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Efficacy of cannabinoids against glioblastoma multiforme: A systematic review

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ABSTRACT

Introduction: The increased incidence of Glioblastoma Multiforme, the most aggressive and most common primary brain tumour, is evident worldwide. Survival rates are reaching only 15 months due to its high recurrence and resistance to current combination therapies including oncotomy, radiotherapy and chemotherapy. Light has been shed in the recent years on the anticancer properties of cannabinoids from *Cannabis sativa.*

Objective: To determine whether cannabinoids alone or in combination with radiotherapy and/or chemotherapy inhibit tumour progression, induce cancer cell death, inhibit metastasis and invasiveness and the mechanisms that underlie these actions.

Method: PubMed and Web of Science were used for a systemic search to find studies on the anticancer effects of natural cannabinoids on glioma cancer cells *in vitro* and/or *in vivo.*

Results: A total of 302 papers were identified, of which 14 studies were found to fit the inclusion criteria. 5 studies were conducted *in vitro,* 2 *in vivo* and 7 were both *in vivo* and *in vitro.* 3 studies examined the efficacy of CBD, THC and TMZ, 1 study examined CBD and radiation, 2 studies examined efficacy of THC only and 3 studies examined the efficacy of CBD only. 1 study examined the efficacy of CBD, THC and radiotherapy, 2 studies examined the combination of CBD and THC and 2 more studies examined the efficacy of CBD and TMZ.

Conclusion: The evidence in this systematic review leads to the conclusion that cannabinoids possess anticancer potencies against glioma cells, however this effect varies with the combinations and dosages used. Studies so far were conducted on cells in culture and on mice as well as a small number of studies that were conducted on humans. Hence in order to have more accurate results, higher quality studies mainly including human clinical trials with larger sample sizes are necessitated urgently for GBM treatment.

Introduction

Cancer

Cancer, is the uncontrolled growth of abnormal cells beyond their normal borders that invades adjoining parts of the body and spread to other organs (Stratton *et al.*, 2009). It is the second leading cause of death universally and 608,570 deaths due to cancer are expected in 2021 (Siegel *et al.*, 2021). The alteration of healthy cells into tumour cells is a multistage procedure, generally progressing from a pre-cancerous lesion to a malignant tumour. A combination of the person's genetic factors and several external agents such as ultraviolet and ionizing radiation, asbestos, components of tobacco smoke and infection from certain viruses, bacteria or parasites lead to the formation of the abnormal cells (WHO, 2018). By altering or avoiding key risk factors such as reducing alcohol consumption, exercising regularly and

Abbreviations: 2-AG, 2-arachidonoyl glycerol; Akt, Protein kinase B; BBB, Blood brain barrier; CB, Cannabinoid receptors; CBD, Cannabidiol; EGFR, Epidermal growth factor receptor; elF2α, α-subunit of the eukaryotic translation initiation factor 2; ER, Endoplasmic reticulum; ERK, Extracellular receptor kinase; GBM, Glioblastoma multiforme; GPCRs, G protein-coupled receptors; GSCs, Glioma stem cells MES, Mesenchymal; HIF-1α, Hypoxia inducible factor 1α; I.P, Intraperitoneal; Id-1, DNA-binding protein inhibitor 1; IDH, Isocitrate dehydrogenase; LC3, Microtubule-associated protein 1A/1B-light chain 3; MAP1LC3B, Microtubule-associated proteins 1A/1B light chain 3B COL4A3BP, collagen type IV α3 binding protein; MMP, Matrix metalloproteinase; MPs, Microparticles; mTORC1, Mechanistic target of rapamycin complex 1; NSC/NPC, Neural stem/ progenitor cells; PDGF, Platelet derived growth factor; PI3K, Phosphoinositide 3 kinase; ROS, Reactive oxygen species; TGF, β1, Transforming growth factor-β1; THC, Tetrahydrocannabinol; TMZ, Temozolomide; VEGF, Vascular endothelial growth factor; YB-1, Y box binding protein 1.

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maintaining a healthy body weight and addressing infection-related risk factors could reduce cancer death by 30%-50% (WHO, 2018).

Glioblastoma

The most recurrent class of malignant intracranial primary brain tumour and one of the most aggressive forms of cancer is Glioblastoma multiforme (GBM) or grade IV astrocytoma, which accounts for 3%-4% of all cancer-related deaths (Homma *et al.*, 2006). GBM, that develops rapidly *de novo*, has a high prevalence of genetic and epigenetic changes with countless possibility produced neoantigens (Heiland *et al.*, 2017). Hence, life expectancy after diagnosis is just 12 to 15 months (Torres *et al.*, 2011).

GBM is characterised by abnormal excess anaplastic glioma cells, diffuse infiltration, tendency for necrosis, robust angiogenesis, potent resistance to apoptosis and excess genomic instability (Furnari *et al.*, 2007). The presence of necrosis indicates a predictive factor for poor survival. The dramatic behaviour of this type of brain tumour is primarily due to its high invasive properties and increased proliferation rate (Torres *et al.*, 2011).

Epidermal growth factor receptor (EGFR) mutations are a common cause of GBM progression and are found in 40% of all GBM cases (Frederick *et al.*, 2000). EGFR amplicons of variant 3 (*EGFRvIII* or ΔEGFR*)* are usually mutated, with deletion of exons 2 to 7 being the most frequent type (Ohgaki and Kleihues, 2007). Lacking the region of the extracellular ligand binding domain from this truncated receptor, leads to a constitutively activated receptor despite being unable to bind ligands (EGF), causing mitogenic effects and enhanced cell proliferation (Narita *et al.*, 2002). In addition, the progression of low-grade astrocytoma to the high-grade GBM is strongly correlated by the presence of mutant p53 (Sidransky *et al.*, 1992).

Current treatments for glioblastoma

Magnetic resonance imaging has been used for the last 20 years, as the standard in brain tumour imaging to define lesion borders such as location of the tumours, shape and size (Carlsson *et al.*, 2014). The current standard treatments for GBM are only palliative and consist surgical resection, which is often incomplete due to the vicinity of the tumour to vital brain structures, followed by a combination of radiotherapy and chemotherapy (Scott *et al.*, 2014). Radiation therapy causes severe destruction of DNA hence cells undergo apoptosis as double-strands break (Wu *et al.*, 2014).

GBM cancers are represented by 'baseline resistance to numerous drugs' due to their anatomical location, which are protected by the Blood-brain barrier (BBB) (Abbott, 2013). The current chemotherapeutic agent used for GBM is Temozolomide (TMZ) (Würstle et al., 2017). This is a small lipophilic molecule, orally-administered monofunctional DNA alkylating agent of the imidazotetrazine class and does not accumulate inside the BBB (Anjum *et al.*, 2017). TMZ causes its cytotoxicity by the ability of methylating DNA and subsequent generation of O6-MeG followed by arrest of the cell cycle at G2/M phase (Zhang and Bradshaw, 2012).

However, the presence of several known genes and proteins can affect the sensitivity of GBM to TMZ (Phillips *et al.*, 2006). Upregulation activity of methylguanine-DNA methyltransferase gene results in a decreased efficacy of TMZ, and methylation of this gene is, at the moment, intended to be one of the most important factors for predicting susceptibility of GBM to treatment with TMZ (Stavrovskaya *et al.*, 2016).

Mutations in isocitrate dehydrogenase 1 and 2 correlate with increased total survival of GBM patients. The reduction of α-ketoglutarate to 2-hydroxyglutarate is favoured by the altered glioma metabolism, which in turn downregulates DNA and histone demethylases, featuring hypermethylation at a big number of loci, which is prognostic of a favourable outcome and predictive chemotherapy response (Parker *et al.*, 2015). Y-box binding protein-1 (YB-1) is another gene found to be overexpressed in primary GBM but not in normal brain tissues. Hence it was found that inhibition of YB-1 caused an enhanced sensitivity to human GBM passaged cells when treated with TMZ (Gao *et al.*, 2009).

Endocannabinoid system

Mammalian tissues contain an endogenous cannabinoid system, a homeostatic regulator of neurotransmitter activity and at least two types of cannabinoid receptors CB1 and CB2 (Pertwee, 2009). CB1 receptors are mainly found in several brain regions such as the forebrain and hippocampus but also exist in peripheral organs including the liver, thyroid gland, skeletal muscle and adipose tissue (Cavuoto *et al.*, 2007; Mackie, 2008). CB2 receptors are found in specific neuron subpopulations, in glioma cells. They are also expressed in circulating immune cells, on macrophage-derived cells such as osteoclasts and hepatic Kupffer cells, as well as in tonsils and spleen (Galiègue et al., 1995; López-Valero et al., 2018b; Louvet et al., 2011).

Two endocannabinoids also exist throughout the body. Anandamide and 2- arachidonoyl glycerol (2-AG) were discovered in 1992 and in 1995, respectively, 30 years after the discovery and isolation of the ingredients of *Cannabis sativa,* Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD). The discovery of THC was followed by the discovery of the CB receptors which were the start-off point for the discovery of the endogenous ligands as well (Maccarrone *et al.*, 2015). THC binds to CB receptors with almost the same affinity as Anandamide (Wu *et al.*, 2012).

Anandamide and 2-AG are synthesized on demand and are degraded rapidly to have a transient, localised effect (Horváth *et al.*, 2012). Anandamide and 2-AG bind with different affinities to the two 7-transmembrane G protein-coupled receptors (GPCRs). Like most other lipid mediators, Anandamide and 2-AG, have more than one series of biosynthetic and degrading pathways as well as enzymes each (Di Marzo and Piscitelli, 2015).

The seven-transmembrane domain protein encoded by both CB1 and CB2 belongs to Gi/o-protein-coupled receptor family. CB1 receptors efficiently couple and activate both Gi and Go, whereas CB2, only Go (Bifulco *et al.*, 2008). This leads to the inhibition of the enzymatic activity of adenylate cyclase, causing the inhibition of cyclic adenosine monophosphate (cAMP) inside cells (Guindon and Hohmann, 2011). This brings about the inhibition of proliferation and migration and induces apoptosis of cancer cells (Khan *et al.*, 2016).

Another receptor was found in recent years, the transient receptor potential vanilloid type 2 from the TRP family that serves as an ionotropic cannabinoid receptor (Lowin and Straub, 2015). It is a six-domain trans-membrane channel, gating the passage of various types of cations (Ca2+) after a stimulation by CBD, which is the most potent agonist (De Petrocellis and Di Marzo, 2010). CBD enhances the uptake of antiproliferative and cytotoxic drugs in cancer cells that express transient receptor potential vanilloid channel (Nabissi *et al.*, 2012).

THC and CBD

C. Sativa has been found to contain 525 natural components that fall under several chemical classes. Cannabinoids fit in the chemical class of terpenophenolics and 104 of them have been identified so far (El-Alfy *et al.*, 2010, Lafaye *et al.*, 2017). THC is the most active component of the plant due to its high potential and profusion in plant preparations (Velasco *et al.*, 2012). THC mimics the endogenous substances, Anandamide and 2-AG, by binding to the CB receptors inducing different pathways, eventually leading to the reduction of tumour growth (Pertwee, 2008).

Other distinguished cannabinoids also exist such as CBD, cannabinol (CBN) and cannabigerol (CBG) that exert anticancer activity however, importantly CBD and CBG are free of psychoactivity (Scott *et al.*, 2014). The non-psychoactive cannabinoids have minor attraction for the CB receptors hence they do not elicit their activity through these receptors. Instead, CBD induces apoptosis by the possible mechanism of induction

of oxidative stress through accumulation of Reactive Oxygen Species (ROS) (Massi *et al.*, 2006).

In 1981, a synthetic analogue of Δ9-THC was licensed for the inhibition of vomiting and nausea-induced from chemotherapy and in 1992 it was used as an appetite stimulant (Pertwee, 2009). In 2005, one more cannabis-based medicine, Sativex, entered the clinic containing similar amounts of Δ9-THC and CBD and is used by adult patients with advanced cancer as a complementary analgesic treatment (Pertwee, 2009). The function of the endocannabinoid system in tumour generation and development has gained a lot of interest in the last decade.

Malfitano *et al.* (2011) showed that overexpression of endocannabinoids and their receptors is correlated with cancer and tumour aggressiveness. In glioma cancer, the upregulation of both CB1 and CB2 receptors has been found to co-exist with a downregulation on the amount of the enzymes used in the endocannabinoid degradation (Guillermo Velasco *et al.*, 2016). Cannabinoids have been shown to block the growth of gliomas in mouse models (Sanchez *et al.*, 2001).

Mechanism of action of cannabinoids in GBM

Studies conducted on malignant gliomas have shown that inhibition of tumour cell migration and invasion occurs due to the cannabinoids (Blázquez *et al.*, 2004). It is also strongly supported that cannabinoids reduce tumour progression via inhibition of tumour angiogenesis, tumour cell apoptotic death and by inhibition of cancer cell proliferation (Blázquez *et al.*, 2008). Cannabinoids can cause cell cycle arrest, inhibit cell proliferation and elicit cell death which leads to prevention of tumour spread, inhibition of oxygen and nutrient supply, and halt in angiogenesis of tumour environment (Pisanti *et al.*, 2013).

The inhibitory effect caused by cannabinoids is linked with a downregulation in kinase activity, oncogenic tyrosine kinase receptor (RTK) expression and phosphorylation (EGFR, nerve growth factor receptor, prolactin and vascular endothelial growth factor receptor (VEGF-R)) (Blázquez *et al.*, 2004). The action of matrix metalloproteinases (MMP) has been found to play a central role in the obtainment of invasive capacities by tumour cells (Duffy *et al.*, 2000). The central association in the disruption of the extracellular matrix and in the peptide cleavage leading to activation of various classes of tumour progression factors has linked MMPs with tumour invasion (Curran and Murray, 2000).

Hence, activation and increased expression of MMPs are linked with poor patient prognosis (Deryugina and Quigley, 2006). Blázquez *et al.* (2008) showed that cannabinoid delivery inhibits MMP-2 expression leading to inhibition of glioma cell invasion. Two major signalling elements upregulated by THC, the sphingolipid ceramide and the stress protein p8, induce this inhibitory effect. Activation of the p8- regulated pathway increases the suppressive interaction of Tribbles pseudokinase 3 with Protein kinase B (PKB or Akt), causing the inhibition of mechanistic target of rapamycin complex 1 (mTORC1) and subsequent occurrence of autophagy-mediated cell death (Velasco *et al.*, 2012).

During autophagy, organelles and other cytoplasmic units are engulfed within autophagosomes which fuse with lysosomes during their maturation. This leads to their degradation by lysosomal enzymes, finally causing cell death (Velasco *et al.*, 2016). Activation of CB1 receptor, by THC administration, induces sphingomyelin hydrolysis and sharp ceramide production within minutes, in glioma cells (Cianchi *et al.*, 2008). Whereas, CB2 receptor activation-induced apoptosis in glioma cells, mostly relies on the prolonged build-up of ceramide, through enhanced *de novo* synthesis which activates the Raf-1-MEK-ERK pathway leading to apoptosis (Pisanti *et al.*, 2013).

Moreover, CBD has been also found to induce cell death of glioma cells through apoptosis, however the exact process by which CBD induces this response has not yet been clearly specified. As CBD acts independently of the CB receptors, it is believed that it increases the production of ROS in cancer cells (Velasco *et al.*, 2016). In addition, CBD has been found to induce downregulation of invasiveness and metastasis along with reduction in tumour growth. A strong correlation exists between DNA-binding protein inhibitor 1 (Id-1) expression and the aggressiveness of brain tumours. The downregulation of the helix-loop-helix transcription factor Id-1 seems to be, at least in part, the mechanism behind these actions caused by CBD (Soroceanu *et al.*, 2013).

Rationale and objectives

Glioblastoma multiforme incidence and mortality have increased dramatically and will continue to rise if novel therapeutic approaches are not developed urgently. The aim of the current systematic review is to determine whether cannabinoids (CBD and/or THC) either combined with TMZ or radiotherapy can inhibit tumour progression in glioma cancer. Whether they can induce cancer cell death, hinder metastasis and invasiveness and the possible mechanisms responsible for these actions is yet to be seen.

Methodology

Search strategy

PubMed was the database used for the initial search that was conducted to find out if there are any recent existing systematic reviews on this topic. Several reviews came up with the latest written in 2017, hence it was decided to continue with this topic as it is essential to have a new systematic review covering the most recent studies. Eventually, Web of Science along with PubMed were used for the research using the key words 'Cannabinoids (Cannabidiol, Δ9-THC, Marijuana and hashish) [Title/Abstract]' and 'cancer [Title/Abstract]' or 'Cannabinoids [Title/Abstract]' and 'Temozolomide [Title/Abstract]' and/or 'Radiotherapy [Title/Abstract]' and 'Glioblastoma Multiforme [Title/Abstract]' or 'Brain tumour [Title/Abstract]'.

Inclusion/exclusion criteria

Both *in vitro* and *in vivo* studies were used as there is an apparent shortage of human-based trials. Studies with involvement of cannabinoids along with TMZ and/or radiotherapy or cannabinoids alone against glioblastoma cancer were used, as well as any mechanism of action leading to regression of malignant cells and inhibition of tumour size growth. Only primary studies that were conducted in 2006 onwards were used in this review.

However, any studies involving the combination of synthetic agonists for CB receptor such as WIN 55,212-2 were excluded, as this systematic review considers only natural products. Studies that were not written in English, did not have free full-text access and did not have a focus on glioblastoma cancer were not used in this review.

Quality assessment

The Toxicological data Reliability Assessment Tool (ToxRTool) was used to assess the quality of included studies. The purpose of this tool is to evaluate toxicological data to increase transparency and assign data to Klimisch categories 1, 2 or 3 by assessing against certain appraised criteria (Klimisch et al., 1997). Two distinct tables exist, one for the *in vivo* (Table 1) studies and one for the *in vitro* (Table 2) studies. Criteria are answered with 'yes' (score 1) or 'no' (score 0). Criterion in red in each group is considered indispensable for reliable data hence 'non-compliance with at least one red criterion leads to Klimisch category 3' despite the overall scoring of the study (Table 3) (Schneider *et al.*, 2009).

Table 1
Listed criteria for *in vivo* studies.

	Criteria
No.	**Criteria Group I: Test substance identification**
1	Was the test substance identified?
2	Is the purity of the substance given?
3	Is information on the source/origin of the substance given?
4	Is all information on the nature and/or physico-chemical properties of the test item given, which you deem indispensable for judging the data (see explanation for examples)?
	Criteria Group II: Test organism characterisation
5	Is the species given?
6	Is the sex of the test organism given?
7	Is information given on the strain of test animals plus, if considered necessary to judge the study, other specifications (see explanation for examples)?
8	Is age or body weight of the test organisms at the start of the study given?
9	For repeated dose toxicity studies only (give point for other study types): Is information given on the housing or feeding conditions?
	Criteria Group III: Study design description
10	Is the administration route given?
11	Are doses administered or concentrations in application media given?
12	Are frequency and duration of exposure as well as time-points of observations explained?
13	Were negative (where required) and positive controls (where required) included (give point also, when absent but not required, see explanations for study types and their respective requirements on controls)?
14	Is the number of animals (in case of experimental human studies: number of test persons) per group given?
15	Are sufficient details of the administration scheme given to judge the study (see explanation for examples)?
16	For inhalation studies and repeated dose toxicity studies only (give point for other study types): Were achieved concentrations analytically verified or was stability of the test substance otherwise ensured or made plausible?
	Criteria Group IV: Study results documentation
17	Are the study endpoint(s) and their method(s) of determination clearly described?
18	Is the description of the study results for all endpoints investigated transparent and complete?
19	Are the statistical methods applied for data analysis given and applied in a transparent manner (give also point, if not necessary/applicable, see explanations)?
	Criteria Group V: Plausibility of study design and results
20	Is the study design chosen appropriate for obtaining the substance-specific data aimed at (see explanations for details)?
21	Are the quantitative study results reliable (see explanations for arguments)?

Table 2
Listed criteria for *in vitro* studies.

	Criteria
No.	**Criteria Group I: Test substance identification**
1	Was the test substance identified?
2	Is the purity of the substance given?
3	Is information on the source/origin of the substance given?
4	Is all information on the nature and/or physico-chemical properties of the test item given, which you deem indispensable for judging the data (see explanation for examples)?
	Criteria Group II: Test system characterisation
5	Is the test system described?
6	Is information given on the source/origin of the test system?
7	Arenecessary information on test system properties, and on conditions of cultivation and maintenance given?
	Criteria Group III: Study design description
8	Is the method of administration given (see explanations for details)?
9	Are doses administered or concentrations in application media given?
10	Are frequency and duration of exposure as well as time-points of observations explained?
11	Were negative controls included (give also point, if not necessary, see explanations)?
12	Were positive controls included (give also point, if not necessary, see explanations)?
13	Is the number of replicates (or complete repetitions of experiment) given?
	Criteria Group IV: Study results documentation
14	Are the study endpoint(s) and their method(s) of determination clearly described?
15	Is the description of the study results for all endpoints investigated transparent and complete?
16	Are the statistical methods for data analysis given and applied in a transparent manner (give also point, if not necessary/applicable, see explanations)?
	Criteria Group V: Plausibility of study design and results
17	Is the study design chosen appropriate for obtaining the substance-specific data aimed at (see explanations for details)?
18	Are the quantitative study results reliable (see explanations for arguments)?

Table 3
Definition of Klimisch categories..

Reliability Categorisation			
	1	2	3
	Reliable without restrictions	Reliable with restrictions	Not reliable
In Vivo	18-21	13-17	<13 or not all **red** criteria met
In Vitro	15-18	11-14	<11 or not all **red** criteria met

Results

Study selection

After an initial search, 302 articles were identified through PubMed and Web of Science (Fig. 1), however many duplicates were present. After removing those duplicates 72 articles were removed and 230 articles were kept. Upon further screening, 209 articles were removed as they did not fit the inclusion criteria set for this systematic review. The remaining 21 full-text articles were evaluated and finally three more papers were excluded. Two papers were published before the set date (2006 onwards) for this review (Blázquez *et al.*, 2004; Massi *et al.*, 2004) and one article related to a synthetic cannabinoid rather than a natural one (Echigo *et al.*, 2012).

Hence, 18 studies were included in total in this systematic review

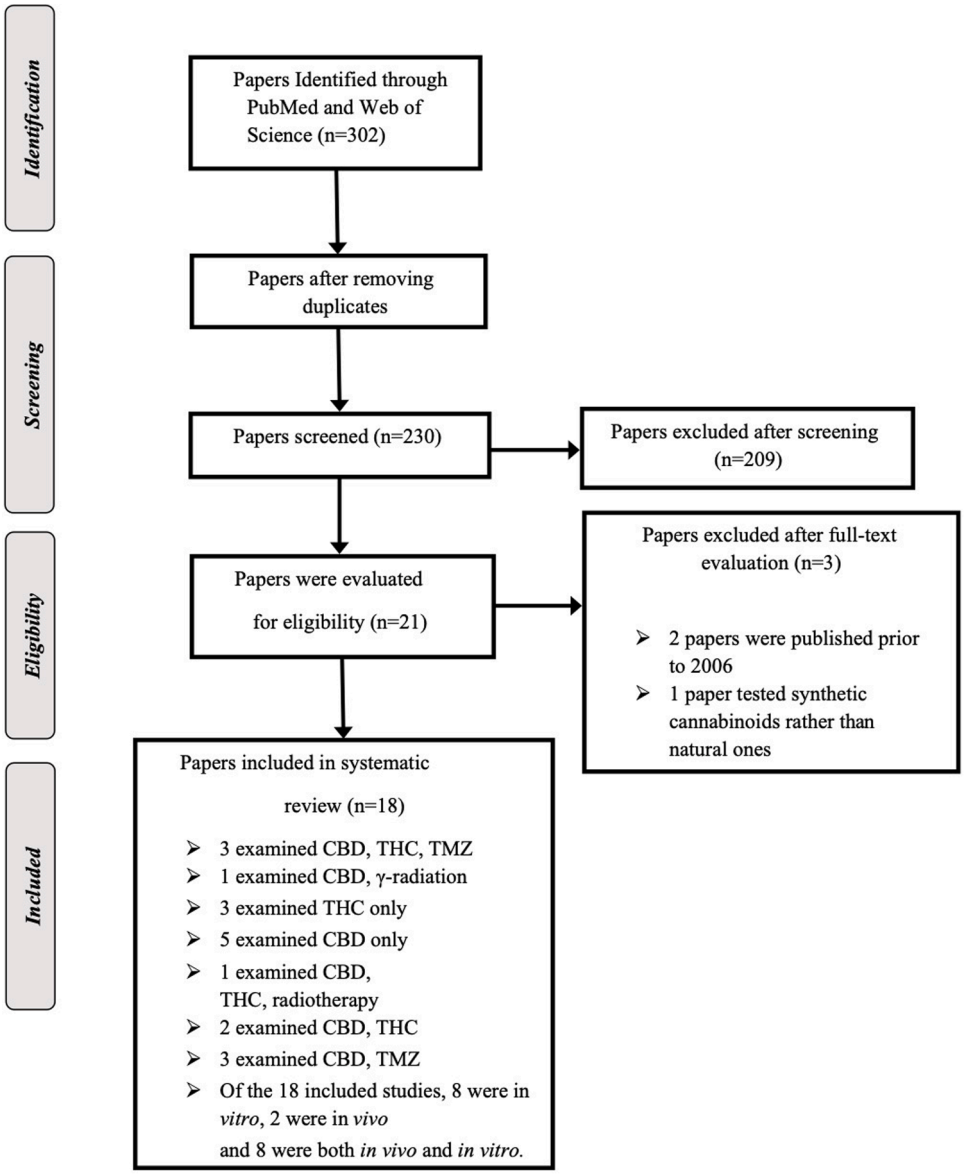

Fig. 1. PRISMA diagram of the selection process.

(Table 6). Three out of the 18 studies included, examined the combined effect of CBD, THC and TMZ on glioma cells (López-Valero *et al.*, 2018a, 2018b; Torres *et al.*, 2011). One study examined the efficacy of CBD with γ-radiation (Ivanov *et al.*, 2017), three studies examined the efficacy of THC only (Hernández-Tiedra *et al.*, 2016; Salazar *et al.*, 2009; Guzman *et al.*, 2006) and five studies examined the efficacy of CBD alone (Singer *et al.*, 2015; Solinas *et al.*, 2013; Soroceanu *et al.*, 2013; Aparicio-Blanco *et al.*, 2019; Nabissi *et al.*, 2015). Three studies combined CBD and TMZ (Deng *et al.*, 2017; Nabissi *et al.*, 2012; Kosgodage *et al.*, 2019). There was only one study that examined the efficacy of combined CBD, THC and radiotherapy (Scott *et al.*, 2014) and two studies that examined CBD and THC only (Hernán Pérez de la Ossa *et al.*, 2013; Marcu *et al.*, 2010).
Quality assessment

In vitro studies

The qualitative analysis of all included *in vitro* studies led to the conclusion that they were all considered reliable without restriction as they were assigned to Klimisch category 1 (Table 4). Thirteen out of 16 received maximum score (18 points) as all criteria were met (Deng *et al.*, 2017; Ivanov *et al.*, 2017; López-Valero *et al.*, 2018a; Marcu *et al.*, 2010; Salazar *et al.*, 2009; Scott *et al.*, 2014; Solinas *et al.*, 2013; Soroceanu *et al.*, 2013; Torres *et al.*, 2011; Guzman *et al.*, 2006; Kosgodage *et al.*, 2019; Aparicio-Blanco *et al.*, 2019; Nabissi *et al.*, 2015). Three studies (Hernández-Tiedra *et al.*, 2016; Nabissi *et al.*, 2012; Singer *et al.*, 2015) gained 17 points as all 3 studies missed criterion 2 (purity of the test substance). All studies, including the *in vivo* ones, received a point for criterion 4 as such information was not needed for the type of studies included in this systematic review.

In vivo studies

Ten studies were scored in the *in vivo* category (Table 5) and were all assigned to Klimisch category 1, except one that was assigned to Klimisch category 3 (Scott *et al.*, 2014) due to not meeting a red criterion (14-number of mice that were assigned to each treatment group), despite meeting the rest criterion (scored 20). Hence this study was considered not reliable. Three studies scored the maximum gathering 21 points (López-Valero *et al.*, 2018a, 2018b; Salazar *et al.*, 2009). Soroceanu *et al.* (2013) scored 20, missing criterion 8 (age/body weight of the test organism at the beginning of the study) and Guzman *et al.* (2006) scored 20 as criterion 7 was missing (information on patients' race). In addition, three studies (Hernán Pérez de la Ossa *et al.*, 2013; Singer *et al.*, 2015; Torres *et al.*, 2011) scored 19 as two of them missed criterion 6

Table 4
Data analysis of *in vitro* studies adapted from ToxRTool.

Studies	Criteria Group I				Criteria Group II			Criteria Group III						Criteria Group IV			Criteria Group V		Total Score	Klimisch category
	1*	2	3	4NR	5	6	7	8	9*	10*	11*	12*	13	14	15	16	17*	18		
Scott *et al.* (2014)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	18	1
Torres *et al.* (2011)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	18	1
Marcu *et al.* (2010)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	18	1
Salazar *et al.* (2009)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	18	1
Deng et al. (2016)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	18	1
Hernandez- Tiedra *et al.* (2016)	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	17	1
Nabissi et al. (2013)	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	17	1
Solinas *et al.* (2013)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	18	1
Lopez- Valero et al. (2018)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	18	1
Soroceanu *et al.* (2013)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	18	1
Singer *et al.* (2015)	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	17	1
Ivanov *et al.* (2017)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	18	1
Guzman *et al.* (2006)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	18	1
Kosgodage *et al.* (2019)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	18	1
Aparicio-Blanco *et al.* (2019)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	18	1
Nabissi *et al.* (2015)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	18	1

Criteria description for *in vitro* studies:
*maximum score is needed for a study to be assigned to Klimisch category 1 or 2; a score of 1 indicates criterion met, a score of 0 indicates criterion not met, and NR indicates criterion not reported.
Criteria Group I: Test substance identification; Criteria Group II: Test organism characterisation; Criteria Group III: Study design description; Criteria Group IV: Study results documentation; and, Criteria Group V: Plausibility of study design and results.

(sex of the test organism) and 8. The other study missed criterion 2 (purity of the test substance) and 8. Finally, Hernández-Tiedra *et al.* (2016) scored 18 as it did not meet criterions 2, 6 and 8.

Data extraction and analysis

CBD, THC and TMZ

Three studies examined the combined efficacy of CBD, THC and TMZ on the human glioma cell line U87MG, either *in vitro* or *in vivo* (Tables 7a, b and c). Torres *et al.* (2011) showed that THC, TMZ and CBD co-administration upon glioma xenografts reduced tumour growth in a greater extent than treatment with individual agent. Resistance of cells to individual treatment of TMZ and THC was shown to be overcome when 5 mg/kg of TMZ and 7.5 mg/kg of THC- botanical drug substance + 7.5 mg/kg of CBD- botanical drug substance (Sativex like) were co-administered, exhibiting a strong antitumoral action. Hence, leading to an enhanced reduction of tumour growth.

López-Valero *et al.* (2018a), showed that administration of THC and CBD in a 1:5 ratio, 5mg/kg THC + 25 mg/kg CBD, produced a stronger reduction on the proliferation of Glioma Initiating Cells than the effect of a 1:1 ratio, enhancing even more the effect of TMZ (5 mg/kg I.P administration). In addition, it was indicated that a slight amount of THC (0.83μM) is essential, in order to observe an enhanced anticancer activity when 4.17 μM of CBD and 100 μM of TMZ are administered.

López-Valero *et al.* (2018b) showed that oral treatment with cannabinoids, 45 mg/kg THC- botanical drug substance + 45 mg/kg CBD- botanical drug substance (containing 32mg/kg THC and 30 mg/kg CBD) and 5mg/kg Intraperitoneal (I.P) TMZ caused total regression to 67% of the animals and reduced the growth of U87MG subcutaneous xenografts in all animals. Oral administration has been shown to permit reaching effective concentrations of cannabinoids at the tumour site, similar with the ones of local administration. I.P delivery of Sativex like 7.5 (7.5mg/kg THC- botanical drug substance + 7.5 mg/kg CBD- botanical drug substance) in U87MG intracranial tumour xenografts inhibited their growth hence I.P administration can target tumours inside brain parenchyma.

CBD and γ-radiation

Ivanov *et al.* (2017) demonstrated that U87MG cells achieved almost 90% apoptotic levels upon treatment with 20μM CBD and 5Gy γ-radiation (Table 8). Interestingly, a pro-apoptotic signal was absent from normal neural cells after CBD-treatment. Oxidative stress upon CBD treatment induced signalling pathways (upregulation of phosphorylated JNK-cJUN, downregulation of active phosphorylated form of AKT and ERK) involved in cell proliferation and survival and hence induced autophagy and apoptotic commitment.

THC

Three studies were found regarding the evaluation of THC's efficacy on glioma cells (Tables 9a, b and c). Hernández-Tiedra *et al.* (2016) showed that 4 μM of THC stimulated autophagy- mediated cancer cell death through the modification of sphingolipid composition of the endoplasmic reticulum (ER) of glioma cells. Hence, transmitted to autolysosomes and autophagosomes leading to lysosomal membrane permeabilization, and finally to activation of mitochondrial apoptotic cell death.

The first clinical study on 9 patients diagnosed with recurrent GBM, performed by (Guzmán *et al.*, 2006), showed that THC delivery was safe and no psychoactive effects were noted. In addition none of the patients experienced significant alterations in biochemical, physical, heamatological and neurological parameters that could be attributed to THC. Regarding the antitumoral action of THC, Patients 1 and 2 (received 1.46 and 1.29 mg total dosage, respectively) had evident reduced tumour-cell proliferation as well as marked decrease of tumour vascularization, rendering THC treatment an effective antitumoral therapy.

Salazar *et al.* (2009) showed that THC-upregulation of tribbles pseudo-kinase 3 leads to the inhibition of Akt/mTORC1 axis with subsequent induction of autophagy and apoptotic cell death. *In vivo* studies showed that THC-treated cells (patient 1 received 1.46 mg of THC for 30 days, patient 2 received 1.29 mg of THC for 26 days) of two patients with recurrent GBM had increased autophagic phenotype observed through biopsy. THC administration can therefore cause an autophagy-mediated cell death in human glioma tumours.

CBD

Five experimental studies investigated the effect of CBD, on glioma cells (Tables 10a, b, c, d and e). Singer *et al.* (2015) showed that Glioma Stem Cells (GSCs) viability and self-renewal capacity were inhibited in a ROS-dependent manner by CBD both *in vitro* (2.6 μM-3.5 μM CBD) and *in vivo* (15 mg/kg CBD intraperitoneal for 5 days/week) leading to an increased survival rate both *in vivo* and *in vitro* (Table 10a). Interestingly, upon administration of CBD, an adaptive reprogramming towards

Table 5
Data analysis of *in vivo* studies adapted from ToxRTool.

Studies	*Criteria Group I*				*Criteria Group II*				*Criteria Group III*						*Criteria Group IV*			*Criteria Group V*	*Total Score*	*Klimisch Category*
	1*	2	3	4NR	5*	6	7	8	9	10*	11*	12*	13*	14*	15	16	17	18	19	20*	21			
Torres *et al.* (2011)	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	19	1	
Scott *et al.* (2014)	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	20	3	
Hernan Perez de la Ossa *et al.* (2013)	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	19	1	
Salazar *et al.* (2009)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	21	1	
Lopez- Valero *et al.* (2018a)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	21	1	
Hernandez- Tiedra *et al.* (2016)	1	0	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	18	1	
Lopez- Valero *et al.* (2018b)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	21	1	
Soroceanu *et al.* (2013)	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	20	1	
Singer *et al.* (2015)	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1		19	1	
Guzman *et al.* (2006)	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	20	1	

Criteria description for *in vivo* studies:
*maximum score is needed for a study to be assigned to Klimisch category 1 or 2; a score of 1 indicates criterion met, a score of 0 indicates criterion not met, and NR indicates criterion not required.
Criteria Group I: Test substance identification; Criteria Group II: Test organism characterisation; Criteria Group III: Study design description; Criteria Group IV: Study results documentation; and, Criteria Group V: Plausibility of study design and results.

resistant mesenchymal (MES) phenotype was established by a sub-population, and was also seen in xenograft tumour tissue.

Solinas *et al.* (2013) proved that 9-12 μM CBD caused a dose-depended decrease in cell invasiveness with the strongest reduction being 90% at 12 μM. Also 5-12 μM of CBD caused a down-regulation of tumour-related proteins (6 proteins in U87MG and 9 proteins in T98G cells) released by glioma cells, leading to the inhibition of signalling pathways, and thus inhibition of tumour growth. Hypoxia-inducible factor-1α (HIF-1α) was also decreased in U87MG cells after a 5-9 μM CBD treatment, leading to the inhibition of angiogenesis.

Soroceanu *et al.* (2013) demonstrated that Id-1 expression is correlated with GBM cell invasiveness and higher tumour grades, as 70% of GBM cells expressed Id-1. Upon treatment with 1-1.5 μM of CBD, Id-1 expression and corresponding cell-invasiveness were both down-regulated in U251 and primary GBM cells. *In vivo* results suggest, after a significant down-regulation of Id-1 expression upon intraperitoneal CBD injection of 15 mg/kg for 5 days a week, a 95% decrease in tumour area was observed, inhibiting GBM progression.

Aparicio-Blanco *et al.* (2019) confirmed the anti-proliferative effect of CBD against GBM cells and thus its anti-tumour effects upon treatment with CBD-loaded lipid nano capsules (LNCs). However, CBD-functionalized (CBD added on its surface)-CBD-loaded LNCs have been shown to achieve a significantly higher glioma targeting effect. Another modification that has been shown to reduce the IC_{50} value of CBD-loaded LNCs is the reduction of particle size of LNCs as it affects the cellular uptake and the drug release rate which leads to a higher cytotoxicity against GBM cells.

Nabissi *et al.* (2015) demonstrated that the GSCs differentiation that led to the activation of the autophagic process and inhibition of GSCs proliferation was caused by transient receptor potential vanilloid 2 activation through CBD treatment. In addition, Aml-1a was found to be upregulated during differentiation of GSCs while its absence led to a restoration of stem cell phenotype. Interestingly, Aml-1a has been found to bind transient receptor potential vanilloid 2 promoters leading to its enhanced transcription. Through these interrelated effects of CBD-stimulated glial differentiation along with inhibition of GSCs proliferation, its anti-tumour effects are confirmed.

CBD and TMZ

Three studies were found on the combined efficacy of CBD and TMZ (Tables 11a, b and c). Nabissi *et al.* (2012) showed that 10 μM of CBD triggered Ca^{2+} influx in transient receptor potential vanilloid 2-expressing glioma cells, increasing TMZ uptake. CBD synergized with the cytotoxic agents, increased chemosensitivity of glioma cells to TMZ and induced apoptosis, after a 1-50 μM CBD administration. Co-administration of 10 μM CBD and 400 μM of TMZ in U87MG cells, significantly reduced the IC_{50} value of TMZ that would be needed to reach cytotoxic effects when administered alone.

Deng *et al.* (2017) proved that CBD caused an inhibition on cell viability and cell proliferation on human GBM cell lines and proved that has antineoplastic activities. 1-10 μM of CBD with 30 μM of TMZ showed a concentration- dependent synergistic antiproliferative and cell-killing response in T98G cells, proving that CBD enhanced its cytotoxic effect.

Kosgodage *et al.* (2019)demonstrated that upon combinatory treatment of 800 μM TMZ and 5 μM CBD, a marked reduction in cell viability was noted which was absent when CBD or TMZ were used individually. The combinatory treatment also caused a reduction in pro-oncogenic miR21 which was significantly greater than the reduction noted when TMZ was used alone. The level of anti-oncogenic miR126 was greatly increased indicating an anti-GBM function through changes in this miRNA, in response to CBD. It was also evident that upon CBD treatment, prohibitin was decreased leading to reduced chemoresistance and thus supporting previous evidence showing that CBD has anti-cancer effects.

Table 6
Summary of included studies' characteristics.

Author and year	*Cannabinoids and treatments used*	*Country of study*	*Study design*	*Outcome measures*
López-Valero *et al.* (2018a)	CBD, THC and TMZ	Spain	Pilot experimental	Anticancer effect on aGICs
López-Valero *et al.* (2018b)	CBD, THC and TMZ	Spain	Pilot experimental	Anticancer efficacy of systemic administration
Ivanov *et al.* (2017)	CBD, γ-radiation	USA	Experimental	Upregulated activity of γ-radiation by CBD
Deng *et al.* (2017)	CBD, TMZ	USA	Experimental	Cell-killing of CBD alone or combined with TMZ
Hernández-Tiedra *et al.* (2016)	THC	Spain, Denmark, UK, Japan	Experimental	Autophagy-mediated cancer cell death
Singer *et al.* (2015)	CBD	USA	Experimental	Therapeutic response to bGSCs
Scott *et al.* (2014)	CBD, THC and radiotherapy	UK	Experimental	Antiproliferative effects
Solinas *et al.* (2013)	CBD	Spain	Experimental	Antiproliferative/anti-invasive properties
Soroceanu *et al.* (2013)	CBD	USA	Experimental	Reduced invasion and tumour growth
Nabissi *et al.* (2012)	CBD	Italy	Experimental	Enhanced activity of chemotherapeutic agents
Hernán Pérez de la Ossa *et al.* (2013)	CBD, THC	Spain	Experimental	Antitumor efficacy
Torres *et al.* (2011)	CBD, THC and TMZ	Spain	Experimental	Synergic antitumoral action
Marcu *et al.* (2010)	CBD, THC	USA	Experimental	Synergic inhibition of cell growth and induction of apoptosis
Salazar *et al.* (2009)	THC	Spain, France, Italy	Experimental	Cell death through autophagy
Guzman *et al.* (2006)	THC	Spain	Pilot experimental	Antiproliferative actions and safety profile
Kosgodage *et al.* (2019)	CBD, TMZ	UK	Experimental	Enhanced activity of TMZ and anticancer effects
Aparicio-Blanco *et al.* (2019)	CBD	Spain	Experimental	Antitumor effects of lipid nano capsules
Nabissi *et al.* (2015)	CBD	Italy	Experimental	Anti-tumor effects on [b]GSCs

aGICs-Glioma Initiating Cells; bGSCs- Glioma Stem Cells

Table 7a
Studies experimenting the combined efficacy of CBD, THC and TMZ.

Author, year and study design	*Aim of study*	*Cell culture and test organism characteristics*	*Concentration/exposure to CBD, THC, TMZ*	*Effects of cannabinoids on TMZ and on tumour growth*	*Outcome*
Torres *et al.* (2011), *In vitro* and *in vivo*	To examine the possible synergic antitumoral action of CBD, THC and TMZ	Human glioma cell lines (U87MG, A172, SW1783, U373MG, T98G, SW1008, LN405, Primary cultures of brain tumours cells (HG19, HG2, HG14), Nude mice were induced by subcutaneous injection of U87 and T98 cells	THC + TMZ (0.9 μmol/l + 75 μmol/l; 24 h) on U87MG cells THC (15 mg/kg) +TMZ (5 mg/kg) on growth of U87MG cell-derived tumour xenografts THC + CBD (0.9 + 0.9 μmol/l; 24 h) on LC3 immunostaining of U87MG cells Single peritumoral injection for 14 days SAT-L [THC-BDS (7.5 mg/kg) + CBD- BDS (7.5 mg/kg)] +TMZ (5 mg/kg)	Survival of certain human glioma cell lines and 2 primary cultures of glioma cells were reduced aLC3-II was increased. *In vivo* results showed that THC + TMZ caused a greater reduction in tumour growth than treatment with individual agents. This was also evident on tumour xenografts Co-administration of CBD, THC and TMZ greatly reduced the growth of U87MG- and T98G cell-derived tumour xenografts A greater resistance was observed in T98G cells (higher MGMT mRNA levels) than in U87MG cells when treated with TMZ or THC	Apoptosis and autophagy were enhanced in a higher extend with combination treatments rather than treatment with individual agents THC + TMZ; THC + CBD; THC + CBD + TMZ; TMZ + SAT-L Resistance of T98G cells was overcome by combined treatment of TMZ + THC or TMZ + SAT-L Leading to a diminished growth of these cells

Conclusion: Treatment with TMZ + SAT-L reduced tumour growth, despite tumours being resistant when these agents were applied individually. When CBD was also added, the triple combination caused a significantly greater reduction in the growth of gliomas.

aLC3-Microtubule-associated protein 1A/1B-light chain 3; bBDS- botanical drug substance

CBD, THC and radiotherapy

Scott *et al.* (2014) (Table 12) showed that CBD and THC, both in their pure form (over 96% purity) and as botanical drug substance reduced cell numbers in a dose-depended manner by triggering autophagy leading to apoptotic death, through inhibition of cell cycle. The most intriguing finding were the *in vivo* results in the orthotopic syngeneic model, where combination of 2 mg/kg of CBD and THC each together with 4 Gy radiotherapy caused a significant reduction in tumour progression. When cannabinoids were administered before irradiation a dramatic reduction was noted.

CBD and THC

Two studies were found to evaluate the combined efficacy of CBD and THC (Tables 13a and b). Hernán Pérez de la Ossa *et al.* (2013) showed that biodegradable polymeric microparticles (MPs) loaded with 37.5 mg CBD and 37.5 mg THC increased apoptotic activity and reduced angiogenesis in U87MG xenografts requiring less repetition of administration process. Hence, tumour growth of glioma xenografts can be reduced at the same extent as the daily treatment of THC-CBD (0.25 mg THC + 0.25 mg CBD) in solution.

Marcu *et al.* (2010) initially showed that individual treatments of THC (IC_{50} values in U87 cells was 3.3 μM) and CBD (IC_{50} values in U87 cells was 0.6 μM) both inhibited the growth of three glioblastoma cell lines, however CBD caused a greater inhibition than THC, in all three cell lines. Inhibitory effects of THC (1.7 μM) on glioma cells were enhanced by CBD (0.4 μM) causing a greater reduction in tumour

Table 7b
Studies experimenting the combined efficacy of CBD, THC and TMZ.

Author, year and study design	*Aim of study*	*Cell culture and test organism characteristics*	*Concentration/exposure to CBD, THC, TMZ*	*Effects of cannabinoids on TMZ and on tumour growth*	*Outcome*
López-Valero *et al.* (2018a) *In vitro* and *in vivo*	To test the effect of co-administration of THC + CBD and TMZ (containing varied ratios of THC and CBD) on glioma models, especially those derived from GICs	Human brain cell line-U87MG, Glioblastoma patient derived- cells aGH2-GICs, 12012-GICs cells) 5-week-old nude mice (essential weight 25g) 6-8 animals for each condition. Mice were injected subcutaneously in right flank with U87MG and intracranially into right cerebral hemisphere with U87MG and GICs	THC: CBD 1:5 ratio [0.83 μM THC + 4.17 μM CBD]; THC: CBD 1:1 ratio [2.5 μM THC + 2.5 μM CBD] and TMZ (100 μM or 20 μM) for 10 days on U87MG (subcutaneous xenografts) Daily oral administration for 20 days of THC: CBD (1:4 ratio) [THC (6.5 mg/kg) + CBD (24.5 mg/kg)] and TMZ (5mg/kg I.P administration) THC: CBD oral administration at 1:5 ratio [THC (5mg/kg) + CBD (25 mg/kg) and TMZ (5mg/kg I.P administration) on glioma xenografts (intracranial injection of 1202 GICs)	A lower decrease in tumour growth was produced upon CBD + TMZ treatment than TMZ alone THC: CBD (1:5 ratio) constrained GICs proliferation and self-renewal of GICs to higher extent than THC: CBD (1:1 ratio) Inhibition growth of subcutaneous U87MG tumour xenografts and enhanced anticancer activity on TMZ was similarly observed with both THC: CBD 1:1 ratio and THC: CBD 1:4 ratio Treatment with THC: CBD (1:4 ratio) and TMZ strongly reduced tumour growth and enhanced survival of mice bearing U87MG intracranial xenografts	When CBD is administered with TMZ, a slight amount of THC is needed in order to produce an enhanced anticancer effect CBD has been found to suspend self-renewal of GICs, causing a longer survival for animals with intracranial xenografts Also, combination of CBD, THC and TMZ activated apoptosis leading to a significant reduction of GICs population *in vitro* Treatments of THC: CBD containing a higher proportion of CBD than THC (1:5 ratio) affected more effectively the population of GICs than THC: CBD at 1:1 ratio

Conclusion: Combinations of TMZ with THC+CBD, containing higher amount of CBD (1:5), have been found to produce stronger antitumoral actions, greater activation of apoptosis and target more efficiently the GICs than a 1:1 ration of THC: CBD.

aGICs-Glioma Initiating Cells

Table 7c
Studies experimenting the combined efficacy of CBD, THC and TMZ

Author, year and study design	*Aim of study*	*Cell culture and test organism characteristics*	*Concentration/exposure to CBD, THC, TMZ*	*Effects of cannabinoids on TMZ and on tumour growth*	*Outcome*
López-Valero *et al.* (2018b) *In vivo*	To evaluate the efficacy of systemic (intraperitoneal (I.P) or oral) administration of THC and CBD in preclinical models of glioma as anticancer agents when administered with TMZ	5-weeks-old male nude mice were induced with U87MG cells in right flank for the generation of subcutaneous xenografts 5-weeks-old male nude mice were injected with U87MG cells into right cerebral hemisphere for the formation of intracranial xenografts	Subcutaneous xenografts: SAT-L 15 (15 mg/kg of THC-aBDS + 15 mg/kg CBD-BDS, containing 10.5mg/kg of THC and 10 mg/kg of CBD) or SAT-L 45 (45 mg/kg THC-BDS + 45 mg/kg CBD-BDS, containing 32 mg/kg THC and 30 mg/kg CBD) +TMZ (5 mg/kg daily I.P administration for 12 days Intracranial xenografts: SAT-L 7.5 (7.5 mg/kg THC-BDS and 7.5 mg/kg CBD-BDS) + TMZ (5mg/kg I.P administration)	I.P delivery of THC inhibited tumour growth, triggered autophagy and apoptosis in U87MG-cell derived subcutaneous tumour xenograft Oral SAT-L 15 + TMZ reduced the subcutaneous xenografts volume in 5/6 mice (83%) in relation to its initial volume and caused total regression at the tumours in 3/6 (50%) of mice. Oral SAT-L 45 + TMZ reduced the tumour volume in all mice (6/6) and caused regression to 4/6 (67%) Oral SAT-L alone or in combination with TMZ caused a remarkable reduction in the tumour's size. The survival of the mice was increased by SAT-L and TMZ and was significantly enhanced when the two treatments were administered together	I.P delivery of THC allowed reaching concentrations of THC and targeted tumours located within the brain parenchyma Volume of glioma xenografts was strongly reduced upon oral treatment of Sativex-like and TMZ leading to a complete reduction in growth of the tumours in > 50% of the animals SAT-L permitted reaching effective concentrations at tumour site with an efficacy similar to that of local administration Oral administration of SAT-L + TMZ strongly reduced tumour growth and increased survival of mice bearing U87MG-derived intracranial xenografts

Conclusion: Systemic administration (preferably oral) of cannabinoids reduced the growth of glioma cells and intensified the anticancer effect of TMZ with a comparable efficacy to local administration.

aBDS-botanical drug substance

growth by increased apoptotic activity, through production of ROS and oxidative stress.

Discussion

The aim of this systematic review was to investigate the efficacy of cannabinoids, either alone or in combination with the current treatments for GBM, in inhibiting cancer cell growth and to determine the mechanisms underlying this activity. The 18 studies included in this systematic review demonstrate that cannabinoids can induce apoptosis through various signalling pathways leading to cell death and regression of cancer growth.

The different types of cells, exposures and dosages are few of the factors that affected the treatment's sensitivity. Torres *et al.* (2011),

Table 8
Study experimenting the combined efficacy of CBD and γ-irradiation treatment

Author, year and study design	*Aim of study*	*Cell culture and test organism characteristics*	*Concentration/exposure to CBD, γ- irradiation*	*Effects of CBD on γ- irradiation and on tumour growth*	*Outcome*
Ivanov *et al.* (2017) *In vitro*	To investigate the enhanced cytotoxic effect of γ-irradiation in GBM by CBD	Human embryonic neural stem cells Human glioblastoma lines: U87MG, U118MG, T98G	20μm CBD and 5 Gy were co- administered for 72 hours Ionizing radiation (5Gy) with (5-15) μM on NSC/NPC	Active JNK1/2 was upregulated, ERK1/2 activity was downregulated and BAX and aTRAIL proapoptotic proteins were upregulated No significant change was observed on bNSC/NPC, only a modest change in apoptotic levels	Apoptotic levels were 50% (48 h) and almost 90% (72 h) in U87MG cells and almost 70% in U118MG Protein levels of TRAIL were increased in U87MG cells leading to their apoptotic cell death In this study it was confirmed that CBD does not cause any pro-apoptotic signalling in normal neural cells
			10 Gy + 20Mm CBD on U87MG and U118MG	JNK was significantly upregulated, MAPK p38 activity was moderately increased	Increased radiation and CBD dose led to a further upregulation of CBD-induced apoptosis (U87MG, U118MG)
				When CBD was added after irradiation, apoptosis was decreased	Indicating that apoptosis is favoured by administration of CBD first and then exposure to radiation
Conclusion: CBD-dependent modulation of cell signalling in combination with radiotherapy led to further increase on the efficiency of GBM treatment, with a protective effect for NSC/NPC.					

aTRAIL -TNF-related apoptosis inducing ligand; bNSCs-neural stem cells; bNPCs- neural progenitor cells.

Table 9a
Studies conducting the efficacy of THC treatment alone against GBM.

Author, year and study design	*Aim of study*	*Cell culture and test organism characteristics*	*Concentration/exposure to THC*	*Effects of THC on tumour growth*	*Outcome*
Hernández-Tiedra *et al.* (2016) *In vitro* and *in vivo*	To investigate the molecular mechanism of THC- induced autophagy-mediated cancer cell death	Human glioma cell line-U87MG A375, SK- MEL28 cells Hsd: AthymicNude-Foxn2 nu mice were injected subcutaneously with U87MG cells	THC (4 μM, 1 h, 3 h and 6 h) on U87MG cells THC, 4 μm for 3 h on U87MG THC treatment, 6 μM for 6 h on U87MG THC, 4 μM for 18 h	Accumulation of aMAP1LC3B-positive dots was observed indicating autophagy mRNA level of various enzymes that are involved in sphingolipid synthesis de novo was upregulated Ceramide levels increased and enhanced the levels of dihydroceramides	Increased levels of dihydroceramide led to a significant modification of ceramide: dihydroceramide ratio of U87MG cells' microsomal fraction THC acts upon the intracellular trafficking of sphingolipids, causing their accumulation in the ER
			THC, 4 μM for 16 h on cytosolic fraction of U87MG THC (15mg/kg, peritumoral administration) on tumours generated by subcutaneous injection of U87MG cells	bCOL4A3BP phosphorylation was increased by THC Increase in cytosolic cCTSB (cathepsin B) and dCTSL (cathepsin L) activity, causing appearance of CTSB in the cytosol of both U87MG and SK-MEL-28 Increased level of C16 dihydroceramide and decreased ratio of ceramide: dihydroceramide was correlated with THC- reduced tumour growth	The conformational change of COL4A3BP promoted by THC, inhibited its ability to transport ceramide from ER to Golgi COL4A3BP was found in the membrane of vesicles with autophagosomes in their morphology Autophagy induction by THC promoted eLMP, leading to the activation of the mitochondrial apoptotic pathway
				Autophagy was enhanced, intensity of CTSB immunostaining was increased	cell death pathway induced through autophagy *in vivo,* is activated by THC
Conclusion: Activation of autophagy-mediated cancer cell death leads to a change in sphingolipid composition of the ER. Triggered upon THC administration leads to LMP, cathepsin release and activation of apoptotic cell death.					

a MAP1LC3B -Microtubule-associated proteins 1A/1B light chain 3B; COL4A3BP- collagen type IV α3 binding protein; cCTSB- cathepsin B; dCTSL- cathepsin L; eLMP -lysosomal membrane permeabilization

showed by immunofluorescence monitoring that THC and TMZ treatment caused LC3-II conjugation suggestive of autophagy. When CBD was also added, activity of autophagy was significantly enhanced exhibiting a strong antitumoral action. A previous study found that CBD induced cell death by the immediate production of ROS and of strong depletion of glutathione, illustrating that each cannabinoid acts through a different mechanism (Massi *et al.*, 2006).

CBD-mediated autophagy was once more proven by Nabissi *et al.* (2015) when the cleaved LCE-II form levels and Beclin-1 protein were found to be increased. The interrelation between transient receptor potential vanilloid 2 and Aml-1a expression, which was overexpressed upon CBD treatment, was also confirmed, and found to play a crucial role towards the differentiation of GSCs as well as on their viability.

However, when the two cannabinoids are combined, they act through the mechanism of THC. This was also demonstrated by Marcu *et al.* (2010) when the combination of THC and CBD caused a CB2-dependent apoptosis. CBD has been found to act both as an agonist in some plasma membrane-associated ion channel receptors, like transient receptor potential vanilloid 1 and transient receptor potential vanilloid 2 (Bisogno *et al.*, 2001; Qin *et al.*, 2008) and act also in a protein- independent manner triggering biologic responses such as, DNA damage and apoptosis through oxidative stress (Solinas *et al.*, 2013).

Nabissi *et al.* (2012) demonstrated that when CBD was combined with TMZ a synergistic GBM-killing activity was observed through the

Table 9b
Studies conducting the efficacy of THC treatment alone against GBM

Author, year and study design	*Aim of study*	*Cell culture and test organism characteristics*	*Concentration/exposure to THC*	*Effects of THC on tumour growth*	*Outcome*
Guzman et al. (2006) *In vitro* and *in vivo*	To assess the antitumoral action of THC in patients with recurrent GBM and to establish the safety of THC administration intracranially	9 patients with GBM who all failed standard therapy (surgery and external beam radiotherapy). Mean age of cohort was 55 years. Size of recurrent tumours was medium-large.	Patient 1: total dose 1.46 mg in 2 cycles Patient 2: total dose 1.29 mg in 4 cycles Patient 3: total dose 3.29 mg in 6 cycles Patient 8: total dose 1.60 mg in 1 cycle Median duration of a cycle was 10 days. Tumor cells obtained from biopsy of Patient 1 were treated with 2.5 μM THC	Patients 1 and 2 had reduced tumour-cell proliferation upon THC treatment which was evident by Ki67 immunostaining but also a marked decrease of tumour vascularization was observed through CD31 immunostaining Patient 3 had a very aggressive recurrent GBM, but upon the first 3 cycles of THC treatment, tumour growth was restrained for about 9 weeks While recurrent GBM of patient 8 was actively growing, her clinical symptoms improved to a great extent (cephalalgia disappeared and motor deficit decreased) It was evident by TUNEL staining that growth of cells was inhibited by THC through at least in part to apoptosis	Through this study it is clearly shown that tumour growth is not facilitated by THC treatment Tumour-cell proliferation was reduced as well as tumour vascularization THC was associated with the containment of a really aggressive tumour for 9 months THC was associated with improvements in clinical symptoms of patients Most importantly a good safety profile for THC was observed The number of viable cells in the cultures was decreased upon treatment with THC

Conclusion: THC delivery in this study was performed without apparent psychoactive effects and it was safe further enhancing the possibility to be used against GBM due to its antiproliferative action on tumour cells.

Table 9c
Studies conducting the efficacy of THC treatment alone against GBM.

Author, year and study design	*Aim of study*	*Cell culture and test organism characteristics*	*Concentration/ exposure to THC*	*Effects of THC on tumour growth*	*Outcome*
Salazar *et al.* (2009) *In vitro* and *in vivo*	To evaluate the molecular mechanism autophagy- mediated apoptotic death through THC promotion, of glioma cells	Cortical astrocytes, primary cultures of brain tumour cells (U87MG, T98G, U373MG) U87MG induced in nude mice by subcutaneous injection Tumour biopsies from 2 recurrent GBM patients treated with THC	THC at a final concentration of 5 μM THC (15 mg/kg/d) administered by peritumoral injection Patient 1 received a total of 1.46 mg of THC for 30 days Patient 2 received a total of 1.29 mg of THC for 26 days. Both treatments were induced intratumorally	Immunostaining of ER showed a striking dilation in ER of U87MG cells, and an increase in the phosphorylation of the aelF2α THC reduced phosphorylation of p70S6 (mTORC1 substrate), leading to the inhibition of the mTORC1 THC treatment increased p8 and TRIB3 expression, increased LC3-II and active caspase-3 immunostaining in tumour xenografts In both patients, TRIB3 immunostaining increased, S6 phosphorylation decreased upon THC administration Amount of cells with autophagic phenotype and caspase 3- immunostaining increased	THC-treated cells appeared with morphological features of autophagosomes Upregulation of p8 and TRB3 through ER-stress is induced by THC An increase in p8 and TRIB3 induced autophagy of tumour cells though the inhibition of Akt/mTORC1 pathway Induction of the cell- death pathway through autophagy seems to be indispensable of cannabinoid antitumoral action THC administration possibly triggers cell death through autophagy in human Tumours

Conclusion: TRIB3 is upregulated by THC, interacting with and decreasing the phosphorylation of Akt which then triggers the inhibition of mTORCH1 leading to autophagy and decreased tumour growth.

aelF2α- α-subunit of the eukaryotic translation initiation factor 2

enhancement of transient receptor potential vanilloid 2 expression, increasing chemosensitivity of cells to TMZ. When Deng *et al.* (2017) reproduced this result however, it was observed that synergistic responses were only seen in a limited range of concentrations. For example, CBD/TMZ administration on PDGF-GBM cells, antagonized their antiproliferative response but an additive-cell killing response was triggered. The reason behind the antagonistic response on their antiproliferative rates is not known, but a possible mechanism could be that CBD works as a negative allosteric modulation. These results indicate that cell killing observed after combined treatment with CBD and TMZ was not dependent on their ability to decrease the cell proliferation.

On the contrary, Kosgodage *et al.* (2019) demonstrated that after a combinatory treatment, consisting of CBD and TMZ on GBM cells derived through biopsies from people with GBM, cell viability was found to be reduced. A reduction in pro-oncogenic miR21 and an increase in miR126 levels were evident upon treatment with the combinatory treatment, indicating anti-GBM functions. Finally, prohibitin protein levels were greatly reduced in cancer cells upon CBD treatment, decreasing chemoresistance and thus confirming the anti-tumor actions of CBD.

Table 10a
Studies examining the efficacy of CBD alone as a treatment against GBM.

Author, year and study design	*Aim of study*	*Cell culture and test organism characteristics*	*Concentration/exposure to CBD*	*Effects of CBD on tumour growth*	*Outcome*
Singer *et al.* (2015) *In vitro* and *in vivo*	To investigate how CBD treatment acts upon aGSCs	U251 cell line, GSC lines 387, 3832 Tumour lines were injected subcutaneously in flank of athymic Nu/Nu mice Tumours were induced in female athymic nu/nu mice by intracranial injection of GSC 3832 or 387	GSCs 3832 and 387 were treated with 3.5 μM and 2.6 μM respectively GSCs (3832, 387) were treated with CBD (2 μM) for 2 days CBD (2 μM) treatment to detect the mechanism behind the resistant GBM phenotype CBD treatment administered intraperitoneal, 15 mg/kg for 5 days a week until the end of the experiment. Treatment started 9 days after injection of tumour	ROS production was increased upon CBD treatment and viability of GSCs was inhibited CBD inhibited expression of Sox2, Id1 and p-STAT3 and upregulates p38 MAPK There was a downregulation in several stemness markers and an upregulation of various antioxidant response gene products, as well as bMES GBM markers Increase in the MES marker CD44 was found in GBM xenografts Inhibition of p-cAKT and increased activity of cleaved caspase-3 was observed	GSC self-renewal and stemness was inhibited in a ROS-dependent manner by CBD A subset of tumour cells upregulated the antioxidant response genes and underwent an adaptive reprogramming leading to a resistant MES phenotype, resuming a more rapid growth after CBD treatment DNA analysis revealed that expression of stem cell regulators was restrained by CBD GBM progression *in vivo* was inhibited and survival was prolonged upon CBD treatment Intracranial growth of primary GSC-derived tumours was inhibited for the first time, *in vivo*

Conclusion: GSCs self-renewal ability was inhibited by CBD in a ROS- dependent manner, as several stemness markers were downregulated, leading to an increased survival rate both *in vitro* and *in vivo*.

aGSCs- Glioma stem cells; b MES -Mesenchymal; c Akt -Protein kinase B

Table 10b
Studies examining the efficacy of CBD alone as a treatment against GBM.

Author, year and study design	*Aim of study*	*Cell culture and test organism characteristics*	*Concentration/exposure to CBD*	*Effects of CBD on tumour growth*	*Outcome*
Solinas *et al.* (2013) *In vitro*	To characterize the anti-invasive/anti proliferative abilities of CBD in two types of glioma cell lines. And evaluate how CBD acts upon pro-tumoral aERK and bPI3K/Akt pathways, as well as on the expression of cHIF-1α	Human Glioma cell lines U87MG and T98G	U87MG cells were treated with CBD (0.5-12 μM), incubated for 24 h at 37 °C T98G cells were treated with 9μm to 12 μM CBD (5-12 μM) treatment for 24 h for the downregulation of various tumour- related proteins CBD (1-9 μM) concentrations on ERK/Akt phosphorylation	A decrease (from 10% to 90%) of U87MG cell invasion was caused upon CBD administration A significant decrease of cell invasiveness was induced upon 9 μM of CBD used on T98G cells, and a strong reduction of invasiveness (90%) was induced by 12 μM Pre-spotted antibodies on nitrocellulose membranes captured the outcome of CBD on the expression pattern of various proteins released by U87MG and T98G cells dMMP-9, eTIMP-4, fVEGF and gTGF-β1 were a few of the proteins that were downregulated by CBD	Invasion on U87MG and T98G cells is inhibited Anti-invasive concentrations of CBD used in the experiments did not cause any toxic effect in cells CBD down-regulated 6 proteins in U87MG cells and 9 proteins in T98G cells, leading to inhibition of signalling pathways CBD inhibited. HIF-1α in U87MG cells, inhibiting its pleiotropic effects In T98G cells HIF-1α protein was present
			CBD (5-9 μM) on HIF-1α levels	Reduction in a dose- dependent manner in the levels of phosphorylated form of ERK1/2 and Akt was observed but without any effect on the total protein level HIF-1α levels were found to be significantly downregulated upon CBD treatment in U87MG cells	

Conclusion: CBD inhibited cell invasion in both U87MG and T98G cells, down-regulated various tumour-related proteins released by glioma cells and inhibited HIF-1α, inhibiting cell proliferation and invasiveness.

aERK-Extracellular signal regulated kinases; bPI3K- phosphoinositide 3 kinase; c HIF-1- Hypoxia- inducible factor-1; d MMP-9-matrix metalloproteinase; eTIMP-4-Tissue inhibitors of metalloproteinase; f VEGF -Vascular endothelial growth factor; gTGF- β1- Transforming growth factor-β

Even though it was found that TMZ activity was not enhanced upon CBD addition only, López-Valero *et al.* (2018a) showed that when at least a certain amount of THC was added in CBD and TMZ, an enhanced inhibition of tumour growth was produced. Hence, a lower concentration of THC is needed and more CBD, leading to less psychotic side effects. CBD has been also shown to reduce psychotic activity of THC (D'Souza *et al.*, 2009).

The reason behind the extremely low survival rates with GBM is the recurrence of glioma Initiating cells. This is due to the resistance towards multiple therapies, leading to their persistence in the brain parenchyma around the tumour cavity (Osuka and Van Meir, 2017). López-Valero *et al.* (2018a) has proved that combination of THC, a higher amount of CBD and TMZ upon glioma Initiating cells have led to the induction of apoptosis and finally to a remarkable reduction of this cell population.

Through a pilot phase I trial of intracranial THC administration on 9 patients, conducted by Guzmán *et al.* (2006), the antitumoral action of

Table 10c
Studies examining the efficacy of CBD alone as a treatment against GBM.

Author, year and study design	*Aim of study*	*Cell culture and test organism characteristics*	*Concentration/ exposure to CBD*	*Effects of CBD on tumour growth*	*Outcome*
Soroceanu *et al.* (2013) *In vitro* and *in vivo*	To determine the correlation between aId- 1 expression and GBM cell invasiveness and whether CBD could inhibit Id-1 expression	Tissue samples obtained from patients with GBM were cultured as neurospheres SF210, U87, SF126, U251 cell lines Parental U251 cells were injected intracranially in female athymic *nu/nu* mice	Primary GBM- derived cells, evaluated by immunofluorescence/ Western blotting for 48 hrs from original culturing in neurosphere medium U251 and primary GBM cells treated for 3 days with CBD (1 or 1.5 μM) CBD treatment (1 μM) on neurosphere formation 5 mice per group treatment, intraperitoneal CBD injection with 15 mg/kg 5 days a week for 28 days	70% (out of 23 primary GBM-derived cultures) expressed Id-1 protein Knockdown of Id-1 could reverse the bMES phenotype SF126 and U251 cells were found to express significant levels of Id-1 and cell invasion was increased by a 5- to 7-fold Id-1 expression was down- regulated and correlated with an inhibition of invasiveness in U251after CBD treatment CBD inhibited p-ERK and p-Akt as well as Id-1 and cSox2 expression in neuropsheres A significant down-regulation of Id-1 expression was produced upon CBD treatment *in vivo,* inhibiting GBM dispersal and reduction in tumorigenicity	Id-1 expression is correlated with GBM invasiveness and with high tumour grades Id-1 protein controls the MES phenotype transition CBD inhibited Id-1 expression and invasiveness of primary GBM cells and U251 cells Id-1 expression was significantly down-regulated *in vivo* A powerful reduction of GBM progression was produced after CBD treatment in mice, leading to a 95% decrease in the tumour area and in one of the five mice, no tumour cells were observed in any of the brain regions analysed

Conclusion: CBD effectively reduced Id-1 expression and aggressiveness in cancer cells as well as *in vivo,* reducing tumorigenicity in mice.

aId-1-Inhibitor of DNA binding 1; bMES -Mesenchymal; cSox2- Sex Determining Region Y-Box

Table 10d
Studies examining the efficacy of CBD alone as a treatment against GBM.

Author, year and study design	Aim of study	Cell culture and test organism characteristics	Concentration/ exposure to CBD	Effects of CBD on tumour growth	Outcome
Aparicio-Blanco et al. (2019) *In vitro*	To observe the antitumor effects of lipid nano capsules decorated and loaded with CBD but also to assess CBD's potential to target CB receptors which are overexpressed in GBM	Human GBM U373MG cells	CBD-loaded LNCs- dissolved in the core of LNCs at 15% CBD/ Labrafac®WL1349 with remaining excipients added progressively Functionalized LNCs at 2 different concentrations of CBD; 10 mg/ml in a 1:4 ratio for a final CBD concentration of 2.5 mg/ml and 15 mg/ml in a 1:3 ration for a final CBD concentration of 5 mg/ml CBD-functionalized-CBD-loaded LNCs were decorated with 5 mg/ml	Both CBD-loaded LNCs and free CBD (IC_{50} = 29.1 μM) caused a decrease, in a concentration-dependent manner, in U373MG cells viability IC_{50} of 50 nm-sized LNCs was outperformed by a 20 nm-sized LNCs (615.4 μM *vs.* 202.6 μM, respectively), which achieved a 3-fold reduction in its IC_{50} value An enhanced cellular uptake, by 3.0-fold, was observed for undecorated LNCs upon a reduction in particle size of LNCs, while a 3.5-fold was observed for CBD-decorated LNCs Confocal microscopy images proved the significantly higher glioma targeting effect achieved by CBD-decorated LNCs compared to undecorated LNCs	An evident anti-proliferative effect against GBM cells was observed upon CBD treatment, confirming its antitumor effects The size of LNCs has been found to play a crucial role regarding the extend of CBD release Adjustments of particle size and CBD-decorated LNCs lead to enhanced in vitro glioma targeting Both a reduction in particle size of LNCs and the functionalization with CBD further reduce the IC_{50} values of CBD-loaded LNCs Human glioma cells were found to have internalized all tested formulations LNCs have been found to be efficient biocompatible and biodegradable carriers for CBD as well as successfully targeting the cannabinoid receptors

Conclusion: CBD antitumor effects against GBM have been corroborated and the highest cytotoxicity was noted with CBD-functionalized CBD-loaded LNCs as well as with the smaller-sized LNCs

aLNC- Lipid nanocapsule

THC as well as its safety profile were evaluated. Interestingly, no significant psychoactive effects appeared during the trial, except in one of the patients who experienced a short-term and mild episode of hypothermia, bulimia and euphoria. Through the findings of this trial, the fair safety profile of THC along with its antiproliferative actions on GBM cells, lead to a promising future where more trials need to be conducted.

A case report conducted by Dall'Stella *et al.* (2019) examined the side effects upon prolonged use of cannabinoids on 2 patients diagnosed with GBM. No significant alteration in blood counts or plasma biochemistry were developed confirming that cardiac or hepatic functions were not significantly affected by prolonged use of CBD. Interestingly, upon use of THC, in order for symptoms of chemotherapy to be alleviated, a reduction of fatigue and an increase in appetite were observed.

López-Valero *et al.* (2018b) showed that a systemic administration (I. P or oral) of THC can effectively minimise the growth of glioma cells *in vivo* and enhance the reaction of TMZ. Supporting the approach that oral administration reaches the relevant concentration delivery of THC and CBD at tumour site. It was suggested from previous reports (Carracedo *et al.*, 2006; Velasco *et al.*, 2016) that the pathway of non-transformed cells is not activated upon cannabinoid treatment. This was also evident in the study of Ivanov *et al.* (2017) where the NSC/NPC investigation led to the conclusion that CBD does not induce any

Table 10e
Studies examining the efficacy of CBD alone as a treatment against GBM

Author, year and study design	*Aim of study*	*Cell culture and test organism characteristics*	*Concentration/ exposure to CBD*	*Effects of CBD on tumour growth*	*Outcome*
Nabissi *et al.* (2015) *In vitro*	To demonstrate the expression levels of [a]Aml-1 in [b]GSCs during their differentiation and to assess if these levels directly interacted with [c]TRPV2 promoters and how CBD affects this interrelation	GSC lines (#1, #30, #83) obtained through biopsies of 3 patients diagnosed with GBM	GSC lines were treated up to 3 days with CBD, from 0.5 to 50 μM The lowest effective dose of CBD was found to be 10 μM thus this was used for the following experiments Involvement of TRPV2 in CBD- mediated effects on GSC lines was tested by pretreating GSC lines for 1 hour with 50 μM of TRPV2 selective antagonist (tranilast) followed by addition of 10 μM CBD	A significant decrease of cell viability was induced by CBD CBD effects were found to be reverted by tranilast indicating that viability was inhibited in a TRPV2-dependent manner by CBD The cleaved LC3-II form levels and the Beclin-1 (autophagy-related protein) were found to be increased by CBD pAKT levels were reduced upon CBD treatment Aml-1a mRNA was found to be overexpressed in all [d]D-GSC lines and subsequently it was proven that Aml-1a mRNA expression was increased by CBD TRPV2 increases were also evident in D- GSCs An increase in GSCs viability and a reduced expression of TRPV2 was observed upon silencing of Aml-1a in D-GSCs	It was observed that CBD inhibited the viability and arrested the cell cycle at G0/ G1 phase CBD has been found to reduce viability of GSC lines through TRPV2 CBD-mediated autophagic actions have been confirmed by the modulation of expression of different genes that regulate apoptotic and autophagic processes, by CBD Enhanced expression of Aml-1a, caused by CBD, in D-GSC lines indicates its contribution in this differentiation TRPV2 gene promoters have been found to be bound by Aml-1a leading to enhanced TRPV2 transcription The above findings were confirmed by the silencing of Aml-1a that led to increased GSCs viability along with reduced expression of TRPV2

Conclusion: CBD has been found to be causing an increase in Aml-1a expression which in turn causes a TRPV2 enhanced expression, linking autophagy activation to differentiation which leads to sensitization of GSCs to apoptotic death

[a] Aml-1 - Acute myeloid leukemia
[b] GSCs- Glioma stem-like cells
[c] TRPV2-Transient receptor potential vanilloid type 2
[d] D-GSCs- differentiated GSCs

Table 11a
Studies examining the efficacy of combined treatment of CBD and TMZ.

Author, year and study design	*Aim of study*	*Cell culture and test organism characteristics*	*Concentration/ exposure to CBD, TMZ*	*Effects of CBD on TMZ and on tumour growth*	*Outcome*
Nabissi *et al.* (2012) *In vitro*	To assess the role of aTRPV2 channel-CBD induced activation in the sensitization of GBM cells to TMZ	U87MG cell line MZC primary glioblastoma cells Normal human astrocytes (NHA)	U87MG, MZC cells incubated with CBD (10 μM) for 3 days CBD treatment (1-50 μM) for 1- 3 days to evaluate viability and apoptosis U87MG, MZC cells treated with TMZ (400 μM) in combination with CBD (10 μM) for 6 h	QRT-PCR was used to evaluate TRPV2 mRNA levels that revealed increased levels after CBD treatment Viability of U87MG, MZC, NHA was reduced upon maximum (> 25 μM) CBD treatment Co-administration (CBD + TMZ) reduced IC_{50} values of TMZ needed to produce cytotoxic effects alone Pro-apoptotic effects of TMZ used individually were enhanced when administered with CBD	Calcium influx was increased in U87MG cells that expressed TRPV2 CBD up-regulated expression of TRPV2 protein in glioma cells Dose- and time-dependent treatment affects viability and apoptotic cell death of glioma cells CBD potentiated TRPV2- dependent glioma cell chemosensitivity Smaller amount of drug is needed to induce apoptotic-cell death when combined with CBD The increased expression and activation of TRPV2 channels leads to increased chemosensitivity of human GBM cells to the cytotoxic effects of the DNA-damaging agent, TMZ upon treatment with CBD

Conclusion: CBD enhanced TRPV2 expression and activation in GBM cells, significantly enhancing drug influx, cytotoxic activity of TMZ maintaining high antineoplastic effects and lower chemotherapeutic doses.

aTRPV2-Transient receptor potential vanilloid type 2

pro-apoptotic signalling in normal neural cells.

This finding is in contrast with the clinical symptoms of TMZ shown upon treatment on normal neural cells, where an enhanced protein expression was observed. This is a symptom seen regularly in human tumour cell lines after exposure to TMZ and might be due to DNA hypomethylation which leads to up-regulated transcription (Vairano *et al.*, 2004). Hence, cannabinoids can potentially be used as anticancer drugs without affecting the viability of healthy cells.

Hernández-Tiedra *et al.* (2016) have established that THC alteration by sphingolipid metabolism drives towards a modification of sphingolipid load in the ER and autophagosomes. A central role in establishing the cell death-promoting outcome is presented by the modification of the autolysosomes too. Nonetheless, Li *et al.* (2014) have found that upon sphingolipid synthesis *de novo,* THC induced downregulation of Akt-MTORC1 axis by tribbles pseudo-kinase 3 promoting autophagy. Furthermore Salazar *et al.* (2009) showed that cannabinoid-induced

Table 11b
Studies examining the efficacy of combined treatment of CBD and TMZ.

Author, year and study design	*Aim of study*	*Cell culture and test organism characteristics*	*Concentration/exposure to CBD, TMZ*	*Effects of CBD on TMZ and on tumour growth*	*Outcome*
Deng *et al.* (2017) *In vitro*	To investigate the cell-killing- and antiproliferative activity of individual administration of CBD and in combination with TMZ	Human GBM cell lines (T98G, U251, U87MG) Primary cells derived from genetically engineered mouse model of GBM with amplified aPDGF signalling and bNPCS	CBD treatment (10-8 to 10-3 M in half log10) for the evaluation of viability and proliferation being observed 72 after treatment CBD (0.3-100 μM) co-administered with TMZ (1 μM to 1 mM) to analyze interactions and effect on glioma cells CBD (1-10 μM) with TMZ (30 μM) in T98G cells for antiproliferative responses	Cell proliferation was inhibited, and cell viability was reduced in all cells after CBD treatment (with maximal efficacy 94.19%-100%) CBD (1 μM) and TMZ (10 μM) caused an interdependent antiproliferative response in T98G cells Additive cell- killing responses were observed when combined low concentrations of CBD (1-10 μM) with TMZ (30 μM)	Mouse PDGF-GBM cells and NPCs were more responsive in antiproliferative and cell-killing activity by TMZ CBD proved that has an antineoplastic activity on these cells CBD significantly reduced cell proliferation and viability in all human GBM cell lines, mouse PDGF-GBM cells and NPCs CBD with TMZ caused an inhibition on cell proliferation by a synergistic antiproliferative response Cell viability was inhibited upon treatment with CBD and TMZ Several concentrations- combinations led to antagonistic effects, mainly in mouse-PDGF

Conclusion: CBD exhibited a synergistic effect when combined with TMZ in a concentration-dependent manner leading to inhibition of cell proliferation and viability.

aPDGF -Platelet derived growth factor; b NPCs -Neural progenitor cells

Table 11c
Studies examining the efficacy of combined treatment of CBD and TMZ

Author, year and study design	Aim of study	Cell culture and test organism characteristics	Concentration/exposure to CBD, TMZ	Effects of CBD on TMZ and on tumour growth	Outcome
Kosgodage et al. (2019) *In vitro*	To investigate the efficacy of CBD alone or with TMZ, in affecting extracellular vesicle profile in GBM cells and whether prohibitin can be reduced in order to enhance treatment effectiveness	LN18, GBM obtained from a male patient with a right temporal lobe glioma and LN229 GBM obtained from a female patient with right frontal parietal-occipital GBM	GBM cells were treated with a combinatory treatment of 800 μM TMZ and 5 μM CBD for 1 hour to assess for modulation in microRNA cargo LN18 and LN229 cells were treated for 1 hour with 800 μM TMZ and 5 μM CBD to assess cell viability LN18 and LN229 cells were treated with 5 μM CBD for 1 hour in order to assess prohibitin protein levels	A significant reduction of pro-oncogenic miR21 was noted in extracellular vesicles released from LN18 and LN229 cells Anti-GBM associated miR126 was remarkably increased after 1 hour of combinatory treatment in extracellular vesicle released from both cells Cell viability upon the combinatory treatment resulted in a 24.2% decrease in LN18 GBM cells and in a 10.9% decrease in LN229 cells Reductions of 11.3-37.7% were observed in prohibitin protein levels in LN18 cells and 15-17% in LN229 cells after 1 hour treatment with CBD	Reduction of pro-oncogenic miR21 was significantly greater when combinatory treatment was used compared to TMZ treatment alone The increased levels of miR126 were evident after the combinatory treatment on both cells indicating an anti-GBM function, in response to CBD, through changes in this miRNA Combinatory treatment caused a reduction in cell viability in both cells while the individual treatment (CBD 5 μM or TMZ 800 μM) failed to cause any reduction in cell viability of LN229 cells Prohibitin protein levels were greatly reduced in both cells compared to DMSO treated cells, leading to reduced chemo-resistant functions

Conclusion: CBD combined with TMZ caused a reduced pro-oncogenic miR21 and an enhanced anti-oncogenic miR126 expression in GBM cells as well as a reduction in prohibitin protein upon CBD treatment.

autophagy is dependent on tribbles pseudo-kinase 3 inhibition of the Akt/mTORC1 which finally leads to reduction of tumour growth agreeing with the previous study.

Singer *et al.* (2015) demonstrated for the first time that CBD treatment can inhibit the stem cell key regulators (Id1, Sox2) in a ROS-dependent manner in GSCs. Evidence on the potent effects of ROS has been established when ROSlow phenotype was correlated with GSCs, a common characteristic that is essential for the conservation of their self-renewal capacity, indicating that ROS-p38 axis causes a powerful blockage effect on tumour growth (Sato *et al.*, 2014).

According to Torres *et al.* (2011) findings, T98G tumour cells were resistant to THC action, but when combined with CBD there was a strong reduction in tumour growth. Nevertheless, Solinas *et al.* (2013) demonstrated that CBD alone affects T98G cell's growth and invasion. Regarding the mechanism behind this effect, Soroceanu *et al.* (2013) were in agreement with the above study, that CBD down-regulated ERK and Akt, the main pathways for glioma cell survival and proliferation, as well as MMP-2 levels correlated with invasiveness.

Another crucial factor of these effects has been shown to be doses of cannabinoids. Scott *et al.* (2014) demonstrated that with higher concentrations of cannabinoids a reduction on pERK was seen, whereas at lower concentrations an increase was observed. Even though cannabinoids have many anticancer potentials, they are non- soluble in water, unstable and their oily-resin in nature causes a difficult development of

Table 12
Studies examining the combined efficacy of CBD, THC and radiotherapy.

Author, year and study design	*Aim of study*	*Cell culture and test organism characteristics*	*Concentration/exposure to CBD, THC, radiotherapy*	*Effects of cannabinoids on radiotherapy and on tumour growth*	*Outcome*
Scott *et al.* (2014) *In vitro* and *in vivo*	To evaluate the antiproliferative properties of THC, CBD and irradiation both in *in vitro* glioma setting and in a murine orthotopic glioma model and determine the potential clinical benefits	Human cancer cell lines (T98G and U87MG) Mouse glioma cell line GL261 (syngeneic to the C57BL/6 mouse) Female, 9 weeks of age C57BL/6 mice were injected with 150 000 GL261 cells	CBD and THC both in their pure form (> 96% purity) and as aBDS containing 60%-72% of the specific cannabinoid, with the remaining mass made up of CBG and CBC CBD and THC (10 μmol/l dose of both) were added for 4 hours before irradiation (< 10 Gy) to the 3 cell lines CBD, THC (2 mg/kg each in 100 μl) and irradiation (4 Gy) treatment MRI scans on days 9, 13, 16, 21	Dose-dependent reductions in cell numbers were observed in all the 3 cell lines T98G cell line was found to be more sensitive to treatments Irradiating cells showed an increase in γ-H2AX foci (marker of DNA-double strand breaks) Co-administered cannabinoids with irradiation, caused a bigger reduction in pAKT and pERK levels This combination caused a much slower tumour growth Final tumour sizes were undoubtedly smaller compared to the result of each treatment individually	A hyper-additive effect of CBD and THC was seen in reduction of cell numbers Autophagic activity was observed, with cleavage of caspase-3 occurrence when cannabinoids were administered before irradiation Cannabinoids delayed the recovery of double-strand breaks and DNA damage was prolonged in cells pre- treated with cannabinoids and then exposed to radiation Autophagy was evident when a cannabinoid was administered with irradiation in high concentrations A dramatic reduction was observed in *in vivo* tumour growth when cannabinoids were administered before irradiation
Conclusion: Cannabinoids and irradiation led to a slower tumour growth, reducing the tumour size. Powerful reductions in tumour volumes were observed when cannabinoids were combined with irradiation.					

a BDS -Botanical drug substance.

Table 13a
Studies experimenting the combined efficacy of CBD and THC.

Author, year and study design	*Aim of study*	*Cell culture and test organism characteristics*	*Concentration/exposure to CBD, THC*	*Effects of cannabinoids on tumour growth*	*Outcome*
Hernán Pérez de la Ossa *et al.* (2013) *In vivo*	To assess the efficacy of CBD- and THC-loaded aMPs as an alternative delivery system, with a controlled release of cannabinoids and their antitumor efficacy in a murine xenograft model of glioma	Microspheres were incubated to observe the release of cannabinoids U87MG human glioma cells were induced by a subcutaneous injection on the right flank of athymic nude mice	Incubated in PBS and kept in shaking incubator (37°C) At different time-intervals, supernatant was quantified for the release of cannabinoids in the medium 75 mg MPs (37.5 mg THC and 37.5 mg CBD) every 5 days for 22 days Another group of tumours treated every day with one peritumoral injection of combined THC and CBD solution (0.25 mg THC and o.25 mg CBD)	During a 20-day observation, 64% and 79% of total CBD and THC respectively was released Cannabinoid-loaded MPs had the same antitumor activity as cannabinoids in solution THC- and CBD MPs enhanced apoptosis, reduced tumour cell proliferation and decreased tumour blood vessel forming	THC- and CBD-MPs diminished tumour vascularization, increased apoptotic activity and reduced cancer cell proliferation Growth of glioma xenografts in tumour- bearing mice is reduced with a similar potency than a daily local administration of cannabinoids in solution An effective concentration of cannabinoids could be reached at the tumour site using less repetition of MPs administration
Conclusion: *in vivo* administration of cannabinoid-loaded MPs activated apoptosis and reduced the growth of tumour cells without letting THC affect brain regions responsible for psycho-activity.					

aMPs-microparticles

efficient production for their route of administration (Grotenhermen, 2003).

In addition, the sublingual Sativex spray contains both THC and CBD as well as ethanol and propylene glycol that act as solubilising agents but cause irritation to the site of administration. Taking into consideration all the above, cannabinoid-loaded MPS came into use as an alternative (Hernán Pérez de la Ossa *et al.*, 2012). Furthermore, by restricting local administration of cannabinoid-loaded MPs in the therapeutically relevant site only, and not to sites that are responsible for psycho- activity, the undesired side effects of THC are absent (Hernán Pérez de la Ossa *et al.*, 2013).

Positive results were reported by the company GW Pharmaceuticals in their orphan drug-designated study that involved 21 confirmed GBM patients. The oral administration (maximum of 12 sprays/day) which included both THC and CBD plus TMZ led to an 83% 1-year survival rate and median survival of over 662 days compared to the control group, which received only TMZ and had a 44% 1-year survival rate and a median survival of 369 days (Schultz and Beyer, 2017). In addition, the most common side effects noted during this study were dizziness, headache, nausea, vomiting and constipation (Schultz and Beyer, 2017).

Recently, Aparicio-Blanco *et al.* (2019) demonstrated that the dose requirements reported in a clinical trial that tested CBD as a possible therapy for GBM, have been met by the high load of CBD attained with LNCs. A great improvement in dosing regimens could be therefore achieved through the extended release profile of CBD detected through CBD-loaded LNCs. This would lead to a reduced number of

Table 13b
Studies experimenting the combined efficacy of CBD and THC.

Author, year and study design	*Aim of study*	*Cell culture and test organism characteristics*	*Concentration/exposure to CBD, THC*	*Effects of cannabinoids on tumour growth*	*Outcome*
Marcu *et al.* (2010) *In vitro*	To evaluate whether CBD can modulate THC's ability to stop glioblastoma cell growth and induce apoptosis	Human GBM cell lines (SF126, U251, U87)	IC_{50} values for THC in SF126, U251 and U87 cells were 2.5 μM, 3.3 μM, 3.3 μM, respectively IC_{50} values for CBD in SF126, U251, U87 were 1.2 μM, 0.6 μM, 0.6 μM, respectively THC and CBD (concentrations of 100 nM) to assess positive or negative interactions for invasiveness THC (1.7 μM): CBD (0.4 μM) ratio on induction of apoptosis	CBD caused a stronger inhibition of cell growth than THC, in all three cell lines CBD and THC when used alone inhibited U251 cell invasiveness, but activity of THC was not enhanced by CBD when combined Combination of cannabinoids treatment in U251 and SF126 cells caused a significant down-regulation of apERK When combined CBD and THC are administered, significant apoptosis is observed Combination of treatment produced a considerable increase in formation of bROS Up-regulation of p8 expression was observed upon combination treatment, as well as an up-regulation of caspase 3, 7, 9 activities leading to apoptosis	THC and CBD caused an inhibition on the growth of glioblastoma lines, with CBD causing a stronger inhibition Inhibitory effects of THC on glioblastoma cell growth are enhanced upon CBD treatment, producing a greater inhibition on cell growth U251 cells experienced a substantial down- regulation of ERK activity upon combination treatment Cell viability was also reduced through induction of apoptosis Combination of CBD and THC caused apoptosis through the increased production of ROS and oxidative stress

Conclusion: CBD enhanced the anticancer activity of THC, up-regulated the activity of various pro-apoptotic proteins causing the obstruction of cell proliferation and induction of cycle arrest and apoptosis.

aERK- extracellular receptor kinase; bROS- Reactive oxygen species

administrations required (as noted in the above clinical trial, up to 12 times/day). Also, the CBD-decorated LNCs can be encapsulated with other antitumor agents, like TMZ, which can lead to a more potent antitumor effect against GBM.

Although none of the studies included here have tested it, it seems that a common route of administration is by inhaling cannabinoids, which have many obvious clinical drawbacks (Dryburgh *et al.*, 2018). However, with similar pharmacokinetics to intravenous administration, inhaled administration's bioavailability ranges from 10%- 35% (Lucas *et al.*, 2018). Oral administration has a poor bioavailability owing to its high lipophilicity, constituting a challenge for further researches (Grotenhermen, 2003).

Conclusion

After the evaluation of the included studies it was apparent that cannabinoids can enhance the activity of radiotherapy, the alkylating agent TMZ and cause apoptotic cell death on tumour cells, leading to regression of cancer. However, further in-depth determination of the exact dosages and exposures should be conducted as it was shown that anticancer activities are dose-dependent. In addition, when triple combinations were used CBD, THC and TMZ or CBD, THC and radiotherapy significant reductions were observed in the viability of the cells as well as increases in apoptotic activity suggesting that cannabinoids should be therapeutically utilized for the tackling of GBM. As it is now evident through the few clinical trials that have been completed, cannabinoids have displayed a fair safety profile without any reported prolonged narcotic effects. A few of the reported side effects include headache, bulimia, euphoria, nausea and vomiting, permitting and encouraging future clinical trials to be performed. While the treatment administration through CBD-decorated and loaded LNCs have been managed in satisfactory dose regimens, future studies should explore its usage further, as it greatly decreases the number of administrations. Furthermore, future clinical trials are essential to evaluate the exact effect of cannabinoids on humans, whilst taking the bioavailability of cannabinoids in the body into consideration also.

Declaration

All data were generated in-house, and no paper mill was used. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy.

Authors contributions

I.K, N.Y and E.P conceived the study; I.K. performed and designed the methodology, investigation, analysis and wrote the paper with contribution from N.Y and E. P; N.Y. and E.P. contributed to conceptualization, writing, editing, reviewing and supervision.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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