Biomedicine & Pharmacotherapy 132 (2020) 110728

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Biological effects of Cannabidiol on normal human healthy cell populations: Systematic review of the literature

Stefano Pagano [a], Maddalena Coniglio [a], Chiara Valenti [a], Maria Isabella Federici [a], Guido Lombardo [a,*], Stefano Cianetti [a], Lorella Marinucci [b]

[a] Department of Biomedical and Surgical Sciences, Odontostomatological University Centre: Chair Prof. Stefano Cianetti, University of Perugia, Perugia, Italy
[b] Department of Experimental Medicine, Section of Biosciences and Medical Embryology, University of Perugia, Perugia, Italy

ARTICLE INFO

Keywords:
Cannabidiol
In vitro study
Cell viability
Apoptosis
Inflammation

ABSTRACT

A systematic review was performed to evaluate the biological effects of Cannabidiol (CBD), one of the major components of Cannabis Sativa, on normal human healthy cell populations in terms of cell viability, proliferation, migration, apoptosis and inflammation. Inclusion criteria were: studies on cell lines and primary cell culture from healthy donors, CBD exposure as variable, no CBD exposure as control and published in English language. Quality assessment was based on ToxR tool, with a score of reliability ranging from 15 to 18.Following the PRISMA statement, three independent reviewers performed both a manual and an electronic search using MEDLINE via PubMed, Scopus, Web of Science and Cochrane. From a total of 9437eligible articles, 29 studies have been selected. The average quality assessment score was 16.48.Theresults showed heterogeneous CBD concentration exposure (0.01–50 μM or 0.1 nmol/mL-15 mg/mL). The definition of a threshold limit would allow the identification of specific effects on expected outcomes. From the data obtained CBD resulted to inhibit cell viability in a dose-dependent manner above 2 μM, while in oral cell populations the inhibitory concentration is higher than 10 μM. Moreover, it was observed a significantly inhibition of cell migration and proliferation. On the contrary, it was highlighted a stimulation of apoptosis only at high doses (from 10 μM).Finally, CBD produced an anti-inflammatory effect, with a reduction of the pro-inflammatory cytokine gene expression and secretion. CBD down-regulated ROS production, although at high concentrations (16 μM) increased ROS-related genes expression. The diffusion of CBD for therapeutic and recreational uses require a precise definition of its potential biological effects. A thorough knowledge of these aspects would allow a safe use of this substance without any possible side effects.

1. Introduction

Drugs intake represents a problem both for the social and the health conditions of a community, in terms of potential acute toxic effects and potential problems related to chronic addiction. In the recent years, an increase in the consumption of Cannabis Sativa, commonly called "Marijuana", and its derivates has been reported, both for therapeutic and recreational use [1].

Cannabis Sativa is the most widely used drug in Europe according to the European Monitoring Center for Drugs and Drug Addiction [2], with almost 20 % of the people between 15 and24 years reporting they have used it at least once in the past year. In 5 years, hemp-grown land has increased 10 times, passing from 400 ha in 2013 to almost 4000 estimated in 2018.

Cannabis Sativa consists of two main more than a hundred components and the most represented ones are: tetrahydrocannabinol (THCor Δ^9-tetrahydrocannabinol), with a psychotropic effect, and cannabidiol (CBD or 2-[(6R)-6-isopropenyl-3-methyl-2-cyclohexen-1-yl]-5-pentyl-1,3-benzene-diol), with non-psychotropic effects. However, Cannabis contains more than 60 active principles. The biosynthesis process of cannabinoids, starting from Cannabis Sativa plant, produces cannabidiolic acid (CBDA) and tetrahydrocannabinolic acid (THCA) [3]. THC

* Corresponding author at: Department of Biomedical and Surgical Sciences, Odontostomatological University Centre, General Hospital Sant'Andrea delle Fratte, place L. Severi 1, 06132 Perugia, Italy.
E-mail addresses: stefano.pagano@unipg.it (S. Pagano), maddalena.coniglio@studenti.unipg.it (M. Coniglio), chiara.valenti@studenti.unipg.it (C. Valenti), mariaisabella.federici@studenti.unipg.it (M.I. Federici), guido.lombardo@unipg.it (G. Lombardo), stefano.cianetti@unipg.it (S. Cianetti), lorella.marinucci@unipg.it (L. Marinucci).

https://doi.org/10.1016/j.biopha.2020.110728
Received 5 August 2020; Received in revised form 29 August 2020; Accepted 2 September 2020
Available online 7 October 2020

and CBD can be obtained by a time-dependent decarboxylation of their precursors CBDA and THCA which occurs in presence of heat. Cannabis Sativa *Light*is a genetically modified variety of Cannabis Sativa that contains high concentrations of CBD and a reduced amount of THC, with concentrations lower than 0,2% [4,5].In the last few years, the progressive legalization of both Cannabis Sativa for therapeutic purposes in Europe and Cannabis Sativa *Light* in Italy (DL n.242 / 2016), led to the openings of many cannabis shops. Nowadays it is easy to find Cannabis Light in the form of products such as oils, foods, herbal teas and cosmetics, which can be purchased without prescription.

THC and CBD are phytocannabinoids and THC interacts as a partial agonist ligand with cannabinoids CB1 and CB2 receptors, which are mainly found in the central nervous system and in the immune system. THC acts as a partial agonist ligand on both receptors. Its pharmacological action is carried out through the binding of CB1 receptor with a presynaptic inhibition of the uptake of various neurotransmitters (in particular, dopamine and glutamate), and the stimulation of periaqueductal gray substance (PAG) and ventromedial rostral marrow (RVM) areas, which inhibit the ascending pathways of pain. CBD has a low affinity for CB1 and CB2 receptors, but acting as an indirect antagonist, potentiates THC by increasing the density of CB1 and extend sits effects by inhibiting cytochrome P450, CYP3A and CYP2C enzymes. CBD also acts as an antagonist of GPR55 (cannabinoid G protein-coupled receptors), which is a putative cannabinoid receptor expressed in the caudate nucleus and in the putamen, and of 5-HT1a receptor, which is associated with antidepressant, anxiolytic, and neuro protective effects [1]. The chemical structure of CBD is characterized by an aromatic ring, a perpendicular terpene ring and a pentyl side chain.

Despite the wide use of CBD, there are few information about its pharmacokinetic parameters in humans. After the administration with an oral spray, CBD turns out to have an half-life of 1.4–10.9 hours and 2–5 days with repeated oral administration, 24 h after intravenous administration and 31 h after smoking [6]. CBD bioavailability is about 6% after oral administration and 31 % after inhalation and there are no other evidences for intravenous formulations. The low oral bioavailability is a consequence of the lipophilic nature of cannabinoids. After oral administration, CBD absorption is slow and irregular and the pharmacological effects result within 30 and 60 min, reach a peak after 2−4 hours, lasting for about 8 h [6]. The maximum plasma concentrations are reached from 0 to 4 h and result faster after smoking or intravenous administrations, increasing in a dose-dependent manner, especially after high-fat meals, because CBD is a lipophilic substance. Furthermore, CBD has poor water solubility (12.6 mg/L), which leads to a low and irregular solubilization process in water-rich environments such as the gastrointestinal tract. Pharmacokinetic CBD parameters show great interindividual variability and understanding properties such as bioavailability and half-life is essential for future therapeutic successful use. CBD has a rapid distribution in the lungs, liver, hearth, brain and also in the hypovascularized tissues. It is important to consider that CBD lipophilicity allows it to be transported through the placenta into breast milk. There are also less information available for tissue distribution or CBD metabolites in living humans and few animal studies are relevant [6]. From *in vivo* studies on animals, CBD oral bioavailability results to be very low (13–19 %) and after an intense first pass metabolism its metabolites are mostly excreted in the kidneys, instead concentrations in plasma and brain are dose-dependent and bioavailability is increased with various lipid formulations [7]. The metabolism of CBD in humans occurs entirely in the liver, it is estimated that around 70–75 % of an orally absorbed dose of CBD can be removed by the hepatic metabolism before reaching the systemic circulation. CBD elimination occurs both renal (1/3 of the metabolites) and faecal (2/3 of the metabolites) and the excretion process is faster in chronic cannabis users. The slow excretion of cannabinoids influences the tolerability profile of the drug, especially considering the effects on cognitive and psychomotor levels [3]. According to the therapeutic use of the drug, the possible reduction of renal and hepatic function must be taken into consideration, because it could lead to an increase in CBD or metabolites plasma levels, related to the duration of the drug side effects, disrupting in particular activities that require attention and psychomotor coordination. However, despite the common use in humans, there are no sufficient evidences about CBD pharmacological properties and for this reason this review aims to be a preliminary study that anticipates further future studies also addressed on *in vivo* experiments.

CBD is a lipophilic substance and its bioavailability is about 6% after oral administration and 31 % after inhalation. CBD has a rapid distribution in the lungs, liver, hearth, brain and also in the hypovascularized tissues. Lipophilicity allows CBD to be transported through the placenta into breast milk. CBD is metabolized in the liver, but the excretion is mainly faecal [3].

The negative effects of THC highlighted in the literature are euphoria, alteration of spatio-temporal perceptions, confusional state, drowsiness, mydriasis, cardiovascular effects such as tachycardia and changes in blood pressure, neuronal degeneration, and increased risk of cancer and fibrosis in patients with chronic hepatitis [8,9]. In the oral cavity, THC causes xerostomia, leukoplakia, candidiasis, gingivitis and periodontal disease [10]. CBD is currently used for its potential therapeutic effects [11]; it is known to have anti-inflammatory [12–14], pain-relieving, antioxidant, immunomodulatory, antidepressant, antiepileptic, anticonvulsant and antineoplastic effects, with only partial data on the effects on oral neoplasms [15–18].

The recent Literature [3] has mainly focused on CBD therapeutic effects, since it is a non-psychoactive component. Some studies [7,10], however, report negative effects which, in the specific case of the oral cavity, are represented by oxidative stress and gingival hyperplasia. The potential toxic effects on healthy cells are not well known but it is important to establish a link with the increased consumption and availability of Cannabis Sativa *Light* products. Theaim of this review was to investigate the safety level of CBD and to understand the dose- and time-dependent effects that CBD could induce, in vitro, on different types of healthy normal cell populations, in terms of viability, proliferation, migration, apoptosis and inflammation, through analysis of functional and morphological alterations.

2. Materials and methods

2.1. Protocol

This descriptive qualitative systematic review was carried out according to the PRISMA statement [19] and checklist (Supplementary Fig.1).A PRISMA flow diagram (Fig. 1) was used to represent the analysis workflow for the inclusion or exclusion of the articles. A search protocol was developed *a priori*, after the confrontation of all the members of the research team. The central question of this systematic review was: In *in vitro* conditions, does CBD exposure, compared to no CBD exposure, cause changes in different types of human cells regarding cell viability, proliferation, migration, apoptosis or inflammation? The research team constructed this question according to the PICOS strategy format [20] (Table 1). The review protocol can be obtained upon request to the lead author.

2.2. Study selection: criteria for eligibility of the studies

The inclusion criteria for eligibility were: (i) studies on normal human cell lines (ii) studies on human primary cells culture from healthy donors; (iii) CBD exposure as a variable; and (iv) published in the English language. *In vitro* or *in vivo* animal studies, clinical studies, case reports, review articles, retrospective studies, editorials, opinions, surveys, guidelines, conferences, commentary articles, as well as *in vitro* studies with human pathological or tumoral cells, were excluded. Studies with no full-text available were also excluded.

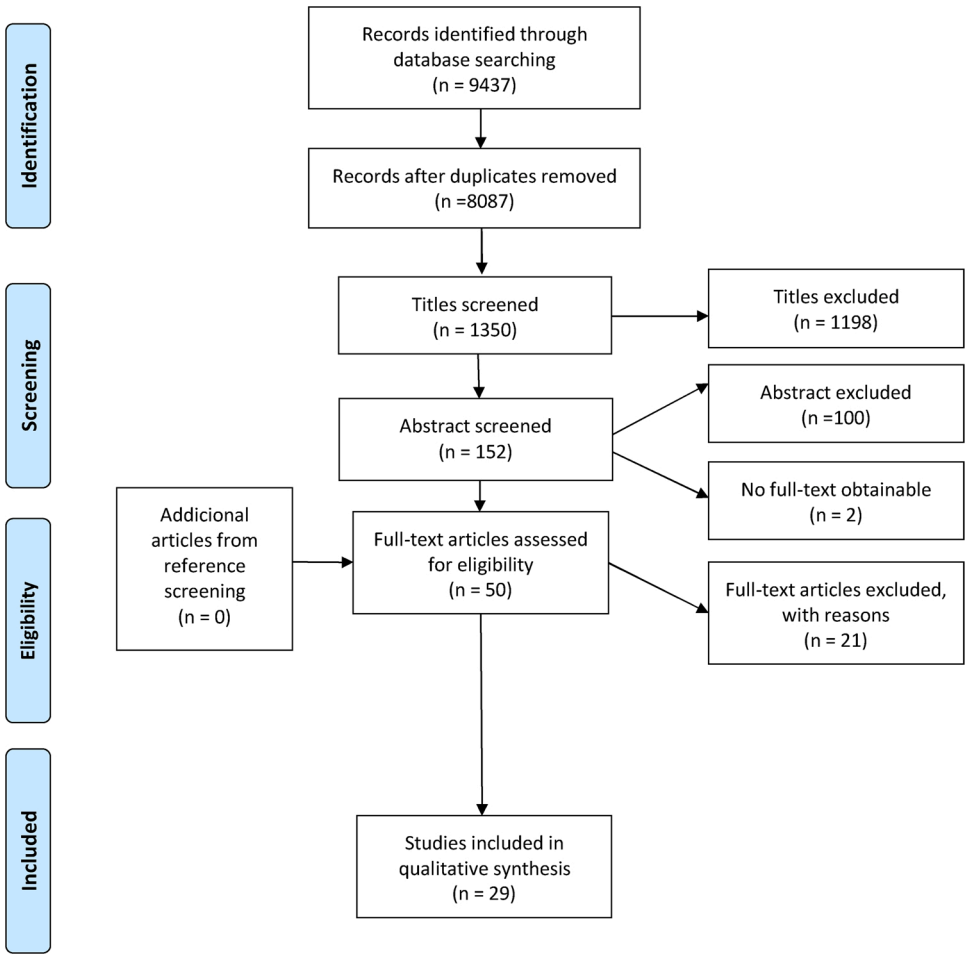

Fig. 1. Flow chart of the screening and selection process of the articles, according to the PRISMA Statement.

Table 1
Focused research question presented using the PICOS framework.

PICOS	
Population/patient	Keratinocytes: epidermal and gingival cells; Fibroblast: gingival, dermal, lung and endometrial stromal cells; Epithelial cells: sebocytes, melanocytes and bronchial cells; Endothelial cells: blood brain barrier, brain, stromal and umbilical vein cells; Stem cells: primary mesenchymal, adipose tissue mesenchymal, gingival mesenchymal and mesenchymal periodontal ligament cells; Blood cells: peripheral blood mononuclear cells and polymorphonuclear cells; Smooth muscle cells: umbilical artery cells; Chondrocytes; Trophoblast.
Intervention/ indicator	CBD exposure
Comparator/ control	No CBD exposure
Outcomes	Cell viability Cell proliferation Cell migration Apoptosis Inflammation
Study design	*In vitro*, experimental

2.3. Information about search methods and strategy for identification of the studies

The search process was performed by three different reviewers. Specific search strategies were developed and conducted up to March 9th 2020, using different electronic databases: MEDLINE via PubMed, Scopus, Web of Science and Cochrane Library (Table 2) were searched without temporal restriction. Adaptations were made to adopt the same terms on the different search engines of the three databases, in combination with database-specific filters.

2.3.1. Electronic searching

As shown in Table 2, the terms of the first column (CBD[All fields] OR Cannabidiol[Mesh Terms]) have been combined with the boolean operator "AND" with each of the terms of the second column.

2.3.2. Manual searching

Additionally, reference lists of any potential studies were examined in an attempt to identify any further relevant publications that could be considered for inclusion. Bibliographies of full-text reading articles were also screened.

2.4. Data collection process: synthesis, extraction and management

All titles of the articles initially retrieved in the search were selected following the eligibility criteria, and duplicates were eliminated. Three independent reviewers (C.M., F.M.I. and V.C.) were involved in the study. The titles were read and those indicating no relevance were excluded. Articles compatible with the inclusion criteria were selected

Table 2
Electronic database and search strategy. (09/03/2020).

#1 terms	#2 terms	Entries:			
		PubMed	Scopus	Web of Science	Cochrane
CBD[All fields] OR Cannabidiol [Mesh Terms]					
	Apoptosis[Mesh Terms];	115	212	87	5
	Blotting, western [Mesh Terms];	27	43	31	0
	Caspase[Mesh Terms];	43	56	35	31
	Cell adhesion [Mesh Terms];	22	48	26	3
	Cell cycle[Mesh Terms];	45	57	37	25
	Cell cytokine [Mesh Terms]	124	195	112	37
	Cell death[Mesh Terms]	106	201	96	3
	Cell differentiation [Mesh Terms]	53	41	22	2
	Cell line[Mesh Terms]	199	253	118	23
	Cell migration [Mesh Terms]	55	76	64	18
	Cell movement [Mesh Terms]	35	29	21	4
	Cell proliferation [Mesh Terms]	102	156	98	41
	Cell survival [Mesh Terms]	93	88	74	14
	Cytotoxicity [Mesh Terms]	15	34	27	21
	Epithelial cell [Mesh Terms]	94	78	63	19
	Extracellular matrix[Mesh Terms]	6	2	1	4
	Fibroblast[Mesh Terms]	27	22	20	16
	Flow cytometry [Mesh Terms]	23	16	19	3
	Gene expression [Mesh Terms]	121	254	136	142
	Inflammation [Mesh Terms]	218	229	206	136
	In vitro[Mesh Terms]	509	626	493	148
	Keratinocyte [Mesh Terms]	10	21	14	8
	MTT[All fields]	13	15	9	7
	Necrosis[Mesh Terms]	74	85	65	64
	Oxidative stress [Mesh Terms]	93	173	102	101
	Real time PCR [Mesh Terms];	26	20	10	14
	ROS[Mesh Terms];	32	42	25	27
	Saliva[Mesh Terms];	41	17	27	13
	Scratch test[All fields];	0	0	0	0
	TNF-alpha[Mesh Terms];	74	85	63	65
	Toxicity[Mesh Terms];	167	149	108	135
	Tumor Necrosis Factor-alpha [Mesh Terms].	58	65	49	42

for further examinations and abstracts were screened. The full texts of potentially eligible studies were then reviewed against the inclusion/exclusion criteria independently by the reviewers and any disagreement resolved by consultation with the other authors. Scientific and technical informations were collected into two evidence tables with Microsoft Office Excel, Table 3 including: Author(s) and year of publication, cell types and cultures (primary or line) investigated, test types (viability, proliferation, wound repopulation, apoptosis and inflammation) and funding source(s), and Table 4 including: Author(s) and year of publication, cell types, cell viability, proliferation, migration, apoptosis and inflammation data (assay used, CBD exposure concentrations, time points and conditions, ToxRtool quality score and principal findings). For data analysis, a narrative approach was adopted.

2.5. Quality assessment in included studies

The ToxRtool (Toxicological data Reliability Assessment Tool) quality assessment for *in vitro* researches was performed for each included study to assess the inherent quality of toxicological data by assigning a score of reliability [21]. The tool consists of a18-pointrating checklist, which considers the description of methodological aspects (identification of test substance and test system, study design and documentation of results). Articles with less than 11 points were considered unreliable, studies with 11–14 points were reliable with possible restrictions, and articles with 15–18 points were considered reliable without restrictions.

3. Results

3.1. Search results and characteristics of selected articles

The flow diagram of screened manuscripts (Fig. 1) shows a total of 9437 potentially eligible studies following the electronic screening strategy search. After reviewer agreement and duplicates removal, which led to the elimination of 8087 articles, title screening was completed on 1350 studies resulting in1198 non-eligible studies being excluded at this stage. Abstract screening was completed on 152studies with 50 progressing to full-text review. Finally, 29 studies were included in the full data analysis. They were published over a 35 year period(from 1985 to 2020).

3.2. Quality assessment according to the ToxRTool

The 29 eligible articles were submitted to the ToxRTool toxicological quality assessment. Table 5 provides full details of the quality assessment for each study. As shown all studies were considered reliable without restrictions (scoring over 15 points) and no one was removed due to high risk of bias. The lowest score was 15 [22–25] and the highest score was 18 [26,27].Regarding the key methodological domains assessed by the ToxR tool, 27 studies failed to achieve in substance identification, test system characterization, study design protocol description or results documentations. Regarding funding sources, only 4 studies did not provide any details about fundings [25,28–30] and all the others received grants from educational, governmental and charitable sources (Table 3).

3.3. Cell types investigated

All studies investigated normal human cell types. Seventeen studies used primary cells, ten studies investigated cell lines and two studies investigated both primary cells and cell lines.

The cell types most studied were oral cells: eight studies investigated CBD effects on oral cells. Among these studies, five investigated primary human gingival mesenchymal stem cells (hGMSCs) [12,24,31–33], one investigated periodontal ligament mesenchymal stem primary cells (hPDLSCs) [34], one used human telomerase-immortalized gingival

Table 3
Characteristics of included studies: cell type, test types and funding sources.

Study	Cell type investigated	Primary cells or cell line	Cell viability	Cell proliferation	Wound repopulation	Apoptosis	Inflammation	Funding
Almada et al. (2020)	Human endometrial stromal (St-T1b) Human decidual cells (HdF)	Primary Cell line	x					Fundação para a Ciência e a Tecnologia (FCT/MCTES)
Aparicio-Blancoet al. (2019)	Human cerebral microvascular endothelial cells(hCMEC/D3)	Cell line	x					Complutense Research Fund and Santander-UCM Research Group Parenteral Administration of Drugs
Burstein et al. (1985)	Lung fibroblast cell (WI-38)	Cell line					x	No details provided
Casares et al (2020)	Normal human epidermal keratinocytes (NHEK) Human keratinocyte cells (HaCaT)	Cell line					x	Medical Research Institute of theUniversity of Dundee, Cancer Research UK andTenovus Scotland, Ministry of the Economy and Competition and European Union FEDER
Chiricostaet al. (2019)	Human gingivalmesenchymal stem cells (hGMSC)	Primary	x			x	x	Ministry of Health, Italy
Gu et al. (2019)	Human gingival keratinocyte cells (TIGKs)	Cell line	x				x	U.S. Department of Health and Human Services
Hwang et al. (2017)	Human epidermal melanocyte (HEMa-LP)	Primary	x					World Class 300 Project, Export Promotion Technology Development Program, Ministry of Agriculture,Republic of Korea
Jastrzabet al. (2019)	Human keratinocyte cells (CDD 1102 KERTr)	Cell line	x			x	x	National Science Centre Poland
Jenny et al. (2009)	Peripheral blood mononuclear cells (PBMC)	Primary	x				x	ÖsterreichischeForschungsförderungsgesellschaftand Bionorica Research (Innsbruck, Austria)
Lanza Cariccioet al. (2018)	Human periodontal ligament stem cells(hPDLSC)	Primary	x			x		IRCCS Centro Neurolesi “Bonino Pulejo”, Messina, Italy
Libro et al. (2016a)	Human gingival mesenchymal stem cells (hGMSC)	Primary				x	x	IRCCS Centro Neurolesi “Bonino-Pulejo”, Messina, Italy
Libro et al. (2016b)	Human gingival mesenchymal stem cells (hGMSC)	Primary				x		IRCCS Centro Neurolesi “Bonino-Pulejo”, Messina, Italy
Luo et al. (2019)	Human cerebral microvascular endothelial cells (hCMEC/D3)	Cell line	x		x			No details provided
Muthumalage& Rahman (2019)	Bronchial epithelial cells (BEAS-2B) Lung fibroblasts cells (HFL-1) Normal bronchial epithelial cells (NHBE)	Primary Cell line	x				x	Toxicology Training Program grant, National Institute of Health and WNY Center for Research on Flavored Tobacco Products
Neradugommaet al. (2019)	Human endometrial stromal cells (THESC) Placental trophoblast cells (HTR8-SV)	Cell line	x		x			National Institute of Drug Abuse, National Institute of the University of Washington: Alcohol and Drug Abuse Institute
Oláh et al. (2014)	Human sebaceous gland cells (SZ95)	Cell line	x	x		x	x	Hungarian and European Union research, Belgian FederalGovernment, the Research Foundation-Flanders, the Research Council of the KU Leuven and the Italian Ministry of Health
Petrosino et al. (2018)	Human keratinocyte cell line (HaCaT)	Primary	x				x	No details provided
Rajanetet al. (2016a)	Human gingival mesenchymal stem cells (hGMSC)	Primary	x	x				Ministry of Health, Italy
Rajanet al. (2016b)	Human gingival mesenchymal stem cells(hGMSC)	Primary					x	Ministry of Health, Italy
Rawal et al. (2011)	Human gingival fibroblasts (HGF)	Primary	x				x	Alumni Endowment Fund, University of Tennessee College ofDentistry
Ruhl et al. (2018)	Adipose tissue mesenchymal stroma cells (atMSC)	Primary		x			x	No details provided
Sangiovanni et al. (2019)	Human keratinocyte cells (HaCat) Human dermal fibroblasts (HDF)	Primary	x				x	Linnea SA; Ministry of Education, University and Research (MIUR), Italy
Schmuhl et al. (2014)	Mesenchymal stem cells (MSC)	Primary	x	x	x			FORUN program of the Medical Faculty, University of Rostock
Schwartz et al. (2018)	Human umbilical artery smooth muscle cells (HUASMC)	Primary	x	x	x	x	x	Deutsche Forschungsgemeinschaft
Solinas et al. (2012)	Human umbilical vein endothelial cells (HUVEC)	Primary		x	x	x	x	GW Pharmaceuticals and AIRC, Italy
Tagneet al. (2019)	Human polymorphonuclear leukocytes (PMN)	Primary	x		x		x	Clinical and Experimental Medicine and Medical Humanities, University of Insubria
Watzl et al. (1991)	Peripheral blood mononuclear cells (PBMC)	Primary					x	ADAMHA grants
Winklmayr et al. (2019)	Human chondrocyte cell line (C28/I2)	Cell line	x			x		No details provided
Wu et al. (2018)	CD14+ cells	Primary				x	x	Ministry of Science and Technology, Executive Yuan, Taiwan

Table 4
Principal findings of studies which investigated effect of CBD on cell viability, cell proliferation, wound repopulation, apoptosis or inflammation.

Study	Cell type	Assay used	Concentrations/time points	CBD preparation	ToxRtool	Principal finding
CELL VIABILITY						
Almada et al. (2020)	St-T1b HdF	MTT assay	2, 5 and 10 μM 6 days	DMSO (< 0,01 %)	17	No effect observed with 2 μM Reduced cell viability with 5 and 10 μM ($p < 0.05$)
Aparicio-Blanco et al. (2019)	hCMEC/D3	MTT assay	15 mg/mL with lipid nanocapsules (3:1 v/v) 1, 4 and 24 h	THC Pharm	15	No effect observed with 15 mg/mL (1, 4 and 24 h)
Chiricosta et al. (2019)	hGMSC	MTT assay	5 μM 24, 48 and 72 h	DMSO (0.1 %)	17	No effect observed with 5 μM (24, 48 and 72 h)
Gu et al. (2019)	TIGK	MTT Assay	0–10 μg/mL 2 h	Cayman Chemical	16	Reduced cell viability with 10 μg/mL ($p < 0.001$)
Hwang et al. (2017)	HEMa-LP	MTT assay	1, 3 and 6 μM 5 days	Methanol (1 mg/mL)	18	No effect observed with 1, 3 and 6 μM (5 days)
Jastrzab et al. (2019)	CDD 1102 KERTr	MTT assay	0, 0.1, 0.5, 1, 2, 4, 10, 25, 50, 100 nmol/mL on cells irradiated or non-irradiated with UVA (30 J/cm^2) and UVB (60 J/cm^2) 24 h	Ethanol	15	No effect observed with 0.1−1 μM Reduced cell viability with 2−100 μM ($p < 0.05$) Reduced cell viability with 0.1−100 μM ($p < 0.05$) on UVA and UVB-treated cells
Jenny et al. (2009)	PBMC	MTT assay Trypan blue exclusion test	0.01−20 μg/mL on cells stimulated or unstimulated with mitogens: 10 μg/mL PHA or 10 μg/mL Con A 48 h	Ethanol	16	Reduced cell viability with 7.5−20 μg/mL (48 h, $p < 0.05$ and $p < 0.005$) on unstimulated cells, with 5−20 μg/mL (48 h, $p < 0.05$ and $p < 0.005$) on Con A-stimulated cells and with 7.5−20 μg/mL (48 h, $p < 0.05$ and $p < 0.005$) on PHA-stimulated cells
Lanza Cariccio et al. (2018)	hPDLSC	MTT assay Trypan blue exclusion test	0.5 μM with MOR (1:1) 24, 48 and 72 h	DMSO (0.1 %)	17	Stimulatory effect observed with 0.5 μM (48 and 72 h with MTT assay and 72 h with Trypan blue test; $p < 0.05$)
Luo et al. (2019)	hCMEC/D3	MTT assay Trypan Blue exclusion assay	0.1, 0.3, 1, 3, 10 μM 24 h	Methanol (<0,3%)	16	Stimulatory effect observed with 0.3−10 μM (24 h, $p < 0.05$ and $p < 0.01$)
Mabou Tagne et al. (2019)	PMN	MTT assay	0.01, 0.1, 1 and 10 μM 24 h	DMSO	17	No effect observed with 0.01, 0.1, 1 and 10 μM (24 h)
Muthumalage & Rahman (2019)	BEAS-2B HFL-1	AO/PI staining	10.6, 21.2, 31.8, 42.4 μM 24 h	Green Roads (100 mg)	16	BEAS-2B: Reduced cell viability with 31.8 μM (24 h, $p < 0.01$) HFL-1: Reduced cell viability with 42.4 μM (24 h, $p < 0.05$)
Neradugomma et al. (2019)	THESC	MTT assay	0.2, 2 and 20 μM on THESCs decidualized or non-decidualized cells with hormones combination 7 days	Methanol (1 mg/mL)	16	Non-decidualized THESC: No effect observed with 0.2, 2 and 20 μM (7 days) Decidualized THESC: Reduced cell viability with 20 μM (7 days, $p < 0.05$)
Oláh et al. (2014)	SZ95	MTT assay	0, 1, 10 μM 48 h	Ethanol	16	No effect observed with 1and 10 μM (48 h)
Petrosino et al. (2018)	HaCaT	MTT assay	1, 5, 10 e 20 μM with polycytidylic acid (100 μg/mL) 6, 12 e 24 h	DMSO	17	No effect observed with 1, 5, 10 and 20 μM (6, 12 and 24 h)
Rajan et al. (2016a)	hGMSC	Haematoxylin-eosin staining	5, 10 and 25 μM 24 h	DMSO (0,1%)	17	No effect observed with 5 μM (24 h) Reduced cell viability with 10 and 25 μm (24 h)
Rawal et al. (2011)	HGF	MTT assay	0.01–30 μM 1−6 days	Methanol (1 mg/mL)	16	No effect observed with 0.01−3 μM (1−6 days)
Ruhl et al. (2018)	atMSC	PrestoBlue assay Crystal violet staining	3 μM on cells stimulated or unstimulated with LPS: 10 μg/mL 2,4 and 7 days	Ethanol (0.01 %)	15	Reduced cell viability at 4 ($p < 0.05$) and at 7 days ($p < 0.001$) with crystal violet staining No effect observed with 3 μM (2, 4 and 7 days) with PrestoBlue assay
Sangiovanni et al. (2019)	HaCat HDF	MTT assay	HaCat: 0.05–5 μM HDF: 0.1–2.5 μM 6 e 24 h	Ethanol	16	No effect observed with 0.05−5 μM and 0.1−2.5 μM (6 and 24 h)
Schmuhl et al. (2014)	MSC	WST-1 assay	3 μM 6 h	Ethanol (0.01 %)	17	No effect observed with 3 μM (6 h)
Schwartz et al. (2018)	HUASMC	Trypan blue exclusion assay	0.1, 1, 3, 6, 10 μM 48 h	Ethanol (0.033 %)	17	No effect observed with 0.1, 1, 3, 6 and 10 μM (6 h)
Winklmayr et al. (2019)	C28/I2	Resazurin assay	3, 10 and 30 μM 2 and 24 h	Ethanol	17	No effect observed with 3, 10 and 30 μM (2 and 24 h)

(*continued on next page*)

Table 4 (*continued*)

Study	Cell type	Assay used	Concentrations/time points	CBD preparation	ToxRtool	Principal finding
CELL PROLIFERATION						
Oláh et al. (2014)	SZ95	CyQUANT proliferation assay	0, 5, 10 μM 72 h	Ethanol	16	Reduced cell proliferation with 5 and 10 μM (72 h, $p < 0.05$)
		Real Time PCR (MK167)	10 μM 24 h			Reduced MK167 expression with 10 μM (24 h, $p < 0.001$)
		NGS Analysis	0, 5, 10 e 25 μM			Increased HELB, RPS6KA1, GINS1, KIT, MAPK8IP1, NUF2, PA2G4P4 and ZHX2 expression (24 h, $p < 0.05$)
Rajan et al. (2016a)	hGMSC	Illumina MiseqDx (HELB, RPS6KA1, GINS1, KIT, MAPK8IP1, NUF2, PA2G4P4, ZHX2, ZNHIT1, SPHK2, RBBP6, PCNA, PCM1, ORC6, ORC5, ORC3, MIS12, MASTL, BCL7B, CDK5, CDK5RAP1, JAM2, PIN4, MCRS1, PLXNB3, PCID2, PCGF2, PCGF1, OGFR, CDKNIA, CDK4 and CCNDPB1)		DMSO (0.1 %)	17	
Schmuhl et al. (2014)	MSC	Crystal violet staining	3 μM administered 3 times a week 14, 21, 28, 35 days	Ethanol (0.01 %)	17	Reduced cell proliferation with 3 μM (14, 28 and 35 days, $p < 0.01$)
Schwartz et al. (2018)	HUASMC	BrdU cell proliferation assay	0.1, 1, 3, 10 μM 48 h 6 μM 6, 24 and 48 h	Ethanol (0.033 %)	17	Reduced cell proliferation with 10 μM (48 h, $p < 0.05$) and with 6 μM (24 and 48 h, $p < 0.05$)
Solinas et al. (2012)	HUVEC	MTT assay	1–19 μM 24 h	Ethanol (0.05 %)	17	Reduced cell proliferation with 9−19 μM
WOUND REPOPULATION: SCRATCH TESTAND MIGRATION						
Luo et al. (2019)	hCMEC/D3	Wound healing migration assay	3 μM 0, 4, 8, 24 h	Methanol (< 0,3%)	16	Increased cell migration with 3 μM (4 and 8 h, $p < 0.05$)
Mabou Tagne et al. (2019)	PMN	Boyden chamber assay	0.01−10 μM with or without IL-8 and fMLP stimulation 90 min	DMSO	17	No effects observed on cell migration without stimulation Inhibition of cell migration with 0.1, 1 and 10 μM on fMLP-stimulated cells (90 min, $p < 0.001$) and with 1 and 10 μM on IL8-stimulated cells (90 min, $p < 0.001$)
Neradugomma et al. (2019)	THESC	Boyden chamber assay	0.5 μM	Methanol (1 mg/mL)	16	Boyden chamber assay: Inhibition of cell migration with 0.5 μM (7 days, $p < 0.05$)
	HTR8-SV	Real time PCR (MMP-2, MMP-9)	7 days			Real time PCR (THESC): Reduced MMP-2 and MMP-9 expression with 0.5 μM (7 days, $p < 0.05$)
Schmuhl et al. (2014)	MSC	Boyden chamber assay	Boyden chamber assay:	Ethanol (0.01 %)	17	Boyden chamber assay and wound healing migration assay: Increased cell migration with 0.1 μM (6 h, $p < 0.01$), 1 μM (6 h, $p < 0.001$) and 3 μM (6 h, $p < 0.001$ and $p < 0.01$)
		Wound healing migration assay	3 μM 6 and 48 h and 0.01, 0.1, 1, 3 μM 6h			
		Western Blotting (p42/44 MAPK)	Wound healing migration assay: 3 μM 0−18 h Western Blotting: 0.01, 0.1, 1, 3 μM 1 h			Western Blotting: Increased p42/44 MAPK level with 3 μM (1 h, $p < 0.001$)
Schwartz et al. (2018)	HUASMC	Boyden chamber assay	6 and 10 μM 60 min	Ethanol (0.033 %)	17	Inhibition of cell migration with 10 μM (60 min, $p < 0.05$)
Solinas et al. (2012)	HUVEC	Modified Boyden chamber, Wound healing migration assay	1−19 μM 24 h	Ethanol (0,05 %)	17	Inhibition of cell migration upon 9 μM (24 h, $p < 0.01$)
APOPTOSIS						
Casares et al. (2020)	NHEK	Real Time PCR (NRF2, HMOX1, GCLC, p62)	NHEK: 10 μM 24 h	DMSO	18	NHEK: Increased HMOX1 expression with 5 μM ($p < 0.01$) and 10 μM (24 h, $p < 0.001$) and p62 expression with 10 μM (24 h, $p < 0.05$)
	HaCaT		HaCat: 1, 5, 10 μM 16 h			HaCat: Increased HMOX1 expression with 10 mM (24 h, $p < 0.0001$) and GCLC and p62 expression with 10 μM (24 h, $p < 0.01$)
Chiricosta et al. (2019)	hGMSC	Real Time PCR (CASP8)	5 μM 48 h	DMSO (0.1 %)	17	Reduced CASP8 expression with 5 μM ($q<0.05$)
Jastrzab et al. (2019)	CDD 1102 KERTr	Western Blotting (Ref-1 and pASK1)	1 μM on cells irradiated or non-irradiated with UVA	Ethanol	15	Reduced Ref-1 and pASK1 levels with 1 μM on irradiated cells with UVA ($p < 0.05$) and UVB ($p < 0.05$)

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Table 4 (*continued*)

Study	Cell type	Assay used	Concentrations/time points	CBD preparation	ToxRtool	Principal finding
			(30 J/cm^2) and UVB (60 J/cm^2) 24 h			
Lanza Cariccio et al. (2018)	hPDLSC	NGS analysis: Anti-apoptotic genes (63), death signaling genes (31), mTOR pathway genes (63), PI3K/AKT/mTOR pathway genes (38)	0.5 μM + MOR (1:1) 48 h	DMSO (0.1 %)	17	Anti-apoptotic effect with 0.5 μM (48 h, q<0.05)
Libro et al. (2016a)	hGMSC	Real Time PCR: TNF receptors (TNFRSF10B, TNFRSF11B, TNFRSF12A and TNFRSF19), caspases initiator (CASP4 and CASP8), pro-apoptotic mediators (BAX, BAD, BID, BCL7B, BCL2L13 and CYCS), apoptotic peptidase (APAF-1)	5 μM 24 h	DMSO	15	Real Time PCR: Reduced pro-apoptotic genes expression with 5 μM (24 h, $p < 0.05$)
		Western Blotting (CASP1)				Western Blotting: Reduced CASP1 level with 5 μM (24 h, $p < 0.05$)
Libro et al. (2016b)	hGMSC	Real Time PCR: aberrant tau phosphorylation (GSK3β, CDK5, DYRK1A, CAMK2A, MAPK1, MAPK12 and MAPK14), PI3K subunits (PIK3CA and PIK3CB), AKT1	5 μM 24 h	DMSO (0.1 %)	17	Real Time PCR: Reduced aberrant tau phosphorylation expression with 5 μM (24 h, $p < 0.05$) Increased PI3K subunits expression with 5 μM (24 h, $p < 0.05$)
		Immunocytochemistry (pPI3K, PI3K, pAKT, AKT, p-GSK3β, GSK3β)				Immunocytochemistry: Reduced protein level with 5 μM (24 h, $p < 0.0001$)
Oláh et al. (2014)	SZ95	DilC1(5) and SYTOX Green staining	DilC1(5) and SYTOX Green staining: 0, 1, 10 μM 24 h and 6 days	Ethanol	16	DilC1(5) and SYTOX Green staining: No effects observed with 0−10μM (24 h and 6 days)
		Microarray analysis	Microarray analysis: 10 μM 24 h			Microarray analysis: Reduced cell cycle gene expression (phase G2/M) with 10 μM (24 h, $p < 0.01$)
Schwartz et al. (2018)	HUASMC	Flow cytometry	6 and 10 μM 48 h	Ethanol (0,33 %)	17	No effect observed with 6 and 10 μM (48 h)
Solinas et al. (2012)	HUVEC	Flow cytometry	1−19 μM 24 h	Ethanol (0,05 %)	17	No effect observed with 1−19 μM (24 h)
		Caspase-Glo 3/7 assay	Caspase-Glo 3/7 assay: 10, 15, 20 and 30 μM 5 h	Ethanol	17	Caspase-Glo 3/7 assay: Increased caspase 3/7 activity with 10−30 μM (5 h, $p = 0.0266$)
Winklmayr et al. (2019)	C28/I2	Flow cytometry	Flow cytometry: 10, 15 and 30 μM 5 h			Flow cytometry: Increased apoptosis with 30 μM (5 h, $p = 0.0184$) and early apoptotic cells with 15 μM (5 h, $p = 0.0015$) and with 30 μM (5 h, $p = 0.0417$)
		Western Blotting (Erk1/2 and pErk1/2)	Western Blotting: 15 μM 3 h			Western Blotting: Increased p-Erk1/2 level (3 h, $p < .0.05$)
		Microscopy	Microscopy:10 and 30 μM 2 h			Microscopy: Increased in the late apoptotic and necrotic cells
Wu et al. (2018)	CD14+	Flow cytometry	Flow cytometry: 1, 4, 8, 12, 16 μM 1 h and 16 μM 5, 15, 30 min, 1 and 2 h	Ethanol (99.8 %)	17	Flow cytometry (phase sub-G0/G1): Increased apoptosis with16 μM (30 min - 2 h, $p < 0.05$) and with 8–16 μM (1 h, $p < 0.05$)
		Western Blotting (Cyt c)	Western Blotting: 16 μM 1 h			Western Blotting: Increased Cyt c level with 16 μM (1 h)
INFLAMMATION						
Burstein et al. (1985)	WI-38	Arachidonate labelling and release	3.2 μM	Ethanol (50 %), methanol (38 %) and water (12 %)	16	Reduced PGE2 synthesis with 3.2 μM (1, 2, 3 days, $p < 0.0005$)
		PG synthesis measurements	0, 1, 2, 3 days			
Casares et al. (2020)	NHEK	Luciferase assay	0, 5, 10 μM Luciferase assay:	DMSO	18	No effects observed with 0, 5 and 10 μM (30 min and 6 h)
	HaCaT	ROS assay (DCFH-DA)	6 h ROS assay: 30 min			
Chiricosta et al. (2019)	hGMSC	NGS analysis (TNFα, IL-1, IL-6 and TGFβ pathways)	5 μM 48 h	DMSO (0.1 %)	17	NGS analysis: Reduced MAP37K7, CLIP3, CASP8, SHARPIN, CHUK, GNB2L1, RPS27A, UBA52 and UBC expression and increased TNFRSF1A, SPPL2A, RIPK1, USP21, CYLD and UBB expression (TNFα pathway); reduced MYD88, MAP3K7, SQSTM1, IL1R1 and CHUK expression and increased MAP3K3 expression (IL-1 pathway); reduced JAK/STAT, IL6ST, STAT3, PIK3CD and TYK2 expression and increased AKT1, PIK3CA and PIK3CB expression (IL-6 pathway); increased FURIN, TGFBR1, SMURF2, STRAP, XPO1, NCOR1, PPP1CB and PPP1R15A

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Table 4 (*continued*)

Study	Cell type	Assay used	Concentrations/time points	CBD preparation	ToxRtool	Principal finding
						expression and reduced SMAD3, WWTR1, TGFBR2, FKBP1A, SMAD4, PPP1CA expression (TGFβ pathway) with 5 μM (48 h, q<0.05)
		Western Blotting (TNFα, NFkB, TGFβ1)				Western Blotting: Reduced NFkB level (48 h, $p < 0.001$) and increased TGFβ1 and TNFα levels with 5 μM (48 h, $p < 0.01$)
Gu. et al. (2019)	TIGK	ELISA assay (IL-6, IL-8, IL-10, IL-12, TNFα)	1 μg /mL on cells stimulated with LPS (0.1 μg/mL) and *P. gingivalis, F. alocis,* and *T. denticola* (MOI 1−50:1) 20 h	Cayman Chemical	16	TNFα: Reduced level with 1 μg /mL on LPS-stimulated (20 h, $p < 0.05$), reduced level with 1 μg / ml on *P. gingivalis*-stimulated cells (20 h, $p < 0.01$ and $p < 0.001$); IL-6, IL-10 and IL-12: Reduced level with 1 μg/mL on LPS-stimulated cells (20 h, $p < 0.05$), reduced level with 1 μg /mL on *P.gingivalis*-stimulated cells (20 h, $p < 0.01$ and $p < 0.05$); IL-8: Reduced level with 1 μg / ml on LPS-stimulated cells (20 h, $p < 0.05$), reduced level with 1 μg / ml on *P. gingivalis*-stimulated cells (20 h, $p < 0.001$ and $p < 0.05$); similar data was found upon *F. alocis* and *T. denticola* stimulation (data not shown)
		Electron Spin Resonance-ESR (ROS)	1 μM on cells irradiated or non-irradiated with	Ethanol	15	ESR, Spectrometry, CE, ELISA and GC/MSMS: Reduction of ROS, GSSG-R and GSH and increase of Cu, Zn-SOD, TxrR and TxR on non-irradiated cells (24 h, $p < 0.05$); reduction of GSH and increase in Cu.Zn-SOD, GSH-Px, GSSG-R, TrX, TxrR, Ref-1, pASK1 and 4-NHE on UVA-treated cells (24 h, $p < 0.05$); increase of ROS, Cu.Zn-SOD, GSH-Px, GSSG-R, TxrR, Ref-1, pASK1 and 4-NHE and reduction of GHS and TrX on UVB-treated cells (24 h, $p < 0.05$)
		Spectrometry (Cu.Zn-SOD, GSH-Px, GSSG-R, TrxR)				
Jastrzab et al. (2019)	CDD 1102 KERTr	Capillary electrophoresis-CE (GSH) ELISA assay (TrX) Gas Chromatograph y /Tandem Mass Spectrometry-GC/MSMS (4-NHE) Liquid chromatography (15d-PGJ2) Western Blotting (pNrf2, HO-1, Keap1, WTX, DPP3, CBP, TNFα, NFkB, IKKα, IKKβ				Liquid chromatography: Increased 15d-PGJ2 level with 1 μM (24 h) Western Blotting: Reduced NFkB (p52 and p65), PGAM5 and NLRP3 levels and increased TNFα, PIkB, IKKα, IKKβ and p62 levels with 1 μM (24 h, $p < 0.05$)
		Microscopy (NrF2 and NFkB)	UVA (30 J/cm^2) and UVB (60 J/cm^2) 24 h			Microscopy: Increased NFkB level in the cytoplasm on irradiated and non-irradiated cells; nuclear translocation of Nrf2 in the cytosol on non-irradiated cells and reduction of translocation in irradiated cells
		ELISA assay (INFγ)	0.01–20 μg/mL on cells stimulated			ELISA: Reduced IFNγ level with 10 μg/mL (48 h, $p < 0.05$)
Jenny et al. (2009)	PBMC	Real Time PCR (IDO and INFγ)	or unstimulated with mitogens: PHA or Con A (10 μg/mL) 48 h	Ethanol	16	Real Time PCR: Reduced IDO and IFNγ expression with 2.5 μg/mL (48 h, $p < 0.005$) and reduced IDO ($p < 0.005$) and IFNγ ($p < 0.05$) with 5 μg/mL
		High performance liquid chromatography-HPLC (kyn/trp)				HPLC: Reduced kyn/trp level with 10 μg/mL on unstimulated cells (48 h, $p < 0.05$) and with 0.1, 1 and 10 μg/mL on LPS-stimulated cells ($p < 0.05$)
Libro et al (2016a)	hGMSC	Real Time PCR (IL6ST, IL-1β, IL-18, IL-1R1, IL-11RA, IL-13R, MYD88, IFNGR1, IFNGR2, MAPK12, MAPK14, STAT3, STAT6, NFKB2, NFKB3/RELA, MMP-3)	5 μM 24 h	DMSO (0.1 %)	15	Reduced pro-inflammatory genes expression with 5 μM (24 h, $p < 0.05$)
Mabou Tagne et al. (2019)	PMN	ROS assay (DCFH-DA)	ROS assay: 0.01–10 μM 30 min	DMSO	17	ROS assay: Reduced ROS level with 1 μM on fMLP-stimulated cells (30 min, $p < 0.05$)
		Real Time PCR (TNFα) ELISA assay (TNFα)	Real Time PCR and ELISA assay: 10 μM 21 h on cells unstimulated or			Real Time PCR and ELISA: Reduced TNFα expression and level with 10 μM on stimulated cells (21 h, $p < 0.001$)

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Table 4 (*continued*)

Study	Cell type	Assay used	Concentrations/time points	CBD preparation	ToxRtool	Principal finding
			stimulated with fMLP or LPS (0.1 μM)			
Muthumalage & Rahman (2019)	BEAS-2B	Proteome profiler arrays (BEAS-2B: CCL1, CCL2/MCP1, CXCL1/KC, CXCL12, IL-8, IL-6, IL-16, IL-21, MIF, serpin E1, TNFSFS; HFL-1: CXCL1, CXCL2, IL-α, IL-6, IL-8, IL-13, IL-16, IL-21, IL-18) ELISA assay (IL-8 and MCP-1)	Proteome profiler: 10.6 μM 24 h	Green Roads (100 mg)	16	Proteome profiler BEAS-2B: Increased IL-8 and serpin E1 levels with 10.6 μM (24 h, $p < 0.05$); HLF-1: Increased
	HFL-1NHBE		ELISA assay: 10.6, 21.2, 31.8, 42.4 μM 24 h			CXCL1, IL-6 and IL-8 secretion with 10.6 μM (24 h, $p < 0.001$)
		Luminex assay (BEAS-2B and NHBE: MCP-1, CXCL1, CXCL2, IL-8, G-CSF, GM-CSF, IL-6; BEAS-2B with CBD vapor: Eotaxin, MCP-1, CXCL1, CXCL2, IL-8, and IL-6)	Luminex assay: 10.6 μM and 60 puff (1000 mg/30 mL) 24 h			ELISA assay BEAS-2B: Increased IL-8 secretion with 21.2 μM (24 h, $p < 0.05$) and 42.4 μM ($p < 0.001$); HFL-1: Increased IL-8 secretion with 31.8 μM (24 h, $p < 0.05$) and with 10.6, 21.2 and 42.4 μM ($p < 0.01$); reduced MCP-1 secretion on both cells Luminex assay: no observed secretion with 10.6 μM on NHBE and BEAS-2B (24 h, $p < 0.001$); increased Eotaxin level (24 h, $p < 0.05$), MCP-1, CXCL1, CXCL2, IL-8, and IL-6 levels ($p < 0.001$) on BEAS-2B with CBD vaporReal Time PCR: Reduced IL-1β (24 h, $p < 0.001$), IL-6 ($p < 0.05$) and TNFα expression ($p < 0.01$), increased NFkB and CAMP expression (24 h, $p < 0.01$) with 10 μM on LPS-stimulated cells
Oláh et al. (2014)	SZ95	Real Time PCR (TNFα, IL-1β, IL-6, NFkB and CAMP/LL37)	10 μM on cells stimulated or unstimulated with:100 μM linoleic acid,	Ethanol	16	
Petrosino et al. (2018)	HaCaT	Microarray analysis	1 μM Testosterone, 10 μg/mL LTA or 5 μg/mL LPS 24 h	DMSO	17	Microarray analysis: Reduced pro-inflammatory gene expression (24 h, $p < 0.01$ or $p < 0.001$)
		Western Blotting (pP65, pIkBa)				Western Blotting: Reduced pp65 and pIkBa levels
		ELISA assay (MCP-2)	1, 5, 10 and 20 μM on cells stimulated or			ELISA assay: Increased MCP-2 secretion above 5 μM (6 and 12 h, $p < 0.001$) and with 1−20 μM (24 h, $p < 0.001$)
		Bio-Plex Pro Assay (IL-1β, IL-2, IL-6, IL-8, G-CSF, GM-CSF, TNFα)	unstimulated with poli I: C (100 mg/mL) 6, 12 and 24 h			Bio-Plex Pro Assay: Reduced IL-6 level with 1−20 μM all (6−24 h, $p < 0.001$), IL-8 level with 10 (6 h, $p < 0.05$) and 20 μM (6 h, $p < 0.05$ and 12, 24 h $p < 0.001$) and TNFα level with 1 μM (6 h, $p < 0.05$) and 5−20 μM (6−24 h, $p < 0.001$)
Rajan et al. (2016 b)	hGMSC	Real Time PCR (MAPKAP1, TRAP1, STK25, STIP1, HSPB1, HERPUD1, NFE2L2, MAPK12, MAPK14, MICAL1, MICAL2 and MICAL3)	5 μM 24 h	DMSO (0.1 %)	17	Increased MAPKAP1, TRPA1, STK25, STIP1, HSPB1, HERPUD1 and NFE2L2 expression and reduced MAPK12, MAPK14, MICAL1, MICAL2 and MICAL3 expression with 5 μM (24 h, $q \leq 0.05$)
Rawal et al. (2011)	HGF	ELISA assay (TGFβ, Fibronectin, MMP-1 and MMP-2)	0.01–30 μM 1−6 days	Methanol (1 mg/mL)	16	Increased TGFβ secretion with 8 μM (1−6 days, $p = 0.001$), with 0.01, 10 and 20 μM (1−6 days, $p < 0.02$), with 0.025 μM (1−6 days, $p = 0.0003$) and with 0.05 μM (1−6 days, $p = 0.0001$); increased fibronectin secretion with 0.5, 2 and 4 μM (6 days, $p < 0.04$) and with 20 μM ($p = 0.001$); increased MMP-1 secretion with 0.5 and 2 μM (6 days, $p < 0.05$) and with 0.1 μM ($p < 0.005$); increased MMP-2 secretion with 0.5 μM (6 days, $p < 0.005$), with 1 μM ($p < 0.05$) and with 2 μM ($p = 0.001$)
Ruhl et al. (2018)	atMSC	ELISA assay (TGFβ1, VEGF, IGF1)	3 μM on cells stimulated or unstimulated with	Ethanol (0.01 %)	15	ELISA assay and Multiplex magnetic bead immunoassay: Increased IL-6 and VEGF secretion with 3 μM on LPS-stimulated cells (48 h, $p < 0.05$)
		Multiplex magnetic bead immunoassay (TNFα, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-12, IL-13, IL-18, GM-CSF, IFNγ) Protein oxidation assay	LPS (10 μg/mL) 48 h			Protein and lipid assays: Reduced oxidation level with 3 μM on LPS-stimulated cells (48 h, $p < 0.05$)

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Table 4 (*continued*)

Study	Cell type	Assay used	Concentrations/time points	CBD preparation	ToxRtool	Principal finding
Sangiovanni et al. (2019)	HaCat	Lipid peroxidation assay ELISA assay (IL-8, VEGFA and MMP-9)	ELISA: 0.05−5 μM 6 h (IL-8) and 24 h (VEGFA and MMP-9)	Ethanol	16	ELISA HDF: No effects observed in IL-8, VEGFA and MMP-9 secretion with 0.05−5 μM (6 and 24 h) HaCat: No effects observed in IL-8 secretion with 0.05−5 μM (6 h)
	HDF	Real Time PCR (chemokines, interleukins, TNF family and VEGFA)	Real Time PCR: 4 μM on cells stimulated or unstimulated with TNFα (10 ng/mL) 6 h			Reduced MMP-9 secretion with 0.5 μM (24 h, $p < 0.05$), with 1 and 2.5 μM ($p < 0.01$) and with 5 μM ($p < 0.001$); reduced VEGFA secretion with 0.1 and 1 μM (24 h, $p < 0.05$) and with 0.5 μM ($p < 0.01$) Real Time PCR: Increased CXCL8, CXCL10, IL-17C, IL-1β, TNF, LTB and VEGFA gene expression on HaCaT.
Schwartz et al. (2018)	HUASMC	Flow cytometry Real Time PCR (HO-1, HO-2) Western Blotting (HO-1, HO-2, TRPV1, GPR55)	Flow cytometry: 6 and 10 μM 4 h Real Time PCR: 0.1, 1, 3, 6, 10 μM 24 h6 μM 6, 24, 48 h Western Blotting: 0.1, 1, 3, 6, 10 μM (HO) 10 μM (TRPV1, GPR55, CB1, CB2) 24 h	Ethanol (0.033 %)	17	Flow cytometry: Increased ROS level with 6 and 10 μM (4 h, $p < 0.05$) Real Time PCR: Increased HO-1 expression with 10 μM (24 h, $p < 0.05$) and with 6 μM (6 and 24 h, $p < 0.05$) Western Blotting: Increased HO-1 level with 6 and 10 μM (24 h, $p < 0.05$)
Solinas et al. (2012)	HUVEC	Proteome profiler arrays Western Blotting (MMP-2 and uPA) ELISA assay (MMP-2)	1−19 μM 24 h	Ethanol	17	Proteome profiler: Increased MMP-9, TIMP1, PAI-1, uPA, CXCL16, ET-1, PDGF-AA and IL-8 levels with 12 μM (24 h, $p < 0.001$) Western Blotting: Reduced MMP-2 level with 1−19 μM (24 h, $p < 0.001$) and reduced uPA level with 9 and 12 μM (24 h, $p < 0.001$) ELISA assay: Reduced MMP-2 secretion with 14 μM (24 h, $p < 0.01$)
Watzl et al. (1991)	PBMC	ELISA assay (IL-1, IL-2, TNFα, IFNγ)	0.01, 0.1, 1, 2.5, 5, 10 and 20 μg/mL on cells unstimulated or stimulated with mitogens: PWM (0.1 μg /mL) for IL-1, Con A (5 μg /mL) for IL-2, LPS (10 μg /mL) for TNFα and PHA (5 μg /mL) for IFNɣ 24 h	DMSO (20 mg/mL)	17	Reduced IL-1 secretion with 2.5, 5, 10 and 20 μg/mL (24 h, $p < 0.05$); no effects observed on IL-2 secretion; reduced TNFα secretion with 1, 2.5, 5 and 10 μg/mL (24 h, $p < 0.05$); reduced IFNɣ secretion with 0.01, 10 and 20 μg/mL (24 h, $p < 0.05$) and with 0.1 μg/mL ($p < 0.01$)
Wu et al. (2018)	CD14+	Flow cytometry Superoxide dismutase assay (SOD) Confocal microscopy (ROS and mitochondrial permeability transition pore-MPTP)	Flow cytometry:16 μM 5, 15 and 30 min, 1 and 2 h Superoxide dismutase assay: 16 μM 30 min Confocal microscopy: 16 μM stimulated with 1 h (ROS) and 5 min (MPTP) on cells unstimulated or Cyclosporin A (1−10 μM)	Ethanol (99.8 %),	17	Flow cytometry: Increased ROS level with 16 μM (1 and 2 h, $p < 0.05$); increased MMP depolarization with 16 μM (5, 15 and 30 min, 1 and 2 h, $p < 0.05$) on un-stimulated cells and reduced MMP depolarization with 16 μM on stimulated cells with (30 min, $p < 0.05$); reduced cardiolipin level with 16 μM (15 and 30 min, 1 and 2 h, $p < 0.05$) and reduced cardiolipin oxidation with 16 μM on stimulated cells (30 min, $p < 0.05$) Superoxide dismutase assay: no effects observed with 16 μM (30 min) Microscopy: ROS subcellular localization, reduction of mitochondrial calcein and MPTP formation with 16 μM

keratinocytes primary cells (TIGKs) [35] while one study investigated primary normal human gingival fibroblasts (HGF) [8].

Epidermal cells were investigated by five studies: two studies reported the effect of CBD on primary human immortal keratinocyte (HaCat) [29,36], one also on primary normal human dermal fibroblasts (HDFs) [36], one on normal human epidermal keratinocytes cell line (NHEK) [26], one on human primaryepidermal melanocytes (HEMa-LP) [27] and one on human immortalized skin keratinocytes (CDD 1102 KERTr) [23]. Four studies investigated primary peripheral blood mononuclear cells (PBMCs) [37–40]. Two studies investigated human brain endothelial cell line (hCMEC/D3) [22,28], one study used primary human umbilical vein endothelial cells (HUVECs) [41]and one used primary human umbilical artery smooth muscle cells (HUASMC) [42]. Two studies investigated lung cells, in particular normal primary human

Table 5
Quality assessment of the selected articles according to the ToxRTool in vitro criteria.

The authors reported no declarations of interest	The authors reported no declarations of interest	The authors reported no declarations of interest	The authors reported no declarations of interest	The authors reported no declarations of interest	The authors reported no declarations of interest	The authors reported no declarations of interest	The authors reported no declarations of interest
Almada, 2020	4	3	5	3	2	**17**	Reliable without restrictions
Aparicio-blanco, 2019	2	3	6	2	2	**15**	Reliable without Restrictions
Burstein, 1985	4	2	5	3	2	**16**	Reliable without Restrictions
Casares, 2020	4	3	6	3	2	**18**	Reliable without Restrictions
Chiricosta, 2019	4	3	5	3	2	**17**	Reliable without Restrictions
Gu, 2019	3	3	5	3	2	**16**	Reliable without Restrictions
Hwang, 2016	4	3	6	3	2	**18**	Reliable without Restrictions
Jastrzab, 2019	3	3	4	3	2	**15**	Reliable without Restrictions
Jenny, 2009	3	3	5	3	2	**16**	Reliable without Restrictions
Lanza Caricio, 2018	4	3	5	3	2	**17**	Reliable without Restrictions
Libro, 2016a	4	3	4	3	1	**15**	Reliable without Restrictions
Libro, 2016b	4	3	5	3	2	**17**	Reliable without Restrictions
Luo, 2019	3	3	5	3	2	**16**	Reliable without Restrictions
Mabou Tagne, 2019	4	3	5	3	2	**17**	Reliable without Restrictions
Muthumalage, 2019	3	3	5	3	2	**16**	Reliable without Restrictions
Neradugomma, 2019	3	3	5	3	2	**16**	Reliable without Restrictions
Oláh, 2014	3	3	5	3	2	**16**	Reliable without Restrictions
Petrosino, 2018	4	3	5	3	2	**17**	Reliable without Restrictions
Rajan, 2016 a	4	3	5	3	2	**17**	Reliable without Restrictions
Rajan, 2016 b	4	3	5	3	2	**17**	Reliable without Restrictions
Rawal, 2012	4	3	5	3	1	**16**	Reliable without Restrictions
Ruhl, 2018	3	3	4	3	2	**15**	Reliable withoutRestrictions
Sangiovanni, 2019	4	3	4	3	2	**16**	Reliable without Restrictions
Schmuhl, 2014	3	3	6	3	2	**17**	Reliable without Restrictions
Schwartz, 2018	3	3	6	3	2	**17**	Reliable without Restrictions
Solinas, 2012	4	3	5	3	2	**17**	Reliable without Restrictions
Watzl, 1991	3	3	6	3	2	**17**	Reliable without Restrictions
Winklmayr, 2019	3	3	6	3	2	**17**	Reliable without Restrictions
Wu, 2018	3	3	6	3	2	**17**	Reliable without restrictions

bronchial epithelial cell line (NHBE), human bronchial epithelial cell line (BEAS-2B) andhuman lung fibroblasts cell line (HFL-1 and WI 38) [9,43].Two studies investigated primary adipose tissue mesenchymal stromal cells (atMSCs) [25,44]. Two studies used human endometrial stromal cell lines (St-T1b and THESCs) [45,46]. One study used also primary human decidual cells (HdFs) [45] and trophoblast placenta cells (HTR8-SV/neo) [46]. Finally, one study used human sebaceous gland immortalized cell line (SZ95) [14], one investigated human chondrocyte cell line (C28/I2) [30]. Table 3 provides details of the cell types.

3.4. CBD exposure

Some works used CBD concentration between 0.01 μM [37] and 50 μM [14], while others investigated the effects induced by CBD solutions ranging from 0.1 nmol/mL [23] to 15 mg/mL [22]. The exposure time varied from 15 min [38] to 35 days [44].

Typically, CBD was administered as diluted pure and added to culture medium. Previously, CBD was dissolved in different solution, specifically in DMSO in eleven studies [12,24,26,29,31–34,37,40,45], in ethanol in ten studies [14,23,25,30,36,38,39,41,42,44], in methanol in

four studies [8,27,28,46] and in a solution of ethanol (50 %), methanol (38 %) and water (12 %) in one study [43]. Two studies dosed CBD solutions provided by pharmaceutical companies [22,35] and one study used CBD oil diluted with methylenechloride [9]. Table 4 provides details of CBD exposure.

3.5. Cell viability

Twenty-one studies investigated cell viability using different types of assays [8,9,12,14,22,23,25,27–31,34–37,39,42,44–46]. Twelve studies used the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay alone [8,12,14,22,23,27,29,35,35,36,37,45,46] and one study used only Trypan blue assay [42]. Three studies performed the MTT assay and also trypan blue assay [28,34,39].One study performed only Resazurin assay [30] and one study used PrestoBlue assay and Crystal violet staining [25].One study investigated cell viability with AO/PI staining [9] and one study used WST-1 assay [44], while one study used Hematoxylin and Eosin staining [31].

Different results were obtained using these assays. There were no significant results on cell viability in eight studies: inhGMSC streated with 5 μM of CBD [12], in hPDLSCs with a co-treatment of CBD and Moringin (1:1, 5 μM) [34], inHEMa-LPs [27] in HaCat cells [29], in HGFs [8], in HUASMCs [42], in PMNs [37] and in MSCs [44]. Cells viability did not change after the treatment compared to the control group. CBDdid not cause any effect at low doses but, on the contrary, caused a significant reduction in cells viability at higher doses: in hGMSCswith 10 e 25 μM [31], in CDD 1102 KERTr cells irradiated with UVA and UVB, concentrations higher than 10 μM reduced the survival rate around 75 % [23], in St-T1b cells and in HdFs with 5 and 10 μM reduced the survival ratearound 50 %, while there were no effects using 2 μM [45]. In TIGKs treated with 0–10 μg/mL of CBD, cell viability was reduced only with 10 μg/mL [35]; in HaCat and in HdF cells treated with 0−5 μM, CBD showed a cytotoxic effects with concentration higher than 2.5 μM [36]. In THESc, CBD did not cause a cytotoxic effect up to 2 μM, but viability was significative reduced with 20 μM [46].In BEAS-2Band in HFL-1 cells, CBD treatment didnot altered viability except at 42.4 μM in HFL-1 cells [9]. In SZ95 cells, viability was reduced both with high dose (50 μM) and with long time of exposure (10 μM for 6 days) [14]. Five works reported a reduction in viability due to CBD treatment in a dose-dependent manner on C28/I2 cells [30]and on PBMCs stimulated with phytohemagglutinin (PHA) [39]. Cell viability was reduced also on at MSCs with a 7 days treatment [25] and finally, a cytotoxic effect was highlighted on hCMEC/D3 cells treated with lipid nanocapsules and CBD solution [22]; on hCMEC/D3 cells, CBD increased cell viability at high concentrations in a dose-dependent manner [28]. Table 4 details the principal results.

3.6. Cell proliferation

Five studies investigated the effect of CBD on cell proliferation [14,31,41,42,44].One study used Crystal violet staining [44], one study used MTT assay [41] and one study performed BrdU cell proliferation assay [42].One study investigated CyQUANT proliferation assay and MK167gene expression with Real Time PCR [14]. One study used NGS Analysis Illumina MiseqDxtoevaluateproliferative gene expression (HELB e RPS6KA1, GINS1, KIT, MAPK8IP1, NUF2, PA2G4P4 e ZHX2, ZNHIT1, SPHK2, RBBP6, PCNA, PCM1, ORC6, ORC5, ORC3, MIS12, MASTL, BCL7B, CDK5, CDK5RAP1, JAM2, PIN4 e MCRS1, PLXNB3, PCID2, PCGF2, PCGF1, OGFR, CDKNIA, CDK4, CCNDPB1) [31].

Three studies reported statistically significant reductions on cell proliferation with CBD treatment [14,41,42]: Olàh et al. [14] observed a inhibited proliferation on SZ95 cells at 1, 5 and 10 μM after 48 h of treatment; Schwartz et al. [42] founded significant inhibition at 6 μM, specifically in a dose-dependent manner after 24 h and 48 h;Solinas et al. [41] observed on HUVECs statistically significant inhibition above 9 μM at 24 h.On MSCs, CBD treatment with 3 μM for 35 days decreased cell proliferation [44]. On hGMSCs cell proliferation was stimulated after treatment with 5, 10 e 25 μM of CBD for 24 h [31]. Table 4 details the principal results.

3.7. Wound repopulation: scratch test and migration

Six studies investigated cell migration using one or more techniques. [28,37,41,42,44,46]. Four studies used Boyden chamber assay [37,42,44,46] and one used modified Boyden chamber [41]. One study investigated also p42/44 MAPK expression with Western Blotting for the evaluation of cell growth [44] and another study used Real Time PCR to assess MMP-2 and MMP-9 gene expression [46].

Three studies used Wound healing migration assay [28,41,44].Four studies reported the inhibition of cell migration [37,41,42,46]. Cell migration was inhibited on THESCs and HTR8-SV cells with a degradation matrix signaling following an increase in MMP-2 and MMP-9 gene expression [46] after 7 days treatment with 0.5 μM of CBD. Cell migration decreased in a dose-dependent manner with concentrations of CBD ranging from 1 to 10 μM at 24 h on HUVECs [41] and ranging from 0.1–10 μM for 90 min on PMNs [37]. This was confirmed by Schwartz et al. [42]: cell migration was significantly inhibited on HUASMCs after 60 min with 10 μM, while there was not a statistically significant inhibition with 6 μM. Two studies reported a significant increased migration on CBD-treated cell: with 3 μM on hCMEC/D3 cells from 0 to 24 h of exposure [28] and on MSCs at 6 and 48 h [44]. Cell migration induced by CBD treatment has been confirmed with p42/44 MAPK activation [44]. Table 4 details the principal results.

3.8. Apoptosis and cell cycle

Eleven studies investigated apoptosis and cell cycle [12,14,23,24,26,30,33,34,38,41,42]. Four studies used Real Time PCR [12,24,26,33] and one used NGS analysis [34] to investigate the expression of apoptotic and cell cycle genes: Caspase, NFR2, BAX, BCL, PIK3/ATK. One study used also DilC1(5) and SYTOX Green staining and Microarray analysis for transcriptome design [14]. In four studies intracellular protein expressions were investigated focusing on Caspase family, PI3K-Akt pathway, Ref-1, pASK1, ERK1/2, pERK1/2, and Cytochrome C with Western Blotting [23,24,30,38] or Immunocytochemistry [33]. Four studies investigated apoptosis and cell cycle with Flow cytometry with Annexin V [30,41,42] or Propidium iodide (PI) [38]. One study used Caspase-Glo 3/7 assay and microscopy evaluation after CBD treatment [30].

Casares et al. [26] affirmed that CBD treatment, after 16 h of exposure, with 10 μMon NHEK and HaCat cells induced NRF2 target genes expression (HMOX1, GCLC, p62). Caspase family genes expression (Caspase 8 and/or Caspase 4) were down-regulated with CBD treatment (5 μM) at 24 h [24] or 48 h [12] and Caspase1 protein expression was decreased [24] on hGMSCs. On hGMSCs, CBD down-regulated other gene expressions: pro-apoptotic mediators (BAX, BAD, BID, BCL7B, BCL2L13, CYCS) and apoptotic protease (APAF-1). CBD activated phosphatidylinositol 3-kinase, intracellular signaling pathway (PIK3CA e PIK3CB) and AKT serine/threonine kinase 1 (AKT1) and the activation of PIK3/AKT/mTOR pathway determined apoptosis triggering [24]. PIK3/AKT/mTOR activation was also shown on hPDLSCs after 48 h of co-treatment with CBD andMoringin (1:1, 0.5 μM) [34] and on hGMSCs treated with 5 μMof CBD for 24 h [33]. On CDD 1102 KERTr cells irradiated with UVA and UVB, the increased level of Ref-1 and pASK1 expressions induced by UV-irradiation has been partially restored with 1 μMof CBD for 24 h, suggesting that CBD protected from oxidative stress and apoptosis [23].CBD did not cause pro-apoptotic effects on HUVECs, since 90 % of these cells were viable [41], and neither on HUASMCs treated with 6 μM and 10 μM for 48 h [42]. Olàh et al. [14] showed that CBD did not induce apoptosis ranging from 0 to 10 μM on SZ95 cells and proved that cell cycle gene expressions was down-regulated after CBD treatment. CBD showed pro-apoptotic effects ranging from 15 to 30 μM

on C28/I2 [30] and ranging from 8 to 16 μM on CD14+, in a time-dependent manner with a significant effect at 16 μM [38]. Table 4 details the principal results.

3.9. Inflammation

Nineteen studies investigated the effect of CBD on cytokines and inflammatory mediators production [8,9,12,14,23–26,29,32,35–43]. Seven studies investigated inflammatory gene expression with Real Time PCR [14,24,32,36,37,39,42], one study used Microarray analysis with Human Whole Genome Oligo Microarray [14], two studies used Proteome profiler cytokine arrays [9,41] and one used NGS analysis [12]. In fourteen studies intracellular and extracellular protein expression were evaluated with Western Blotting [12,14,23,41,42], ELISA assay [8,9,23,25,29,35,36,39–41], Bio-Plex Pro assay [29], Multiplex magnetic bead immunoassay [25], Luciferase assays [26]and Luminex assay [9]. One study investigated Arachidonate labelling/release and PG synthesis [43]. Four studies investigated ROS production: two studies used ROS assay (DCFH-DA) [26,37], one study used Flow cytometry, confocal microscopy and Superoxide Dismutase assay [38], while one study used Electron spin resonance (ESR) [23]. Ruhl et al. [25] used also Protein oxidation assay (DNPH) and Lipid peroxidation assay(MDA). Jastrzab et al. [23] used also spectrometry, gas and liquid chromatography to investigate inflammatory species: Cu, Zn-SOD, GSH-Px, GSSG-R, TrxR 15d-PGJ2 and 4-NHE. Jenny et al. [39] used also High performance liquid chromatography (HPLC) to evaluate kynurenine and tryptophan.

The most evaluated gene and protein expressions were: Tumor Necrosis Factor α (TNFα), Interleukin (IL) and chemokine pathway(CXCL), TGFβ pathway,Interferon γ (IFNγ), mitogen-activated protein kinases (MAPKs), complex NFκBand Matrix metallopeptidase release. Burstein et al. [43] asserted that on WI-38 cells with 3.2 μMof CBD exposure for 0, 1, 2, 3 days, the synthesis of prostaglandin E2 (PGE2) decreased in a time-dependent manner after 24 h of exposure. On hGMSCs CBD treatment with 5 μM for 48 h showed anti-inflammatory activity on TGF-pathway and IL-1 [12] and down-regulated interleukin(IL-6ST, IL-1β e IL-18), Toll-like (MYD88), Interferonγ (IFNGR1 e IFNGR2), mitogen-activated protein kinases (MAPK1, MAPK12 and MAPK14), transcription factors (STAT3 and STAT6), NFκBcomplex (NFKB2, NFKB3/RELA) and MMP-3 after 24 h [24]. Inhibition of inflammation occurred on TIGKs treated with CBD w/o LPS, through the suppression of IL-6, IL-10 and TNFα [35]. Protein expressions of IL-8 e MMP-9, evaluated with ELISA assay, did not change after CBD treatment on HDF cells, while on HaCat cells MMP-9 expression has been 50 % inhibited in concentration-dependent manner with 5 μM. Moreover, on HaCat cells twenty-six gene expressions had an increase five times higher: chemokine (CXCL8 e CXCL10), interleukin (IL-17C e IL-1β), TNF family (TNF and LTB) and VEGFA [36]. Olàh et al. [14] showed that 10 μM of CBD for 24 h inhibited TNFα, IL-1β, increased IL-6 (LPS-induced) and down-regulated inflammatory gene expressions (cytokine, TLR9 pathway, NFkB) on sebaceous gland cell. CBD suppressed IL-1, TNFα and IFNγ secretion, while for IL-2 did not caused significant changes [40].On PBMCsthe co-treatment of PHA and CBD (0.1 μg/mL) induced an increase inIFNγ secretion and inhibited mitogen-stimulated expression of IDO and IFNγ [39]. Solinas et al. [41] affirmed that on HUVECs CBD treatment down-regulated MMP-9, TIMP1, PAI-1, uPA, CXCL16, ET-1, PDGF-AA and IL-8 protein expressions, while regulated extracellular MMP-2 expression in a dose-dependent manner but did not change intracellular MMP-2 expression. Petrosino et al. [29] showed that CBD inhibitedMCP-2 protein expression in concentration-dependent manner, while only IL-8 levels were reduced by the highest tested CBD concentrations (10 and 20 μM). Significantly high levels of IL-6 and TNFα were detected in a dose-dependent manner. No effect was observed for other cytokines (IL-1β, IL-2, G-CSF and GM-CSF). Rawal et al. [8] evaluated that both low concentrations of CBD (0.01−0.05 μM) and higher (4–30 μM) have increased TGFβ production in short times of exposure, while with long exposures there was no effects or lower production of TGFβ. Lower concentrations of CBD have increased MMP and fibronectin production [8]. Muthumalage & Rahman [9] affirmed that on BEAS-2B cells, CBD significantly increased IL-8 and E1 serpin secretion, and on HLF-1 cells CBD increased CXCL1, IL-6, IL-8 and MCP-1/CC2 levels. On NHBE cells treated with 10.6 μM, CBD did not induce secretion of inflammatory mediators. CBD significantly attenuated CXCL1, G-CSF, and IL-6 on TNFα stimulated cells. On at MSCs, CBD did not influence the inflammation pathway after a 48 h of treatment [25].Regarding ROS production, Rajan et al. [32]noted that CBD significantly regulated genes associated with oxidative stress, up-regulating MAPK (MAPKAP1, TRAP1, STK25, STIP1, HSPB1, HERPUD1 and NFE2L2) and down-regulating MAPK12, MAPK14, MICAL1, MICAL2 and MICAL3. Wu et al. [38] affirmed that16 μM of CBDenhance ROS production in a time-dependent manner. Other authors such as Casares et al. [26] and Jastrzab et al. [23] showed that CBD did not induce ROS formation but was able to reduce ROS levels in a concentration-dependent manner. Schwartz et al. [42] noticed that CBD was able to mediate ROS generation inducing HO-1 gene and protein expression. Finally, Tagne et al. [37] showed that co-incubation offMLP and CBD did not cause changes in ROS production, while pre-incubation with CBD decreased ROS production generated by fMLPdose-dependently. Table 4 details the principal results.

4. Discussion

Cannabis Sativa is composed by a large number of chemical components, which have different biological properties useful for medical applications. Focusing on CBD, the second major component of Cannabis Sativa, it has already been demonstrated that it cannot be associated with psychoactive effects or functional motorial alterations [47].There are several reports that show potential pharmacological effects of this substance related to inflammation, epilepsy, neurodegenerative pathologies, autoimmune diseases as multiple sclerosis, arthritis and neoplasms [13,48,49].CBD is considered as an anticonvulsant, antioxidant, neuroprotective, analgesic and antiemetic agent from various authors [11]. All these pharmacological effects result in the necessity of focusing the attention on the level of biocompatibility of the organic molecule. Even if the literature is richwitharticles about THC effects, there are only few reviews on the specific effects of CBD [10,11]. Therefore, the aim of this review was to define a general and complete scheme of the biological effects of CBD on normal human healthy cell populations.

The analysis of the Literature highlighted a great variability among the selected studies, particularly about the cell type investigated, but also regarding the assays performed and the CBD exposure conditions applied.

Our ToxRTool quality assessment demonstrated several common limitations deficiencies in the included studies: 14 studies did not give a clear identification of the substance used in terms of purity, origin and properties, 1 study did not give clear information about the test system characteristics, origin and properties, 21 articles lacked in the description of the study design in terms of administration methods, doses and concentrations used, frequency and duration of exposure, negative and positive controls and number of replicates, 1 study did not clearly describe the results in terms of endpoints description and determination and statistical methods for data analysis, and 1 article did not give plausibility for the study design chosen, meaning that there was a risk of potential bias. We noted that many studies did not mention the funding details, an aspect that cannot be neglected considering the potential involvement of the Cannabis Sativa Light industry in the research funding.

In this context it is worth to specify that the challenge with any *in vitro* research is to interpret the findings in a significant way considering the clinical relevance. The first aspect considered is the cell viability. In general, CBD influenced the vitality of normal human healthy cells in a

dose-dependent manner, with a significant reduction at dosesabove 2 μM of administration, in particular oral cells populations suffered a reduction in viability with CBD doses above 10 μM [23,31,45].

It should be remembered that pathological processes are more complicated than simple cell viability assays and in this work we also reviewed studies that reported on cell proliferation, migration, apoptosis and inflammatory mediator production.

CBD inhibited the proliferation in a significant way on various normal healthy cell populations. The only study that investigated oral cells [31] had results both in proliferative and anti-proliferative way in relation to different genes analyzed. We believe that these aspects should be further investigated: the stimulation of cell proliferation can cause various types of proliferating diseases, such as fibrosis and cancer.

The reduced proliferation is closely related to a parallel reduction of migration, as expected from the results in the studies reviewed. It should be noted that studies with oral cells, which could confirm this hypothesis, did not evaluated cell migration, even if there was a tendency of CBD to inhibit the migration on health cell populations.

Finally, in all the cell types investigated, including oral cell populations, CBD has not shown a stimulation of the pro-apoptotic genes and proteins pathways at low doses, while at particularly high doses it has significantly stimulated apoptosis.

At low concentrations and with extended administration times, CBD caused changes in the normal cell activity, both in a morphological and defensive way and cells resulted not proliferatively active, but neither dead. Cell damages could actually be caused by high concentrations and so, we believe that CBD therapeutic use must be related to low doses of administration. As CBD is the constituent of many products, the overall concentration in human tissues should be evaluated considering all the different small doses administered, that could derive from different types of products, the synergism of which would lead to exceeding the non-toxicity level, blocking cell proliferation without causing cell death.

Production of inflammatory mediators, including cytokines, appeared to be down-regulated by exposure to CBD at a wide range of concentrations. CBD showed an anti-inflammatory effect in a time and concentration dependent manner, since it did not stimulate genes and proteins of the inflammation pathways. However, in terms of production of reactive oxygen species, CBD at high doses of administration significantly stimulated gene expression of the genes involved in cellular oxidative stress, although at the same time it down-regulated ROS production. This justifies the therapeutic use of CBD, as widely demonstrated in literature [3,11].

CBD at all concentrations inhibited inflammation and down-regulated ROS production, although at high concentrations increased ROS-related genes expression. The expression of these genes involved in inflammation demonstrated that at high doses or with repeated administrations CBD could induce an inflammation process.

In order to improve the quality and value of research on CBD and based on the results of this review, we developed some recommendations for future research projects. CBD should be used in realistic exposure conditions, with concentrations within the physiological ranges on 3D tissue model systems. A clear study design should be applied, reporting guidelines or checklists, sample size calculations, randomization, allocation and blinding. Finally, any study limitations should be discussed and the funding reported.

4.1. Limitations of the study

There are some limitations of our systematic review. The ToxRtool allowed us the qualitative analysis of the works selected by the three independent reviewers, but it is necessary to specify that it does not support in any way the selection of the eligible articles, however all the works selected have a high reliability range. We extended the investigation on the effects of CBD to all normal human healthy cell populations. Including exclusively oral cell cultures would be an important consideration for any future study, in terms of selectivity of effects and interactions with CBD. In this review the investigation was limited to in vitro cell populations, because this work results as a preliminary study for future investigations on 3D tissue models and also on in vivo experiments. It is necessary to consider the pharmacokinetics of the compound related to in vivo conditions for a completeness and an accurate definition of the safety threshold CBD administration. It is important to understand CBD pharmacokinetic properties not only for its therapeutic use in various clinical pathologies, but also its possible negative effects related to doses regimens and administration. We limited our systematic review to the analysis of normal health cells in five domains (cell viability, proliferation, wound repopulation, apoptosis and inflammation), but these evaluations could be considered also in the perspective of a comparison between carcinogenic and health cells.

5. Conclusions

The results of this review show a biological effect of Cannabidiol on human cell populations. The definition of a safety threshold level would represent an important aid for both the clinician and the consumer. The analysis of the literature has shown that CBD inhibited cell viability in a dose-dependent manner. From the data obtained, cell proliferation and cell migration were generally reduced at each CBD concentration used. Low doses of CBD did not result in a pro-apoptotic stimulation, while high doses (>10 μM) have significantly stimulated apoptosis. The down-regulation of inflammatory mediators and cytokines with both low and high concentrations testify the therapeutic use of CBD in various medical fields. The anti-inflammatory effect turned out to vary in a time and concentration dependent manner because high concentrations of CBD (>10 μM) caused an increase in ROS genes expressions. These findings confirm the hypothesis that CBD therapeutic use can be limited and defined only by a low dose administration. Considering instead exclusively oral cell populations dosages higher than 10 μM cause a reduction of cell viability and an anti-proliferative, anti-migratory, pro-apoptotic and anti-inflammatory effects on oral cells.

Funding information

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

The authors reported no declarations of interest.

Acknowledgments

The authors sincerely thank Dr. J. Pitocchi for English revision.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.biopha.2020.110728.

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