoptose", combinados aos operadores booleanos AND e OR, publicados até agosto de 2021. Resultados: Foram encontrados seis estudos, sendo estes, realizados *in vivo*, *in vitro*, *ex vivo*, ou ensaio clínico. O fitocanabinoide mais estudado foi o CBD e todos os estudos selecionados analisaram apenas linfomas não Hodgkin. Todos os ensaios demonstraram a efetividade do uso dos fitocanabinoides no tratamento de linfomas não Hodgkin, sendo o efeito antitumoral demonstrado através da indução de apoptose, citotoxicidade, antiproliferação, e no ensaio clínico por redirecionamento das células malignas para órgãos secundários. Conclusões: A maioria dos resultados obtidos são provindos de pesquisas *in vitro* ou com animais, sendo detectada uma carência de estudos clínicos, além disso, são necessários mais estudos em relação aos mecanismos envolvidos na ação dos fitocanabinoides contra linfomas e outros cânceres, visto que ainda são pouco conhecidos.

Keywords: agente anticâncer; canabidiol; neoplasias hematológicas.

Introduction

In Brazil, it is estimated that hematological neoplasms are responsible for about 7 % of cancer deaths in the country[1]. Among these neoplasms, lymphomas stand out, which are cancers that originate in the lymphatic system, affecting especially the defense cells, and due to their great heterogeneity they can be classified into two large groups, Hodgkin's lymphomas and non-Hodgkin's lymphomas[2].

Hodgkin's lymphoma is characterized by the orderly presence of large and easily identifiable neoplastic cells in the affected lymph node, called Reed-Sternberg cells, immersed in a substrate with an inflammatory appearance. Non-Hodgkin's lymphoma, in contrast, is the most common type, and is characterized by spreading in a disorderly manner, without having a characteristic cell type, presenting great morphological and genetic heterogeneity, and with a lower content of inflammatory cells. In non-Hodgkin's lymphomas, most cells are clonal, leading to somatic mutations in lymphoid progenitor cells, and the malignant cell has a B, T or NK cell (natural killer) phenotype[3].

According to data from the José Alencar Gomes da Silva National Cancer Institute (INCA)[4], it is estimated that in Brazil, each year there will be about 2,640 cases of Hodgkin's lymphoma, with 1,590 in men and 1,050 in women, totaling 532 deaths between both sexes. While non-Hodgkin's lymphoma will total about 12,030 cases, being 6,580 in men and 5,450 in women, reaching 4,923 deaths.

Currently, the treatment of lymphomas is based on chemotherapy, immunotherapy in association with chemotherapy, and/or radiotherapy[5]. These forms of treatment can cause toxic effects, as they affect both altered and healthy cells[6]. Thus, the study of new substances with antitumor potential that prevent the development of lymphomas is extremely important, and compounds derived from *Cannabis* have promising potential in the area.

Cannabis sativa has been studied for many years, where more than 400 bioactive constituents have been isolated from this plant, and from this total, more than 100 phytocannabinoids. Among *Cannabis* derivatives, non-psychoactive cannabidiol (CBD) and psychoactive Δ^9-tetrahydrocannabinol (Δ^9-THC) stand out, both acting on the endocannabinoid system. *Cannabis* derivatives have a wide

therapeutic application, including against different types of cancers, such as pancreas, breast, cervix, prostate, leukemia, lymphomas, among others[7]. These antitumor effects are based on several mechanisms, including induction of cell cycle arrest, promotion of apoptosis, inhibition of cell proliferation, migration and angiogenesis in tumor cells[8].

In this context, the objective of this review was to evaluate the existing scientific evidence to date regarding the antitumor potential of *Cannabis sativa* derivatives (phytocannabinoids) to assist in the treatment of lymphomas.

Materials and Methods

Sample and type of study

The present study is an integrative literature review, a method that according to Mendes et al. (2008)[9], allows the formulation of general conclusions regarding a certain area of study, through the synthesis of several published studies. The construction of this type of study is based on six steps: **I)** Identify the topic of study; **II)** Conduct a search in the scientific literature and establish inclusion and exclusion criteria for articles; **III)** Definition of the information extracted from the selected articles and their categorization; **IV)** Evaluation of selected studies; **V)** Interpretation and analysis of results; and **VI)** Presentation and discussion of the results obtained[9].

Research design

This integrative review addressed studies that consider the use of *Cannabis sativa* derivatives as therapy against lymphomas. To this end, the guiding question of the study was: "Do *Cannabis* derivatives have therapeutic potential against lymphomas?".

According to the guiding question, the "PICO" was established, with the study population (*Population*) being people with lymphoma, laboratory animals with lymphoma, cells collected from patients with lymphoma, and tumor cells in *in vitro* experiments; the intervention (*Intervention*) being the treatment carried out with compounds derived from *Cannabis sativa*; the control group (*Control*) was no treatment with *C. sativa* derivatives; and the outcome (*Outcomes*) was the antitumor effect against lymphomas (apoptosis, antiproliferation, and cytotoxicity).

Inclusion and Exclusion Criteria

Experimental laboratory trials (*in vitro*, *in vivo* and *ex vivo*) and clinical trials, published until August 2021, in English, Portuguese, or Spanish and answering the guiding question of the study, were considered eligible. Case reports, literature reviews, dissertations, theses, monographs, books, book chapters, editorials, manuals, news, reports and comments were excluded.

Procedures

The search for studies was carried out in four databases: PubMed, Virtual Health Library (*Biblioteca Virtual da Saúde* - BVS), Scientific Electronic Library Online (SciELO), Periodical CAPES and Science Direct, from July 31, 2021 to August 16, 2021. To carry out the search, DeCS (Health Sciences Descriptors) and MeSH (Medical Subject Headings) descriptors, in English: "*Cannabis*", "cannabidiol", "lymphoma" and "apoptosis", and in Portuguese: "*Cannabis*" "cannabidiol", "linfoma" and "apoptose", combined together with the Boolean operators "AND" and "OR" ("*Cannabis* OR cannabidiol" AND "lymphoma" AND "apoptosis") ("*Cannabis* OR canabidiol" AND "linfoma" AND "apoptose") were used.

After performing the searches in the four aforementioned databases, duplicate publications were excluded. Subsequently, the titles and abstracts were read, excluding articles that did not fit the theme of this review. The remaining articles were read in full, excluding those that did not fit as experimental studies or that did not answer the guiding question of this review. Finally, in order to locate articles that had not been found in the databases used, searches were

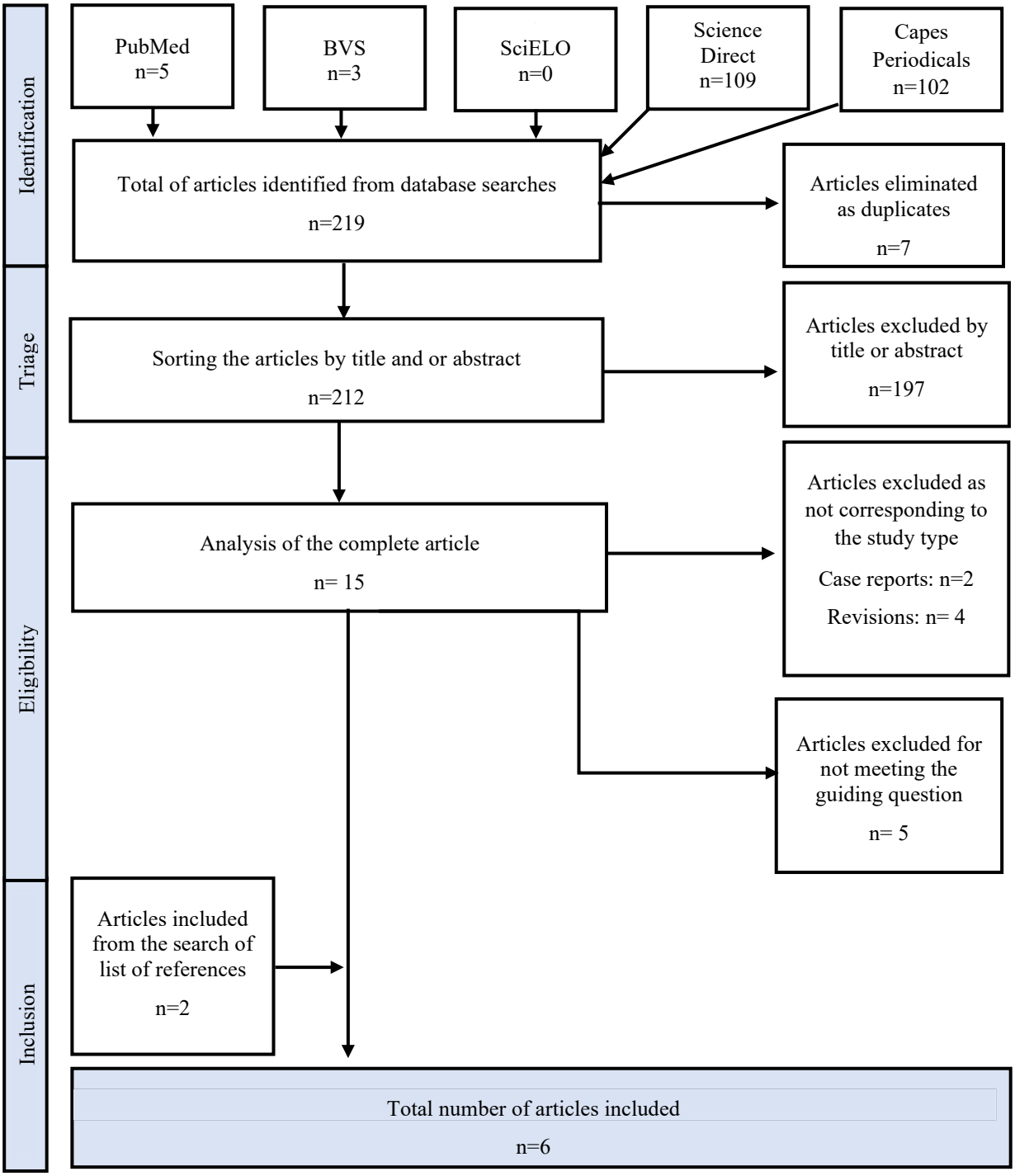
carried out in the reference lists of the selected articles. This selection was performed blindly and independently by two authors of this review.

After reading the articles in full that met the inclusion criteria, some data were collected, shown later in Tables 1 and 2. Table 1 covers general information about all studies included in this review (Author, Year and Country, Database, Title, and Purpose of the study). And in Table 2, more detailed information regarding the *in vitro*, *in vivo*, *ex vivo*, and clinical trials (Author and Year, Study type, Study population, Type of exposure, Control group, Effectiveness of *Cannabis sativa* derivatives (anti-tumor effect) and their limitations).

Results

In total, 219 articles were found in all analyzed databases. Of this total, seven were excluded for being in duplicate, 197 after reading the title and/or abstract, and 11 according to the eligibility criteria (six for not corresponding to the type of study: two case reports and four reviews; and five did not answer the guiding question of the research), leaving four articles. Two articles were also included from the search in the reference list of articles selected from studies that also answered the guiding question of this review, thus totaling six studies included. Figure 1 shows the step-by-step process of carrying out this selection of articles.

Figure 1: Flowchart that presents the methodology used in this integrative review.



Studies were found in several countries, including Romania, Israel, Canada, the United States, Japan, Sweden, Ireland, and Colombia (Table 1). However, with the search parameters used, no study was found in Brazil. Regarding the database, one article was selected from Pubmed (16.7 %), two from Science Direct (33.3 %), one from CAPES Periodical (16.7 %), and two from searches in the reference lists of selected articles (33.3 %).

All studies included in this review, shown in Table 1, analyzed only non-Hodgkin's lymphomas.

Table 1: General characteristics of the articles included in this review.

Author & year	Country	Database	Title	Objective
MAZUZ *et al*. (2020)[10]	Israel, Canada	PubMed	Synergistic cytotoxic activity of cannabinoids from *Cannabis* sativa against cutaneous T-cell lymphoma (CTCL) in-vitro and ex-vivo	Identify active compounds from whole *Cannabis* extracts and their synergistic mixtures, and evaluate their respective cytotoxic activity against CTCL cells (cutaneous T-cell lymphoma).
TOGANO *et al.*(2019)[11]	United States, Japan	Science Direct	The evaluation of cannabidiol's effect on the immunotherapy of Burkitt lymphoma (BL)	Assess whether CBD has an effect on the AF1q/ICAM-1 regulatory axis in Burkitt lymphoma.
MELÉN *et al.* (2019)[12]	Sweden	Science Direct	A Clinical Trial of *Cannabis* as Targeted Therapy for Indolent Leukemic Lymphoma	Demonstrate the effects of Sativex, which contains a mixture of components from the *Cannabis sativa* plant with exact proportions of THC and the partial CB1 antagonist CBD, in patients with indolent B-cell lymphoma.
XU (2018)[13]	Canada	Capes Periodicals	Does CBD induce apoptosis in diffuse large B cell lymphoma?	Assess the ability of CBD, a C*annabis*-derived compound, to induce apoptosis in diffuse large B-cell lymphoma (DLBCL).

MCKALLIP *et al.* (2006)[14]	Colombia, United States	List of references	Cannabidiol-Induced Apoptosis in Human Leukemia Cells: A Novel Role of cannabidiol in the Regulation of $p22^{phox}$ and Nox4 Expression	Examine the effects of the non-psychoactive cannabinoid, CBD on inducing apoptosis in leukemia and lymphoma cells.
MCKALIPP *et al.* (2002)[15]	United States	List of references	Targeting CB2 cannabinoid receptors as a novel therapy to treat malignant lymphoblastic disease	Investigate whether the binding of CB2 receptors could lead to apoptosis in tumors of the immune system (leukemia and lymphoma) and whether the receptor studied could be used in the treatment of cancer. Furthermore, to demonstrate that THC can inhibit the growth of murine lymphoma cells *in vivo* by inducing apoptosis and cure approximately 25 % of tumor-bearing mice.

Source: Research data, 2021.

Legend:

THC: Δ^9-tetrahydrocannabinol; CTCL: cutaneous T-cell lymphoma; CB2: type 2 cannabinoid receptor; BL: Burkitt lymphoma; CBD: cannabidiol; DLBCL: diffuse large B-cell lymphoma; ICAM-1: intracellular adhesion molecule.

Table 2 presents detailed information on studies performed *in vitro* (CTCL, Jiyoye and Mutu I, EL-4, and LSA cells), *in vivo* (C57B mice), *ex vivo* (SPBL and DLBCL cells), and a clinical trial. It is noteworthy that some of the studies addressed more than one type of trial (Table 2).

Table 2 shows that the main phytocannabinoids studied regarding their antitumor potential against lymphomas were cannabidiol (83.3 %) and Δ^9-tetrahydrocannabinol (50 %). In addition to these, cannabidivarin (16.6 %), cannabichromene (16.6 %), and cannabigerol (16.6 %) were also evaluated, but with less frequency.

All studies analyzed expressed the effectiveness of *Cannabis sativa* derivatives against non-Hodgkin's lymphomas. This antitumor potential was demonstrated in the studies included in this review, mainly through apoptosis, cytotoxicity, and reduction of cell proliferation in the analyzed samples (Table 2).

Most of the studies shown in Table 2 were experimental *in vitro assays*[10,11,13,14,15]. In these studies several cell lines were used, and in all assays the apoptotic, cytotoxic, or antiproliferative potential of *Cannabis* derivatives was demonstrated to these cells. Two studies[10,11] shown in this same table, relate the effects observed *in vitro* with some biochemical aspects, such as the cell cycle arrest in the G2 or S phases, the negative regulation of the PI3K pathway (phosphatidylnusitol 3-kinase), responsible for growth, cell survival and proliferation[10], and the increase in the levels of ICAM-1, which acts in cell apoptosis[11].

Regarding *in vivo* studies[14,15], they analyzed cases of acute lymphoblastic leukemia, which could progress to lymphoma, and also demonstrated the antitumor potential of *Cannabis* derivatives used in the treatment of mice. The study by McKallip et al. (2006)[14] analyzed the effect of cannabidiol, while McKallip et al. (2002)[15] analyzed Δ^9-tetrahydrocannabidiol, and apoptotic potential was demonstrated in both studies. McKallip et al. (2002)[15] also described the increase in the survival of mice treated with Δ^9-THC, and the complete cure of these animals.

These two previously mentioned studies also analyzed the action of cannabinoid receptors in the mechanisms of apoptosis. McKallip et al. (2002)[15] associated CB1 and CB2 with the induction of increased apoptosis, while McKallip et al. (2006)[14] only associated CB2 with these same effects. The study by McKallip et al. (2006)[14] also demonstrated the action of CB2 in the activation of caspases, consequently leading to apoptosis, and also demonstrated an increase in the levels of reactive oxygen species (ROS), which may corroborate the process of cell death (Table 2).

Two studies carried out *ex vivo*[10,13] are discussed in Table 2. Mazuz et al. (2020)[10] evaluated the activity of different fractions of *Cannabis* in a cutaneous T-cell lymphoma (CTCL), and the analysis was performed with cells affected by mycosis fungoides (MF), the most common type of cutaneous non-Hodgkin's lymphoma, in the Sézary stage. SPBL cells were collected from seven hospitalized patients, and exposed to phytocannabinoid fractions (CBD and Δ^9-THC), which led to apoptosis of these cells, being significantly selective for the cancer cell population, further implying a possible therapeutic use.

Xu (2018)[13] analyzed the ability to induce apoptosis of diffuse B-cell lymphoma (DLBCL) cells after exposure to CBD, in order to determine the degree to which this phytocannabinoid would be able to induce cell death in cancerous cells. In addition, the author evaluated the viability of different cell types exposed to this same phytocannabinoid together with DLBCL cells, aiming to analyze the ability of DLBCL cells to recover after exposure to CBD, thus measuring the ability of cancer to recur. The results of this study demonstrated the high degree of induction of apoptosis of cancer cells and the reduction of cell viability as the concentration of CBD was increased, these findings being important to determine the efficacy of the phytocannabinoid for the treatment of lymphoma, as well as to show that after the use of CBD there was no recurrence of cancer.

The only clinical trial included in this review was performed by Melén et al. (2019)[12] (Table 2). In this study, 23 patients (18-80 years) with indolent B-cell lymphoma were treated with Sativex, a drug containing CBD and Δ^9-THC. After drug administration, blood samples were collected from the patients and the analyzes showed that the treatment provided a reduction in the levels of malignant lymphocytes circulating in the blood. However, the study reported that there was no activation of caspase 3, suggesting the non-occurrence of induction of apoptosis, but the redistribution of malignant blood cells to secondary lymphoid organs. When targeted to secondary lymphoid organs, malignant lymphoma cells may receive pro-survival signals, and caution in the use of Sativex in patients with indolent leukemic lymphomas is extremely important.

Adverse effects observed after Sativex administration were grade 1, such as dry mouth (78 %), vertigo (70 %), hallucinations (30 %), confusion (17 %), nausea (4 %), vomiting (4 %), hypotension (9 %), euphoria (17 %), and stomach pain (4 %). These effects can be easily controlled; therefore the administration of a single dose of Sativex is considered safe, even in elderly patients (Table 2).

Table 2: Characteristics of the selected studies on the treatment potential of *Cannabis sativa* derivatives against lymphomas.

STUDY		METHOD			RESULTS	
Author & year	**Study type**	**Study population**	**Intervention**	**Control group**	**Effectiveness (anti-tumoral effect)**	**Limitations**
MAZUZ *et al*. (2020)[10]	Experimental trial *in vitro* & *ex vivo*	*In vitro*: CTCL cells: My-La, HuT-78 *Ex vivo*: SPBL cells (collected from 7 hospitalized patients)	Extract with a high content of CBD, separated into 5 fractions: S4 (CBD, THC, CBG, CBDV, a-bisabolol) (5 µg / mL) S5 (CBD, THC, CBG, CBC) (6 µg / mL) S4+S5 (CBD, THC, CBG, CBC) (5 and 6 µg / mL) Cytotoxicity and Apoptosis assessment, Cell Cycle Arrest: 48 h	Cells exposed to doxorubicin (+) and methanol (solvent)	Interaction of the different phytocannabinoids present in the fraction extracts, by synergistic effect, causes high cytotoxicity for cell lines Cell cycle arrest phase G2-M (My-La) and S phase (HuT-78), inhibiting cell proliferation My-La (65.4 %) and HuT-78 (85.9 %) cell apoptosis Via PI3K negatively affected in My-La and Hut-78	Not specified
TOGANO *et al.*(2019)[11]	Experimental trial *in vitro*	Jiyoye and Mutu I cells, from human Burkitt lymphoma	Cells transduced with a lentiviral vector with high expression of the AF1q protein, exposed to CBD for 24 h	Cells transduced with empty lentiviral vector (-) and cells transduced with lentiviral vector with high expression of AF1q (+) not exposed to CBD	Significant increase in ICAM-1 expression, without interference in AF1q expression Increased sensitivity of BL cells to cytotoxicity CBD appears to effectively overcome immunotherapeutic resistance mediated by upregulation of ICAM-1 expression	Limited clinical data, as the results were obtained using cell lines
MELÉN *et al*. (2019)[12]	Clinical trial	23 patients, aged 18 to 80 years, with indolent B-cell lymphoma with no indication for treatment	Single administration of Sativex, in the form of an oral mucosal spray, the maximum dose being 18.9 mg of THC and 17.5 mg of CBD	Blood samples taken the day before treatment, when patients had not yet had contact with Sativex	Significant reduction in the amount of malignant lymphocytes in peripheral blood after treatment Reduction of clonal B cells after treatment One week after treatment, non-malignant lymphocytes returned to baseline	More studies are needed to understand the affected pathways in this type of lymphoma

STUDY		METHOD			RESULTS	
Author & year	**Study type**	**Study population**	**Intervention**	**Control group**	**Effectiveness (anti-tumoral effect)**	**Limitations**
					levels, and clonal B cells increased significantly. No evidence of caspase 3 activation pathway, suggesting redirection of lymphoma cells to secondary lymphoid organs, where they receive a pro-survival signal	
XU (2018)[13]	Experimental trial *ex vivo* & *in vitro*	*Ex vivo*: DLBCL cells extracted from 24 patient samples *In vitro:* 24 cell lines for cell viability assays (CJ, LP, RC, TMD-8, WP, LY-19, MZ, 8LR, HT, MS, Toledo, BJAB, u2392, DS, Pheifer, McA, SUDHL-4, HF, SUDHL-6, HBL-1, DB, EJ, Val	Cells exposed to concentrations of 1.5 µM, 3.1 µM, 6.25 µM, 12.5 µM, 25 µM, 50 µM, and 100 uM of CBD, for 48 h	Cells exposed to 0 uM of CBD	*Ex vivo*: Cell apoptosis as the concentration increases, reaching almost 100 % *In vitro*: Reduction of cell viability as the dose of CBD is increased	Experiment execution errors (presence of air bubbles in the pipette or collection of cells in treatments of lower concentrations) The methods of apoptotic induction by CBD were not examined, making it impossible to compare with the literature Need for more research in the area, as lack of knowledge limits the use of CBD as a drug for DLBCL Time limitations restricted the number of apoptosis trials performed, obstructing the statistical analysis due to lack of extensive data Experiment conducted *in vitro*, not being

STUDY		METHOD			RESULTS	
Author & year	**Study type**	**Study population**	**Intervention**	**Control group**	**Effectiveness (anti-tumoral effect)**	**Limitations**
						possible to fully replicate the results in the human body environment
MCKALLIP *et al.* (2006)[14]	Experimental trial *in vitro* & *in vivo*	*In vitro*: Murine lymphoma EL-4 cells *In vivo*: 6 adult female C57BL mice, injected with EL-4 tumor cells	*In vitro*: Cells exposed to different concentrations of CBD (1.25 µM, 2.5 µM, 5 µM, 10 µM) for 24 h *In vivo*: Mice injected with various doses of CBD (12.5 or 25 mg/kg) on day 10 of tumor, and peritoneal fluid aspirated for cell analysis	*In vitro*: Cells not exposed to CBD (0 µM) *In vivo*: C57BL mice injected with PBS alone	*In vitro*: Significant reduction in cell viability at concentrations above 2.5 uM CBD Induction of apoptosis in tumor cells *In vivo*: Reduction in the number of viable cells in the peritoneal cavity as the dose of CBD was increased Significant induction of tumor cell apoptosis	Not specified
MCKALIPP *et al.* (2002)[15]	Experimental trial *in vitro* & *in vivo*	*In vitro:* EL-4 and LSA murine lymphoma cells *In vivo*: 6 adult female e C57BL mice, injected with EL-4 tumor cells	I*n vitro:* Cells exposed to various concentrations of THC (1, 10, 20 µM), for 24 h *In vivo:* i) Mice injected with different concentrations of THC (1, 3, 5 mg/kg) on the 10th day of tumor and peritoneal fluid aspirated for cell analysis ii) Administration of daily injection of 5 mg/kg of THC for 14 days for analysis of mouse survival	I*n vitro:* Cells exposed to various concentrations of THC for 2 to 24 h *In vivo:* i and ii) Mice injected with PBS alone	I*n vitro:* Reduction of cell viability and induction of apoptosis at concentrations above 10 µM *In vivo:* i) Reduction in the numbem of viable tumor cells and significant induction of apoptosis, above 3 mg/kg ii) Significant increase in mouse survival (25 %); Mice were completely cured as they were resistant to reintroduction with the specific tumor	Not specified

Source: Research data, 2021.

Legend:

THC: Δ^9-tetrahydrocannabinol; CTCL: cutaneous T-cell lymphoma; CB1: type 1 cannabinoid receptor; CB2: type 2 cannabinoid receptor; SPBL: peripheral blood lymphocytes from patients with Sézary; CBG: cannabigerol; CBDV: cannabidivarin; CBC: cannabichromene; LP: prolymphocytic leukemia; RC: "Red Child" cells; BL: Burkitt lymphoma; CBD: cannabidiol; DLBCL: diffuse large B-cell lymphoma; µM: micromolar; h: hours; ICAM-

1: intracellular adhesion molecule; MCL: mantle cell lymphoma; CLL: chronic lymphatic leukemia; JEKKO-1: mantle cell lymphoma cell; B-CLL: B-cell chronic lymphocytic leukemia; SK-MM2-2: plasma leukemic cells; PI3K: via phosphatidylnusitol 3-kinase.

Discussion

The use of *Cannabis sativa* is increasingly gaining ground in the therapeutic area, and there are already several studies on the therapeutic potential of this plant for the treatment of different diseases, including leukemias, which are pathologies similar to lymphomas[7,16]. All the studies discussed suggested that the phytocannabinoids derived from *Cannabis sativa* are effective in the treatment of non-Hodgkin's lymphoma, however, the mechanisms of this antitumor activity are still not well known.

In the present review, different types of studies were exposed, both preclinical: *in vitro*, *in vivo*, and *ex vivo*, and clinical. Most studies provided preclinical data, and only one clinical trial was found[12]. Preclinical studies, carried out in cells (*in vitro*), laboratory animals (*in vivo*), and in cells collected from the patient, but outside the patient's body (*ex vivo*) are extremely important to have a prior knowledge about the efficacy and toxicity of substances before they are administered to humans. However, preclinical trials are performed in a highly controlled manner and the data obtained may have some limitations. *In vitro* studies, for example, do not provide information about the interaction of the substance under analysis with all molecules in the human body, being tested only in isolation, which does not occur in the human body, since the molecules, tissues and organs are in constant communication. In *in vivo* studies, there are uncertainties associated with the extrapolation of data obtained in animal models to humans, and the doses administered, as well as the therapeutic window observed in animals, may be different from those in humans. In order to really know about the effectiveness of a compound with therapeutic application to humans, clinical studies are necessary, since it is essential to really understand how the substance interacts with the organism as a whole[17]. This demonstrates the need for further studies in the area of this research, mainly clinical studies.

Only studies referring to non-Hodgkin's lymphoma were found, probably because this is the type of lymphoma with the highest incidence in the population[4]. However, a case report by Huniadi et al. (2021)[18], demonstrated that a pregnant patient with Hodgkin's lymphoma achieved significant tumor reduction after the use of *Cannabis sativa*. In this case, despite the effectiveness demonstrated against lymphoma, the effects on the fetus must be taken into account[18].

The effects of phytocannabinoids in the body are triggered by the activation of type I (CB1) and type II (CB2) cannabinoid receptors, which are G[19] protein-coupled receptors. CB1 receptors are present in greater amounts in the cortex, hippocampus, cerebellum and basal ganglia, and are related to important brain functions such as memory, learning, peripheral nerve pathways, heart, among others. CB2 receptors in contrast, predominate in the immune system, especially in cells such as lymphocytes[20], and also have actions in inflammatory and neuropathic pain[21].

Among the most studied phytocannabinoids are cannabidiol and Δ^9-tetrahydrocannabidiol. CBD has greater activity at the CB2 receptor, present in lymphocytes[20], and possibly for this reason it was the most used phytocannabinoid in the studies of this review. Another reason would be that CBD is a non-psychoactive phytocannabinoid[22], which may also have contributed to the greater number of studies with it.

The molecular mechanism of action of cannabidiol against tumors is still not well understood. THC and CBD have

similar structures, but different mechanisms that end up resulting in cell death induction or cell growth interruption[23]. A study performed on breast cancer cells demonstrated the migration of tumor cells *in vitro*. This same study also demonstrated that CBD in conjunction with THC induces programmed cell death in glioma cells[24]. A study carried out evaluating prostate cancer cells also demonstrated the apoptosis of the cells analyzed after exposure to CBD, also citing that the effect of CBD may depend on the type of tumor cell under study, once again showing the need for research in this area[25].

Velasco et al. (2016)[8] described in their study that cannabinoids are capable of inducing apoptosis, autophagy, cell cycle arrest, and inhibiting angiogenesis and cell proliferation in tumors, which are the likely mechanisms by which the phytocannabinoids described in this review have demonstrated effectiveness against the lymphomas analyzed. Some of these mechanisms have been described in other studies that analyzed the action of endocannabinoids and synthetic cannabinoids against lymphomas. Flygare et al. (2005)[26] demonstrated the antiproliferative effects of the endocannabinoid anandamide (ANA) and the synthetic cannabinoid WIN 55,212-2 on mantle lymphoma cells, a type of non-Hodgkin's lymphoma, these effects being mediated by the CB1 receptor (*in vitro*). This same study[26] also demonstrated the induction of apoptosis of L144 and L102 cells, obtained from biopsies of patients with mantle lymphoma caused by endocannabinoids (AEA) and synthetic cannabinoids (SR141716) (*ex vivo*). Gustafsson et al. (2006)[27] demonstrated in their study the effect on the cell cycle, where the endocannabinoid AEA was able to induce a significant reduction in lymphoma-derived tumor growth in rats (*in vivo*).

There is also evidence that cannabinoids are able to modulate signaling pathways responsible for the growth and spread of cancer[3]. Among these biochemical mechanisms, we can mention the inhibition of the PI3K pathway (phosphatidylinositol kinase 3), which is an important pathway related to different functions, such as proliferation, cell survival, and participation in the G1-S phase of the cell cycle[28]. Another mechanism was the generation of reactive oxygen species, which may play an important role in inducing apoptosis in T cells[29], and a study carried out by Massi et al. (2004)[30] demonstrated that apoptosis of human glioma cells after exposure to CBD was mediated by the formation of reactive oxygen species (ROS). Activation of caspases plays a crucial role during apoptosis, especially caspase 3, which fragments the DFF inhibitory subunit ("DNA fragmentation factor"), releasing the active subunit of this molecule ("caspase-activated DNAse"), which has the function of migrating to the nucleus and fragmenting the DNA, leading to the formation of oligonucleosomal fragments, which are characteristic of apoptosis[31].

Finally, it is worth mentioning the limitations found in the studies presented in this review. Regarding the *in vitro* studies, the results were obtained using cell lines, and it is not possible to fully replicate the results in the human body; existence of few researches in the area; the mechanisms involved in the induction of apoptosis were not verified in order to be able to compare with what is reported in the literature; possibility of human error during the experiments; and that it would take more time to carry out the tests to have significant statistical data. Regarding the *in vivo* and *ex vivo* studies, the limitations were not specified in the analyzed articles. And regarding the clinical trial, the detailed limitation was the need for more studies to understand the affected pathways in the analyzed lymphoma. Regarding the present review, the limitation found was the absence of experimental studies (laboratory/clinical) related to Hodgkin's lymphomas.

Conclusion

The antitumor potential of *Cannabis sativa* derivatives against lymphomas was demonstrated in this review in *in vitro*, *in vivo*, *ex vivo*, and clinical studies, with experimental studies only referring to non-Hodgkin's lymphoma, while for Hodgkin's lymphoma only case reports were found.

The antitumor potential in relation to non-Hodgkin's lymphomas was described as a result of apoptosis, cytotoxicity, and reduced cell proliferation in the studies carried out, and some biochemical mechanisms were identified as responsible for the occurrence of these events, such as the cell cycle arrest in G2 or S phases, caspase 3 activation, PI3K pathway inhibition, and ROS generation.

Studies of new compounds with anticancer activity are extremely relevant and further clinical studies are essential, since most of the studies available today are *in vitro* studies. In addition, further studies are needed to clarify the pathways involved in the antitumor mechanism of these phytocannabinoids against lymphomas and other cancers, as they are still poorly understood.

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