Phytocannabinoids for Cancer Therapeutics: Recent Updates and Future Prospects

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Abstract: Phytocannabinoids (pCBs) are lipid-soluble phytochemicals present in the plant, *Cannabis sativa* L. and non-cannabis plants which have a long history in recreation and traditional medicine. The plant and the constituents isolated were central in the discovery of the endocannabinoid system (ECS), the most new target for drug discovery. The ECS includes two G-protein-coupled receptors; the cannabinoid receptors-1 and -2 (CB1 and CB2) for marijuana's psychoactive principle Δ^9-tetrahydrocannabinol (Δ^9-THC), their endogenous small lipid ligands; namely anandamide (AEA) and 2-arachidonoylglycerol (2-AG), also known as endocannabinoids and the enzymes for endocannabinoid biosynthesis and degradation such as fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL). The ECS has been suggested as a pro-homeostatic and pleiotropic signaling system activated in a time- and tissue-specific way during pathological conditions including cancer. Targeting the CB1 receptors becomes a concern because of adverse psychotropic reactions. Hence, targeting the CB2 receptors or the endocannabinoid metabolizing enzymes by pCBs obtained from plants lacking psychotropic adverse reactions has garnered interest in drug discovery. These pCBs derived from plants appear safe and effective with a wider access and availability. In the recent years, several pCBs derived other than non-cannabinoid plants have been reported to bind to and functionally interact with cannabinoid receptors and appear promising candidate for drug development including cancer therapeutics. Several of them also targets the endocannabinoid metabolizing enzymes that control endocannabinoid levels. In this article, we summarize and critically discuss the updates and future prospects of the pCBs as novel and promising candidates for cancer therapeutics.

Keywords: Anticancer, endocannabinoid system, antitumor, cannabis, cannabidiol, tetrahydrocannabinol, phytocannabinoids.

1. INTRODUCTION

The cannabis plant popularly known as Marijuana has been known for its medicinal properties since antiquity. The euphoric and psychoactive effects of the cannabis plant along with its irrational use had halted the pharmaceutical development of potential leads from this ancient plant. Recently, the cannabinoids have drawn the attention of the scientific community and currently the interest in diverse therapeutic effects of cannabinoids have been renewed [1, 2]. The potential of cannabinoids to affect the cell survival and death decision pathways is the emerging and exciting area of their therapeutic utility [1, 3, 4]. Currently, the cannabinoids have successfully demonstrated their utility in the management of these side effects in patients undergoing cancer chemotherapy [2, 5, 6]. The role of plant cannabinoids also known as phytocannabinoids (pCBs) is conferred in cancer prevention and treatment [1, 10]. The pCBs like Δ^9-Tetrahydrocannabinol (Δ^9-THC), cannabidiol (CBD), cannabigerol (CBG), cannabichromene (CBC), cannabinol (CBN), Δ^9-tetrahydrocannabinolic acid (Δ^9-THCA), Δ^9-tetrahydrocan-

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nabivarin (Δ^9-THCV), cannabidivarin (CBDV) and cannabidiolic acid (CBDA) have demonstrated anti-proliferative and pro-apoptotic effects in variety of tumor cell lines including breast carcinoma, prostate carcinoma, gastric adenocarcinoma, lung carcinoma, colon carcinoma, C6 rat glioma, rat basophilic leukemia and transformed thyroid cells [1, 2, 7-11] (see Fig. (**1**). for structures of pCBs).

The accumulating evidences suggest potential benefits of cannabinoids as anticancer agents. In the light of currently available literature on pCBs, many mechanisms of action of pCBs for anticancer activity have been identified including the anti-proliferation, induction of apoptosis, inhibition of angiogenesis, cell cycle arrest, differentiation and autophagy or the combinations of these mechanisms [2, 14]. Δ^9-THC is the main psychoactive phytocannabinoid isolated from *cannabis sativa* and is widely studied for its therapeutic potential in various disease conditions. Although, Δ^9-THC and cannabis have the fascinating biological activities, its use in therapeutics is limited due to socioeconomic, medicolegal, regulatory and safety concerns in most of the countries [15]. This led an impetus to discover the Δ^9-THC like compounds having no or minimal psychoactivity. It has been resulted into the identification of safer compounds like CBD, CBG, CBC, Δ^9-THCV, CBDV, Δ^9-THCA and CBDA. Among these compounds, CBD and CBG appear devoid of psychotropic properties, hence more attention has been focused on the safe compound, CBD. The pharmacological properties of CBD make it more attractive molecule for the drug discovery. Δ^9-THC and CBD have interesting biological activities in cancer, diabetes, inflammation and central nervous system (CNS) diseases [2, 7, 14, 16]. The various milestones achieved in the pCBs and cannabis drug research are summarized in Table **1**.

1.1. Phytocannabinoids and Cannabinoid Receptors

Plants containing secondary metabolites like cannabinoids usually belong to similar chemotaxa and exert potent pharmacological effects. The *Cannabis sativa* and its products are the subject of research interest due to their recreational, medicinal and toxicological properties [12, 15]. Beside its medicinal and recreational uses, Cannabis has been reported for thousands of years for its fiber and seeds. Cannabis produces a durable fiber used for the manufacturing of rope and fabrics. The seeds of cannabis are rich in unsaturated fatty acids [17, 18]. Cannabis has a long history as a

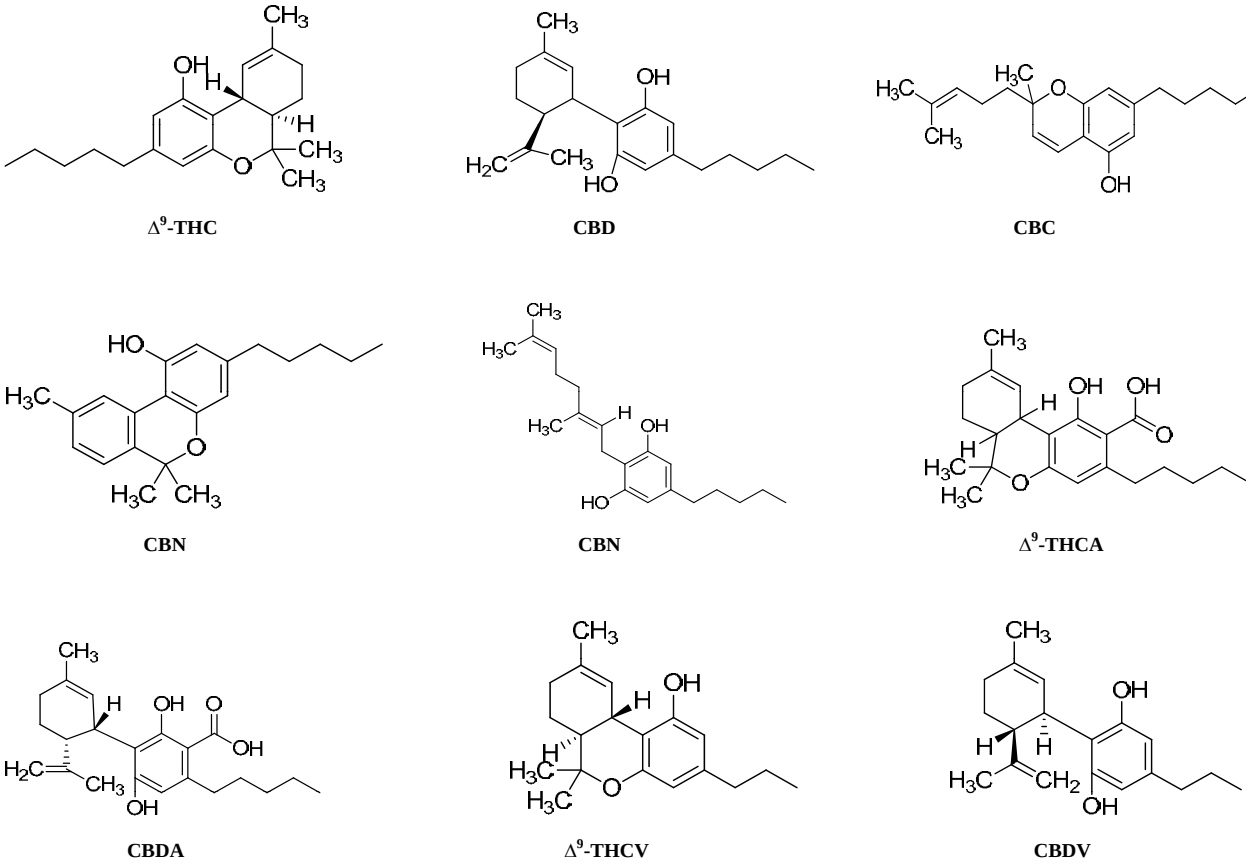

Fig. (1). Chemical structures of principal phytocannabinoids from cannabis plant.

Table 1. Milestones in phytocannabinoid and cannabis research.

Year	Milestone Discovery	References
~60 A.D.	Documentation of medicinal uses of cannabis by Dioscorides and Galen in their writings	[13]
1788	Existence of Cannabis in New England Dispensatory containing major elements of Dioscorides herbal pharmacopoeia	[13]
1840	Introduction of medicinal use of cannabis in the united kingdom (UK)	[13, 169]
1896	The designation of novel cannabinoid from cannabis as a cannabinol (CBN)	[7, 22, 170]
1932	Exclusion of cannabis from the British Pharmacopoeia owing to its psychoactive side effects	[13, 171-173]
1940	Isolation of cannabidiol (CBD) and elucidation of CBN structure by Adams and colleagues	[7, 22, 174]
1942	Isolation of Δ^9-THC from *cannabis sativa* resin by Wollner and group	[22, 175]
1955	Discovery of the first cannabinoid acid, cannabidiolic acid (CBDA), by Krejci and Santavy	[7, 176]
1963	Structural elucidation and determination of CBD stereochemistry by Mechoulam and Shvo	[7, 22, 177]
1964	Isolation and assignment of appropriate structure of Δ^9-THC along with identification of non-psychotropic cannabinoid, cannabigerol (CBG) by Gaoni and Mechoulam	[7, 22, 178, 179]
1965	First attempt to extract Δ^9 –THCA by Korte and coworkers	[7]
1966	Another non-psychotropic cannabinoid, Cannabichromene (CBC), was independently reported by Claussen and coworkers and Gaoni and Mechoulam	[7, 180, 181]
1967	Isolation of Δ^9 –THCA in its pure form by Nishioka and coworkers	[7]
1969	Isolation of cannabidivarin (CBDV) from cannabis by Vollner and coworkers	[7, 182]
1969	Isolation of Δ^9 –THCA by Mechoulam and coworkers	[7, 183]
1970	Detection of Δ^9 - THCVA by Edward Gil and colleagues	[7]
1971	Isolation of Δ^9-THC The isolation and structural elucidation of Δ^9 –THC along with other neutral cannabinoids from cannabis	[13, 184]
1975	First report on the cannabinoid's ability to reduce tumor growth and viability of lung cancer cells established through *in vitro* and *in vivo* experiments by Munson and colleagues	[185, 186]
1975	CBGA was shown to be the first biogenic cannabinoid formed in the plant	[22, 187]
1980	Development of synthetic cannabinoid receptor ligands	[13, 31]
1990	Identification of specific Δ^9-THC binding sites in the human CNS	[31]
1990	Identification and cloning of the first cannabinoid receptor, CB1	[32]
1993	Identification of another cannabinoid receptor, CB2	[33]
1995	Discovery of the endocannabinoid (eCB) system (ECS)	[188]
2005	Development of sativex, which is an approximately 1:1 mixture of Δ^9-THC: CBD	[189]
2005	Sativex as the first drug derived from cannabis plant extracts licensed in various countries for use in multiple sclerosis	[190, 191]
2006	Phase I clinical study to assess the safety and efficacy of Δ^9-THC in the patients with refractory glioblastoma multiforme	[163]
2010	Δ^9-tetrahydrocannabivarin was identified as a CB2 receptor partial agonist which is also a CB1 receptor antagonist	[19]
2011	Launch of Sativex in Denmark and Germany	[164]
2013 to 2015	A clinical trial of Sativex in combination with dose-intense Temozolomide in patients with recurrent glioblastoma is underway sponsored by GW Research Ltd.	[164]

medicinal plant and has laid down the foundation of endocannabinoid system (ECS) [19]. The herb *Cannabis sativa* produces over 421 chemical compounds that contains about 100 terpenophenolic compounds called as pCBs [7, 13]. Cannabinoids contains alkylresorcinol and monoterpene moieties in their structures [20]. Originally, the term cannabinoids is restricted to a group of substances found only in cannabis plant. But, now it is extended to include the substances which are specifically recognized by the cannabinoid systems [21]. Primarily, the Δ^9-THC and similar naturally occurring derivatives are the only plant products known to have direct interaction with cannabinoid receptors. Also, several non-cannabinoid plant products have been reported to act as cannabinoid receptor ligands. Therefore, Gertsch *et al.* [19] defined the term pCBs as any plant-derived natural product capable of either directly interacting with cannabinoid receptors or sharing chemical similarity with cannabinoids or both. The compounds with high binding affinities with cannabinoid receptors and acting as either agonists, antagonists or inverse agonists are referred as direct cannabinoid receptor ligands. The compounds which either interact with ECS proteins or act on allosteric cannabinoid receptor sites are called as indirect cannabinoid receptor ligands [19].

The identification of endogenous cannabinoid receptor ligands called endocannabinoids in the mammalian tissue leads to the establishment of term "Phytocannabinoids" so as to differentiate them from endocannabinoids [20]. Broadly the cannabinoids are classified into the three major classes viz. phytocannabinoids (plant derived), endogenous cannabinoids (endocannabinoids) and synthetic cannabinoids (synthocannabinoids) [21]. Cannabis contains several chemical constituents among which are the pCBs. The pCBs are sub-classified into various types like CBG type, CBC type, CBD type, Δ^9-THC type, Δ^8-THC type, Cannabicyclol (CBL) type, Cannabielsoin (CBE) type, CBN and Cannabinodiol (CBND), cannabitriol (CBT) type and other miscellaneous types [21, 22]. The pCBs can be administered by various routes including oral, sublingual, intravenous, rectal, inhalation or smoking. The transdermal delivery of Δ^9-THC has been successfully proved for therapeutic benefits [21, 23].

The pCBs exerts their actions in mammalian tissues through two main types of receptors called as cannabinoid receptors-1 (CB1) and cannabinoid receptors-2 (CB2). The CB1 receptors are mainly found in the CNS, with low to moderate expression in periphery [21, 24-26]. Cannabinoid receptors are responsible for different pharmacological properties when activated by ligands such as an agonist, antagonist, or inverse agonist. The stimulation of CB1 receptors by agonists is associated with unwanted side effects mainly psychoactivity, whereas the stimulation of CB1 inverse agonists led to loss of appetite, which in useful in the treatment of obesity [17, 27]. Therefore, CB1 receptors are abundant metabotropic receptors involved in multiple physiological and behavioral events whereas CB2 receptors probably participate in the immunosuppressive and antinociceptive effects of cannabinoids [21, 24, 25]. The CB2 receptors are highly localized on the number of immune cells such as splenocytes, macrophages, monocytes, microglia and B and T-cells, with much lower and more restricted distribution in the CNS [1, 26, 28]. Both receptor subtypes contain seven transmembrane spanning domains and coupled with Gi/o proteins, negatively to adenylyl cyclase (AC) and in positive way to the mitogen activated protein kinases (MAPKs) [21, 26, 29, 30]. The development of synthetic cannabinoid (CB) receptor ligands resulted into the identification of specific Δ^9-THC binding sites in the human CNS [31]. Later, first CB1 receptor was identified and cloned in 1990 by Mastusda [13, 32]. These studies led to emergence of term ECS system; which encompasses CB receptors, endocannabinoids and proteins involved in their synthesis and breakdown. Afterwards, the peripheral, cannabinoid CB2 receptor was identified in 1993 [13, 33]. The pCBs , Δ^9-THC and CBD are the main constituents of cannabis with varying degrees of affinities for cannabinoid receptors. The Δ^9-THC is an agonist for both CB1 and CB2 cannabinoid receptors, whereas CBD and THCV modulate the effects of Δ^9-THC via direct blockade of CB1 receptors, and acts as first generation CB1 inverse agonists, such as rimonabant [21, 28]. Next to their regulatory role in human physiology, cannabinoid receptors (CB1 and CB2) are also associated with the proliferation, motility, invasion, adhesion and apoptosis of cancer cells. The CB1 and CB2 receptor activation are linked with various key events in cancer induction and progression such as modulation of adenyl cyclase and cyclic AMP levels, affecting ion channels, regulation of MAPKs, extracellular signal regulated kinase 1 and 2 (ERK1/2), p38 and c-Jun N terminal kinase (JNK) [10, 34, 35].

Following the discovery of cannabinoid receptors, several studies suggested the involvement of additional receptors beyond CB1 and CB2 through which cannabinoid agonists execute their actions. It suggests the possibility that more isoforms of cannabinoid receptors (still to be identified) can exist. The possible candidates

for these orphan G-protein-coupled receptors include GPR119, GPR55, and GPR18. Among these three receptors, it is strongly recommended to rename the GPR55 as cannabinoid receptor, although further studies are expected to approve this designation for GPR55. Another putative cannabinoid receptor includes the transient receptor potential cation channel subfamily V member 1 (TRPV1) receptor. The experimental evidences established that the cannabinoids have ability to modulate this ion channel. International union of basic and clinical pharmacology is engaged in the search of additional evidences before designating TRPV1 as a putative ionotropic cannabinoid CB3 receptor [36, 37]. All these established and proposed cannabinoid receptors (CB1, CB2, TRPV1 and GPR55) are found to be overexpressed in various cancer cells (see Table **2**).

1.2. Phytocannabinoid Biosynthesis

The pCBs are chemically categorized as either cannabinoid acids or neutral. The compounds having carboxylic group are cannabinoid acids while those not having carboxylic acid are called as neutral cannabinoids. In the cannabis plants, cannabinoids are biosynthesized and accumulated as cannabinoid acids and nonenzymatic decarboxylation of these acids yields the neutral cannabinoids [20]. The pCBs are present in the resinous secretion obtained from the trichomes of female *cannabis sativa* plant. The pCBs are synthesized within the plant from phenolic precursor through the agency of various enzymes like synthase, transferase and oxidation reactions [13].

The biosynthetic pathway of major pCBs is as represented in (Fig. **1**). The cannabigerolic acid (CBGA), is the alkylation product of olivetolic acid and geranylpyrophosphate by the enzyme geranylpyrophosphate: Geranyl olivatolate transferase (GOT). CBGA is a common precursor for the biosynthesis of three major cannabinoid acids namely THCA, CBDA and CBCA. This biosynthesis is catalyzed by oxidoreductase enzymes like THCA synthase, CBDA synthase and CBCA synthase [20]. The THCA is the most abundant cannabinoid produced in most of the plants and is precursor of Δ^9-THC. The THC and CBD are C21 terpenophenols with pentyl alkyl tails, whereas THCV is a C19 propyl-tailed analog of THC. Primarily, these compounds are biosynthesized as carboxylic acid which upon heating, drying or exposure to light undergo decarboxylation and produces the neutral pCBs [28]. The THCA upon heating or exposure to UV light is responsible for the biosynthesis of major compound Δ^9-THC and produces the Δ^8-THC in negligible quantity. The tetrahydrocannabinol-C4 (THC-C4) and THCV are short chain versions of THCA containing four and three carbon chains, respectively. On the other hand, oxidation of THCA produces the CBNA and subsequent heating or UV exposure produces CBN. Similarly, CBDA leads to production of CBD, Whereas, CBCA produces CBC (see Fig. **2**).

1.3. Phytocannabinoids

1.3.1. Δ^9-Tetrahydrocannabinol (Δ^9-THC; THC)

Marijuana contains more than 100 compounds which are unique to the plant and are known as pCBs [38]. Δ^9-THC, CBD and CBN are the typical representatives of phytocannabinoid group [39]. Among these compounds, the two pCBs Δ^9-THC and CBD are the most studied for their medicinal properties [40]. Δ^9-THC is the chief psychoactive component present in cannabis, whereas CBD is the major non-psychoactive component of cannabis [41]. The consumers of marijuana experienced the subjective effects like euphoria which is due to Δ^9-THC content of cannabis. This property of Δ^9-THC leads to its label as a psychoactive agent and is used as a reference standard for estimating the psychotropic power of various cannabinoid preparations [38-40]. Δ^9-THC activates the endocannabinoid receptors and acts through CB1 and CB2 receptors. Δ^9-THC also has ability to modulate the endocannabinoids like anandamide (AEA) and 2-arachidonoylglycerol (2-AG) [41].

Δ^9-THC is available into various pharmaceutical forms, which includes a synthetic THC, Dronabiol (Marinol®) and a synthetic THC mimetic, Nabilone (Cesamet®) are the FDA-approved cannabinoids. The oro-mucosal spray comprising of Δ^9-THC and CBD extract, Nabiximols (Sativex) is approved in Canada and United Kingdom whereas the clinical testing was proceeding in United States and Europe [42]. The natural and synthetic cannabinoids are commonly administered through the oral route and as an inhalation. The other developed route for administrations are sublingual, intravenous, transdermal, ophthalmic, intrathecal and rectal [42].

Ligresti *et al.* [5] reported the anti-proliferative activity of Δ^9-THC in MDA-MB-231 cells with IC_{50} value of 24 μM [5]. Whereas, another study by Caffarel *et al.* [43] stated the similar effect of Δ^9-THC in MDA-MB-231 cells at lower concentration with IC_{50} value of 5 μM. The alterations in IC_{50} of Δ^9-THC are assigned to differences in purity of the compounds or culture conditions [11]. The same study, reported the anti-proliferative activity of CBD with IC_{50} value of 11 μM

Table 2. Expression of cannabinoid receptors in various cancer cell lines.

Cell line/ Type of Cancer	Receptor Expression	References
MCF-7, T-47D, MDA-MB-231, TSA-E1, MDA-MB-468 (Breast cancer cells)	CB1	[5, 10, 43, 47, 79, 192, 193, 194]
MCF-7, T-47D, MDA-MB-231,MDA-MB-468, EVSA-T, SkBr3 (Breast cancer cells)	CB2	[5, 10, 43, 47, 48, 79, 192]
MDA-MB-231 (High expression) and MCF-7 (Low expression) (Breast cancer cells)	GPR55	[10, 195]
PC-3, DU-145, LNcap, CWR22RV1, CA-HPV-10 (Prostate cancer cells)	CB1, CB2	[10, 44, 73, 79, 88, 91, 92, 196, 197, 198]
PC-3, DU-145 (Prostate cancer cells)	GPR55	[10, 199]
Non small cell lung cancer (NSCLC) cells	CB1, CB2	[200]
MG-63 (Osteogenic human osteosarcoma cell line)	CB2	[201]
B16, A 375 (Melanoma cell line)	CB1, CB2	[93, 202]
Jurkat (Human T lymphocyte cell line)	CB2	[203]
C6.9 glioma cells	CB1, CB2	[204, 205]
HL-60 (Human promyelocytic leukemia cells)	CB2	[206]
U373MG, GL-5 (Human glioblastoma astrocytoma cells)	CB1	[207]
Panc1, MiaPca2; Capan2, BxPc3 (Human pancreatic tumor cell lines)	CB1, CB2	[83]
Gos3, U87, A172, SW1783, U118, T98 U373, SW1088, CCF, LN405 (Human glioma cell lines)	CB1, CB2	[208]
Human hepatocellular carcinoma (HLC)	CB1, CB2	[209]
Human glioblastoma multiform tumors	CB1, CB2	[163]
Mantle cell lymphoma (MCL)	CB1, CB2	[210]
Human Kidney-2 (HK2) Cells	CB1, CB2, TRPV1, GPR55	[211]

in MDA-MB-231 cells [5]. Δ^9-THC was reported to inhibit the cell proliferation and induced the apoptosis in ER-negative breast cancer cells. The effects of Δ^9-THC are ascribed to blocking of transition from one phase of cell cycle to another, especially the G2-M [11, 43]. The Cdc2 is major cyclin dependent kinase which controls the entry of cells in mitosis [43, 44]. The down regulation of Cdc2 leads to cell cycle arrest and subsequent decrease in proliferation and apoptosis [5, 11, 44]. Another mechanism behind apoptosis induction by Δ^9-THC is the activation of the transcription factor JunD to accomplish the action towards apoptosis in ER-negative breast cancer cells [10, 43, 45, 46].

In animal model of mouse breast cancer, McKallip *et al.* [47] observed an increase in the local tumor size along with increased number and size of metastasis after 21 days systemic administration of Δ^9-THC at the dose of 25 mg/kg, i.p. in xenotransplant model of 4T1 paw cells in BALB/c mice [7, 44, 48]. The variable results were obtained, when same study was replicated in SCID-NOD mice which are devoid of immune response. McKallip *et al.* [47] observed no effect of Δ^9-THC on tumor volume alteration as compared to control animals treated with vehicle. The effect of Δ^9-THC was observed after intraperitoneal administration at the dose of 25 mg/kg for 19 days [11]. The authors reported that Δ^9-THC enhanced the growth of 4T1-derived xenografts and metastases by the inhibition of the anti-tumor immune response mediated through CB2 receptors [47, 48]. In contrast, a study by Preet *et al.* [49] established that a 21 days treatment with Δ^9-THC (5 mg/kg) reduced non-small cell lung cancer tumor weight and volume in SCID mice compared to vehicle control. Similarly, a study by Caffarel *et al.* [50] reported that Δ^9-THC (0.5 mg/animal/day, peritumorally) treatment for 90 days resulted decrease in size of mammary tumors, occurrence of new spontaneous tu-

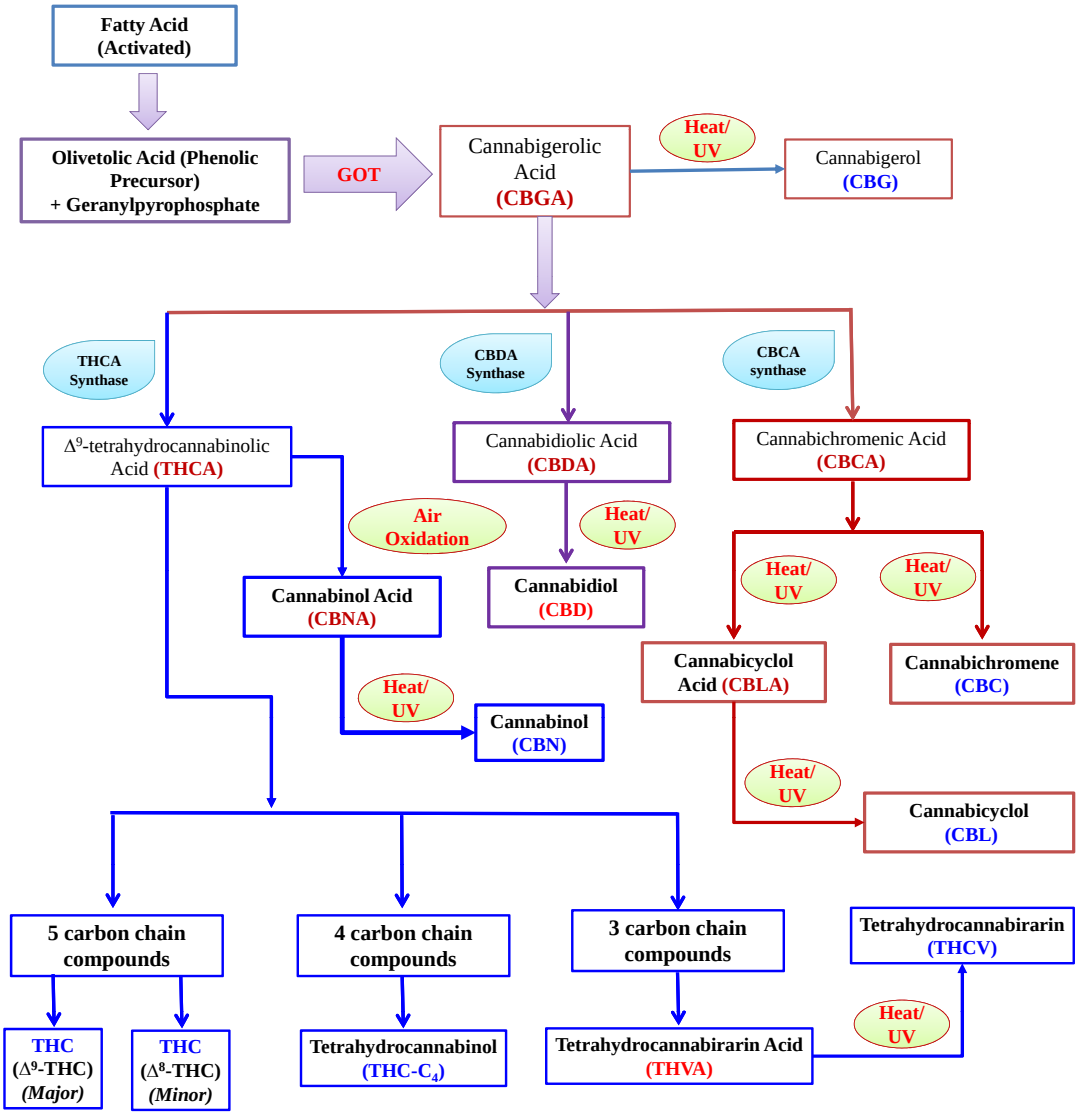

Fig. (2). Biosynthesis of phytocannabinoids.

mors and the degree of metastasis to the lungs in MMTV-neu mice that showed spontaneous development of mammary tumors [37, 50]. An MMTV-neu mouse is well established and clinically relevant model of HER2-positive metastatic breast cancer [48, 51]. Thus, the doses of Δ^9-THC used by McKallip *et al.* were comparatively higher than those that have anti-proliferative effects in other *in vivo* models of cancer. This observation prompted Alexander *et al.* [11] to propose that the stimulant effect of Δ^9-THC on tumor progression is not a true indication of the appropriate anticancer effects of cannabinoids [11]. However, convincing evidences are available to demonstrate the anti-tumor actions of cannabinoids in various types of cancer; few studies demonstrated the pro-tumor effects of cannabinoids [48]. Δ^9-THC is reported to have stimulatory effects on proliferation of MCF-7 cells *in vitro* [52, 53]. The cannabinoid receptors were reported to be expressed at very low or undetectable concentrations in the cells demonstrating pro-tumor effects of cannabinoids [47, 52, 53]. The reverse-transcription polymerase chain reaction detected very low levels of CB1 expression in MCF-7 and MDA-MB-231 breast cancer cell lines, whereas CB2 expression was so lower that it escapes the detection [37, 47]. The failure of Δ^9-THC to demonstrate effect on CB1 receptors in EVSA-T cells could be due to lack of CB1 receptors in this cell line [44]. Thus, caffarel *et al.* [48] suggested the need of extensive studies in breast cancer cell lines to explore the correlation between the lack of CB1/CB2 receptor expression and resistance to cannabinoid anti-tumor action.

Several studies highlighted the involvement of the immune system behind the anticancer activity of the

pCBs. Zhu *et al.* [54] described the CB2 mediated increase in the growth of xenografted lung tumors. On the contrary, it has been established that cannabinoids can inhibit the melanoma xenografts growth in immune-competent mice more effectively as compared to those in the immune-deficient mice [48, 55]. The human epithelial growth factor receptor type 2 (HER2) overexpressing tumors when injected either in immune-deficient mice or in immune-competent syngenic FVB mice and treated with Δ^9-THC, resulted in the significant reduction of tumor growth. This effect is attributed to the inhibition of the serine/threonine protein kinase (Akt), extracellular-signal-regulated kinase (ERK) and C-X-C chemokine receptor type 4 (CXCR4) [50, 56]. Additionally, Δ^9-THC decreased the incidence of tumors and enhanced overall animal survival on prolonged treatment in immune-competent rats [57].

The serine/threonine protein kinase Akt, commonly referred as protein kinase B (PKB) plays a critical role in the regulation of basic cell function and cell proliferation. Thus, it's an important mediator of cell survival in mammalian cells and involved in apoptosis [58, 59]. The inactivation of B-cell lymphoma 2 (Bcl-2)-associated death promoter (BAD), caspase-9 and forkhead transcription factors along with degradation of IκB and subsequent stimulation of nuclear factor kappa beta (NF-κB) are the important events in PKB mediated anti-apoptotic activity. The lipid products of phosphoinositide 3-kinase (PI3K) are responsible for mediating the major pathway of PKB activation [58]. PI3K is a protein kinase family member involved in regulatory functions like cell growth, cell survival and malignant transformations. Akt is one of the main downstream effectors of PI3K and its stimulation is linked to its phosphorylation at Thr308 and Ser473 [60, 61].

The signaling through PI3K/Akt is modulated by cannabinoid receptor activation [11]. A study by Sanchez and co-workers [60] demonstrated the involvement of the PI3K/PKB pathway in the mechanism of action of cannabinoids in human prostate epithelial PC-3 cells. The treatment of PC-3 cells with Δ^9-THC and R-(+)-methanandamide (MET), resulted in the enhanced phosphorylation of PKB in Thr308 and Ser473 and activation of PI3K/PKB pathway. The inhibition of Δ^9-THC affects this pathway by CB1 receptor antagonist (SR141716), CB2 receptor antagonist (SR144528) and by the PI3K inhibitor (LY294002) demonstrated the involvement of both CB1 and CB2 receptors behind the anticancer activity of Δ^9-THC in prostate cancer [60]. Similarly, Gomez *et al.* [58] reported the stimulation of Akt activity by phytoconstituents, Δ^9-THC along with endocannabinoid, AEA and synthetic cannabinoid, CP55,940 in chinese hamster ovary (CHO) cells. Although, the observed effects of Δ^9-THC were concentration and time dependent, these effects are selectively blocked by SR141716 (selective CB1 receptor antagonist), pertussis toxin and wortmannin denoting the involvement of CB1 receptors and PI3K [58].

In contrast, few studies also reported the down regulation of PI3K, protein kinase B (Akt), and ERK signaling pathways along with activation of pro-apoptotic BAD protein and induction of apoptosis by the cannabinoids [59]. The authors proposed that the inhibition of prosurvival pathways, including PI3K/Akt may underlie the antitumor actions of pCBs [59]. In agreement with this, Greenhough *et al.* [62] reported that Δ^9-THC treatment at micromolar concentration resulted in CB1 mediated inhibition of PI3K-Akt and mitogen-activated protein kinase/ERK (MAPK/ERK) survival signaling cascades which are frequently deregulated in colorectal tumors. The inhibition of ERK and Akt activity by Δ^9-THC was accompanied by activation of the pro-apoptotic B-cell lymphoma 2 (Bcl-2) family member BAD. These data suggests an important role for CB1 receptors and BAD in the regulation of Δ^9-THC induced apoptosis in colorectal cancer cells. Several studies suggested that inhibition of survival kinase cascades targeting Bcl-2 family protein BAD, Akt and Erk1/2 may result in the onset of apoptosis due to formation of complex between Bcl-2 family cell death suppressors and pro-apoptotic protein Bad [59].

Sanchez *et al.* [60] have observed dual effect of Δ^9-THC in prostate cancer PC-3 cells. Δ^9-THC at the nanomolar concentration stimulated nerve growth factor (NGF) production, whereas increase in concentrations at micromolar quantities causes inhibition of NGF and induction of cellular apoptosis. This induction of NGF synthesis by Δ^9-THC was found to be dependent on PI3K and p44/42 MAP kinase activation and is responsible for the regulation of prostate cell growth [60]. These reports on effects (activation or inhibition) of pCBs on PI3K/Akt pathway are divergent, more detailed studies are necessary to confirm the exact mechanisms by which pCBs modify the PI3K/Akt pathway for their anticancer activity.

The cannabinoids showed their ability to inhibit tissue invasiveness at least in part through the modulation of tissue inhibitors of matrix metalloproteinases (TIMPs). The TIMP expression is known to be a potent suppressor of tumor growth and angiogenesis. Whereas, decreased TIMP expression is highly corre-

lated with cancer invasiveness [37, 63, 64]. The human cervical cancer (HeLa) cells when treated with Δ^9-THC at strikingly low concentration of 0.01 μM caused a time- and concentration-dependent suppression of HeLa cell invasion which is accompanied by increased expression of TIMP-1. The effect of Δ^9-THC on stimulation of TIMP-1 expression and suppression of cell invasion were reversed by pretreatment of cells with antagonists to CB1 or CB2 receptors, with inhibitors of MAPKs indicating involvement of cannabinoid receptors in Δ^9-THC action [65]. Recently, same group of authors reported the up-regulation of intracellular adhesion molecule-1 (ICAM-1) and TIMP-1 by another phytocannabinoid, CBD (0.001-3 μM) in the lung cancer cell lines A549, H358, and H460 [66]. On the contrary, Blazquez *et al.* [67] reported that the local administration of Δ^9-THC down-regulated TIMP-1 expression in mice bearing subcutaneous gliomas. Furthermore, Δ^9-THC was observed to depressed TIMP-1 expression in cultures of various human glioma cell lines (C6.9 and C6.4) as well as in primary tumor cells obtained from a glioblastoma multiforme patient [37]. In the light of current literature, it is observed that the effect of cannabinoids on TIMP expression levels is controversial. However, several studies have documented an increase in TIMP-1 levels by cannabinoid administration, the effects of cannabinoids on invasion seems to be cancer and cannabinoid-specific [37, 66]. Recent study by Ramer *et al.* [68] suggested a role of the anti-angiogenic factor TIMP-1 in intercellular tumor-endothelial cell communication responsible for anti-angiogenic features of endothelial cells. The treatment of conditioned media (CM) from A549 lung cancer cells, either with Δ^9-THC or CBD decreased the migration along with tube and sprout formation of HUVEC cells [68].

The process of new blood vessel formation called angiogenesis has a role in the development and progression of pathogenic processes including tumor growth [69, 70]. Many studies demonstrated the ability of cannabinoids to modulate the response of cells to various growth factors particularly, vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) which could be the reason for their anti-angiogenic property [3, 45, 60, 69, 71-74]. Various cannabinoids including Δ^9-THC having ability to bind with cannabinoid receptors (CB1 and/or CB2) inhibit vascular endothelial cell survival and migration [69, 75].

The direct effect of cannabinoids on endothelial cells and inhibition of pro-angiogenic regulator including vascular endothelial growth factor (VEGF) induces dual attack on the tumor blood vessel development [37, 75]. Blázquez *et al.* [75] demonstrated the effect of various cannabinoids including Δ^9-THC on the level of major vascularization factor VEGF [37]. The cannabinoids appeared to have consistent effects on the vascularization pathway, causing *in vivo* decrease in tumor vascularization. The endothelial cell lines, human umbilical vein endothelial cells (HUVEC) and ECV304 showed direct susceptibility to various cannabinoids including Δ^9-THC (1 μM). Moreover, intratumoral administration of the cannabinoid, Δ^9-THC to two patients with glioblastoma multiforme (grade IV astrocytoma) decreased VEGF levels and VEGFR-2 activation in the tumors [75]. Preet *et al.* [49] reported the decreased VEGF levels after Δ^9-THC treatment in A549 and SW1573 lung cancer cell lines. This finding is correlated with decreased vascularization of xenotransplanted A549 tumors in immune deficient mice [37]. Δ^9-THC decreased tumor size, number of tumor and lung metastases along with inhibition of cell proliferation and angiogenesis in an engineered animal model of ErbB2 (tyrosine kinase receptor)-driven metastatic breast cancer. This inhibition of cell proliferation was observed to mediated by CB2 receptors [44, 50]. Furthermore, Δ^9-THC leads to impaired angiogenesis as revealed by decrease in number of tumor blood vessels [48].

The tobacco smoking and alcohol consumption are the main triggers for the development of oral cancer [10]. Although, chemotherapy, radiation therapy and surgery are the mainstay of cancer treatment, more safer and effective compounds are still desirable. A study by Whyte *et al.* [76] reported that Δ^9-THC and Δ^8-THC inhibited mitochondrial oxygen consumption rate via receptor independent manner in oral cancer cells [10]. Similar study by Lopes *et al.* [77] showed that apoptosis was induced by Δ^9-THC in oral squamous cell carcinoma (OSCC), which is a malignant form of oral cancer. Several studies reported the effect of Δ^9-THC in various cancer cell lines and *in vivo* models. Δ^9-THC has been reported to have a biphasic effect in various cancer cell lines. In this regard, the dual effects of Δ^9-THC were observed to be concentration dependent, in which increase in cancer cell proliferation was observed at lower concentrations, whereas Δ^9-THC at higher concentration exerted anti-proliferative effects [11]. Although Δ^9-THC has demonstrated its efficacy in glioma, breast, prostate, lymphoma, pancreatic and other types of cancers, it has showed proliferative effects in some breast, bronchial, hepatoma, and lung carcinoma cells [37]. On the basis

of available literature, Cridge *et al.* [37] stated that there is ambiguity, whether Δ^9-THC causes pro or anti-proliferative effects in cancer cells especially the breast cancer cells.

The treatment of androgen-independent prostate cancer cells (PC3) with Δ^9-THC at the concentrations from 0.05 to 0.1 μM resulted into increased cell proliferation [73]. Hart *et al.* [74] described that Δ^9-THC at concentrations between 0.1 and 0.3 μM elicited a 1.2 fold increase in NCl-H292 lung cancer cells and about 2 fold increase in U373-MG glioblastoma cells. However, the authors observed an apoptosis in about 30-80% of cells followed by Δ^9-THC treatment at concentrations between 4-10 μM [11, 37, 74]. A work by McKallip *et al.* [47] showed the proliferation of MCF-7 and MDA-MB-231 cells after treatment with 5 μM of Δ^9-THC. The results of this study were found to be in agreement with the recent study in which Takeda *et al.* [53] reported the proliferative response in MCF-7 cells in response to Δ^9-THC treatment at the same concentration.

Von Bueren *et al.* [78] showed that Δ^9-THC failed to induce the cell proliferation at concentrations up to 1 mM in MCF-7 cells rather at concentrations above 1 μM it inhibited 17β-estradiol induced proliferation in MCF7 as well as MCF7-AR1 cells [10, 45, 72, 73, 78, 79]. Δ^9-THC when studied at the concentration between 1-2 μM showed significant decrease in cell viability and proliferation in variety of glioma cell lines including C6, SF126, U87-MG, U251, SF188 and U373-MG. It indicates the consistent effect of Δ^9-THC in glioma irrespective of cell line [37, 80, 81]. Δ^9-THC (3 μM) is observed to significantly inhibit the EL-4, LSA, and P815 cell growth in serum free media, whereas same activity was demonstrated in calf serum (5%) media at 10 μM concentration [37, 82].

Δ^9-THC also showed the dual effect in pancreatic cell lines including Panc1, Capan2 MiaPaCa, BxPc3 cells. Although, the proliferation of pancreatic cells was observed when cells were treated by Δ^9-THC at concentrations below 1 μM. However, the treatment of similar cells with Δ^9-THC at concentrations above 2 μM resulted in inhibition of cell proliferation. These *in vitro* results are further supported by *in vivo* study in which Carracedo *et al.* [83] observed a significant reduction in tumor growth after 15 days treatment of Δ^9-THC (15 mg/kg) of nude mice to which tumors were developed after subcutaneous implantation of MiaPaCa2 cells [37, 83]. In contrast to above findings, the pCBs like CBD, Δ^9-THC and CBN showed stimulant effect on the MCF-7 cell proliferation irrespective of the concentrations used (1-20 mM) [44, 52, 84]. But, the Guindon *et al.* [44] pointed towards the conflict which may affect the interpretation of data. Watanabe *et al.* [84] and Takeda *et al.* [52] does not demonstrated the expression of cannabinoid receptors in MCF cells, whereas the another groups showed the CB1 and CB2 receptor expression in the MCF cells [44, 47, 85]. On the basis of the current literature on the anticancer activity of pCBs, it can be proposed that the biological response of pCBs depends on drug concentration and cellular context. The nanomolar concentrations of pCBs promote the cell proliferation while the relatively higher concentrations of these compounds induce the apoptosis. These important observations need the critical attention before the therapeutic application of pCBs as an anticancer therapy [74].

The pCBs are reported to exhibit anti-tumor or anticancer activities through the multiple mechanisms. Repetitive studies by Nomura *et al.* [86, 87] established that endocannabinoid, 2-AG may behave as a precursor for long chain fatty acid and involved in the cancer pathology. The role of the ECS system in the control of invasion has been studied by Nithipatikom *et al.* [88] in prostate cell lines. The authors reported the increased invasivity after inhibition of the synthesis of the 2-AG. Furthermore, overexpression of the enzyme; fatty acid amide hydrolase (FAAH), which is involved in metabolism of principal endocannabinoid, AEA, is also reported to have involved in increased invasiveness of prostate cell lines [89, 90]. On the basis of the results of these studies, Fowler [90] suggested the ability of cannabinoids to modulate the ECS is a novel mechanism through which these compound exert their actions. However, the promising results for novel anticancer strategies are exhibited preclinically, still the complete clinical success is anticipated [90].

Δ^9-THC was observed to stimulate the cell proliferation at nanomolar concentrations *in vitro* in lung carcinoma cell line NCI-H292. This effect was dependent on EGFR-mediated activation of ERK1/2 as well as PKB/Akt signaling. Whereas, the treatment of A549 human lung cancer cell line with Δ^9-THC at micromolar concentration leads to inhibition of epidermal growth factor-induced phosphorylation of ERK1/2, c-Jun-NH2-kinase1/2, and Akt along with inhibition of chemotaxis and chemoinvasion. Furthermore, Δ^9-THC suppressed the metastasis and tumor growth in severe combined immune deficient mice [37, 49, 91]. The various pCBs, endocannabinoids and synthetic cannabinoids including Δ^9-THC and CBD have exercised *in vitro* and *in vivo* anti-proliferative, apoptotic and

anti-invasive effects in different prostate cancer cells. The induction of apoptosis occurred either, though receptor independent or receptor dependent mechanisms. Whereas, stimulated PI3K/Akt pathway along with Raf-1/ERK1/2 and NGF induction in PC-3 after cannabinoid receptor stimulation by endocannabinoid were reported by the same group in independent study [10, 88, 92].

The ceramide accumulation is essential for cell death and it has anti-inflammatory and anticancer effects [10]. The blockade of ceramide synthesis and the expression of the stress protein p8 resulted in the loss of Δ^9-THC effects, which implies the essential role of ceramide behind Δ^9-THC induced cell death [93, 94]. Additionally, the effects of Δ^9-THC were proposed to be mediated by its direct action on adenylate cyclase dependent cAMP level, down-regulation of protein kinase A (PKA) and decreased gene transcription [37, 44, 91, 95]. The down regulated protein synthesis is associated with decreased expression of the high-affinity NGF tyrosine-kinase receptor A and subsequent inhibition of growth promoters [37, 48].

The effects of Δ^9-THC in the ErbB-2 breast cancer have been studied by several groups through *in vivo* and *in vitro* studies. The authors reported, anti-proliferative, anti-angiogenic, anti-invasive and pro-apoptotic actions of Δ^9-THC [10]. These effects of Δ^9-THC along with synthetic cannabinoid are proved to be mediated by modulation of AKT, phospho-S6 ribosomal protein, matrix metalloproteinases- 2 and 9 (MMP-2 and 9) expressions [50, 51, 96]. Cafferel *et al.* [50] proposed that the Δ^9-THC actions relies on the induction of cancer cell death through apoptosis and abrogation of cellular proliferation by inhibition of Akt, which is an tumorigenic kinases [48]. A parallel study by Ellert-Miklaszewska *et al.* [59] reported that Δ^9-THC leads to sustained activation of ERK1/2 and inhibition of AKT [10]. Δ^9-THC also resulted in the impaired tumor angiogenesis as evident by reduction in the number of tumor blood vessels [48, 50]. Furthermore, *in vivo* efficacy of Δ^9-THC in animal model of glioma was assigned to its ability of inhibiting MMP-2 expression [10, 75, 94].

The p53 is a component of ERK pathway, which has a critical role in maintaining a balance between cell-cycle arrest and apoptosis [37, 97]. Δ^9-THC was observed to activate the p53 through CB1 receptor which leads to activation of the apoptotic cascade containing B-cell lymphoma (Bcl)-2 and Bcl-2-associated X protein [37, 98]. Δ^9-THC exhibit its effects through the stimulation of ER stress response mediated by eukaryotic translation initiation factor 2 alpha (eIF2α) phosphorylation. The activation of ER stress leads to tribbles homolog 3-dependent (TRB3-dependent) inhibition of the Akt/mammalian target of rapamycin complex 1 (mTORC1) axis which leads to subsequent autophagy and apoptosis [10, 99]. The ability of compound to alter the invasion and metastasis is linked to its inhibitory effect on tumor development. Cannabinoids has ability to modulate the markers of cell migration, adhesion, invasion and metastasis. The pCBs, Δ^9-THC along with other cannabinoids is known to inhibit cellular migration in variety of cell lines [37, 100]. The decreased expressions of breast cancer-associated antigen 1, androgen receptor and prostate specific antigen in hormone responsive cells may be the mechanism behind actions of Δ^9-THC in breast and prostate cells [37].

The nuclear peroxisome proliferator-activated receptors (PPARs) are considered to be the targets of Δ^9-THC [18, 101]. Earlier study by Takeda *et al.* [102] reported, the significant increase in fatty acid 2 hydroxylase (FA2H) expression by Δ^9-THC in human breast cancer MDA-MB-231 cells. In continuation with this earlier study, the same group reported that Δ^9-THC leads to concentration-dependent activation of basal transcriptional activity of PPARα and concomitant up-regulation of PPARα/FA2H suggesting the involvement of PPARα in the Δ^9-THC induced up-regulation of FA2H in MDA-MB-231 cells [103].

In a study, Fowler [90] pointed that the *in vivo* inhibition of tumor growth either by Δ^9-THC or CBD is submaximal. Therefore, it would be better to use the combinations of different pCBs or combined therapy with cannabinoids and other anticancer agents [90]. Tores *et al.* [104] reported the blocked of tumor growth rate and weight in nude mice inoculated with U87MG glioma cells after 15 days peritumoral treatment with combination of Δ^9-THC and temozolomide. In the xenograft tumor model of nude mice inoculated with LNCaP or DU-145 prostate cancer cells, when the animals are treated with the combination a cannabis extract enriched in CBD and bicalutamide resulted into the better prognosis [105]. Recently, Scott and coworkers [106] evaluated the effect of Δ^9-THC and CBD alone or in combination with radiotherapy in several glioma cell lines, T98G, U87MG, and GL261. The authors demonstrated that the use of Δ^9-THC or CBD either in pure form or as a botanical drug substance (BDS) leads to time and dose-dependent decrease in cell viability. In this regard, THC-BDS was found to be more efficacious as compared to pure THC (THC-P)

whereas CBD-BDS was found to be less efficacious as compared to pure CBD (CBD-P). Furthermore, the *in vivo* treatment of THC and CBD along with radiation in an orthotopic murine model for glioma resulted in the dramatic reduction in tumor volumes [106]. A recent study by Armstrong *et al.* [107] reported that Δ^9-THC reduced the viability of melanoma cell CHL-1, A375, and SK-MEL-28 in a dose-dependent manner while having little effect on primary melanocytes at doses up to 6 μM. Furthermore, the treatment of melanoma cells with Δ^9-THC resulted in the activation of autophagy and apoptosis. These *in vitro* observations are confirmed through *in vivo* study in which authors observed that administration of Sativex-like (a laboratory preparation comprising equal amounts of Δ^9-THC and CBD) to mice bearing BRAF wild type melanoma xenografts substantially inhibit the viability of melanoma, proliferation and tumor growth simultaneously with an increase in autophagy and apoptosis [107]. These results suggest that further studies are needed in this direction to establish the advantage of combinations over the single compounds.

In another recent study, Fowler [90] reviewed the pharmacokinetic aspects of pCBs especially the Δ^9-THC and CBD. The Δ^9-THC binds with CB1 and CB2 receptors with Ki of about 25 and 35 nM respectively, suggested the role of cannabinoid receptors in Δ^9-THC action [108]. Administration of a cannabis extract containing Δ^9-THC (10 mg) and CBD (5.4 mg) have yielded the C_{max} of 13 nM for Δ^9-THC and 3.0 nM for and CBD [109]. Huestis *et al.* [110] reported the high volume of distribution for Δ^9-THC [110]. Therefore, Fowler [90], concluded that the actual concentrations of Δ^9-THC may be higher in tumors than these observed values and further attributed the *in vivo* efficacy of pCBs at micromolar concentrations only after intratumoral administration [90]. Adams [111] suggested that changes in oxygen tension in cell culture experiments have effect on the improvement of anticancer effect of a molecule. In this respect, no single study has been executed on Δ^9-THC considering the Adam's perspective. However, two recent studies on CBD has been performed in which authors evaluated the impact of oxygen tension on effects of CBD [90, 112, 113].

The abundant preclinical data on anticancer potential of phytoconstituents established the efficacy of pCBs in various types of cancer through *in vitro* and *in vivo* studies are available. A few selected studies on effect of pCBs in various types of cancer cells or animal models are summarized in (Table **3** and Table **4**).

1.3.2. Cannabidiol (CBD)

CBD is the principal non-psychotropic component of *Cannabis sativa* present in the glandular hairs of the plant [114, 115]. However, the pharmacological effects of this cannabinoid have shown to be exerted through G-protein-coupled cannabinoid receptors (CB1 and CB2). CBD has very low affinity for cannabinoid receptors and at low concentrations, CBD decreases G-protein activity [115]. Also, CBD acts as an inverse agonist at CB2 receptor which is believed to contribute the anti-inflammatory properties of CBD [114-118]. CBD has been demonstrated to act *in vitro* with high potency as an antagonist of CB1 receptors in mouse tissues [117-119]. Additionally, CBD displays inverse agonism at human CB2 receptors [117]. Despite the fascinating activities of the main constituent of cannabis, Δ^9-THC, its clinical use has been hampered because it activates the cannabinoid receptors to an extent that causes psychotropic adverse effects. Therefore, quest to search of Δ^9-THC like compounds without CNS adversity is continuing. In this regard, CBD may be the suitable alternative as it lacks the psychoactivity of Δ^9-THC. Due to this reason, the interest in this non-psychoactive phytocannabinoid has been amplified recently [118].

Contrasting to Δ^9-THC, CBD does not activate CB1 and CB2 receptors, which contributes to its non-psychotropic activity [41]. However, number of studies demonstrating the pharmacological mechanisms of CBD have been proposed, the role of CBD in cannabinoid receptor mediated signaling pathway is unclear. The finding that low affinity of CBD for both CB1 and CB2 receptors, leads to the studies investigating the cannabinoid receptor independent mechanism of action for CBD [114]. CBD is the multi-target molecule having ability to modulate the number of biological targets, beyond the ECS [41, 115]. Currently, several molecular targets for CBD have been described. CBD blocked the orphan G-protein-coupled receptor (GPR55), the transient receptor potential of melastatin type 8 (TRPM8) channel and equilibrative nucleoside transporter (ENT) [41]. The transient receptor potential of vanilloid type 1 (TRPV1) and 2 (TRPV2) channels and nuclear peroxisome proliferator-activated receptor-γ (PPAR-γ) are activated by CBD [90, 120]. It is also responsible for preventing the cellular uptake of adenosine and endocannabinoid; AEA along with inhibition of degrading enzyme, FAAH [41, 90, 115, 121]. CBD binds with 5-hydroxytryptamine receptor-1A ($5\text{-}HT_{1A}$) receptor and proposed to be modulator of opioid receptors at the higher doses [114]. CBD also reduces the

Table 3. ***In vitro*** **actions of phytocannabinoids in various cell types.**

Cell line /cell type	Compound	Effect	Mechanism	Reference
HeLa S3 cells (Cervical cancer cells)	Δ^9-THC	Decreased proliferation (antiproliferative)	-	[212]
C6.9 glioma cells	Δ^9-THC	Induction of apoptosis (apoptotic)	Induction of apoptosis by CB1 receptor independent stimulation of sphingomyelin breakdown	[204]
Human prostate PC-3 cells	Δ^9-THC	Induction of apoptosis (apoptotic)	Cannabinoid receptor-independent, non receptor mediated pathway	[92]
Murine tumors EL-4, LSA, and P815	Δ^9-THC	Decreased cell viability; increased apoptosis (apoptotic)	-	[82]
Chinese hamster ovary (CHO) cells	Δ^9-THC	Activation of the serine-threonine protein kinase, Akt	Effect mediated through the CB1 receptors	[58]
C6 glioma cells	Δ^9-THC	Induction of apoptosis (apoptotic); ceramide accumulation and activation of Raf1/MEK pathway	Cannabinoid receptors mediated apoptosis	[205]
Jurkat, Molt-4, and Sup-T1(Human leukemia and lymphoma cell lines)	Δ^9-THC	Decreased cell viability; increased apoptosis (apoptotic)	Effect mediated through the CB2 receptors	[82]
Human prostate epithelial PC-3 cells	Δ^9-THC	Increased phosphorylation of Akt; cannabinoid mediated activation of PI3K/PKB pathway	CB1 and CB2 receptors mediated action	[73]
EVSA-T(Breast cancer cells)	Δ^9-THC	Blockade of cell cycle progression; decreased cell progression; induction of apoptosis (apoptotic)	Activation of CB2 receptors	[43]
MiaPaCa2 and Panc1(Pancreatic cancer cell lines)	Δ^9-THC	Induction of apoptosis (apoptotic); increased ceramide levels, up-regulation of stress protein p8	CB2 receptor mediated effects	[83]
B16 and A375 melanoma cells	Δ^9-THC	Inhibition of melanoma cell proliferation (antiproliferative); induced apoptosis (apoptotic)	Inhibition of the prosurvival protein Akt; cell cycle arrest at the G_1-S transition	[55]
The colorectal carcinoma cell lines SW480, HCT-15, HT29, Caco-2, HCT116, LS174T and SW620 cells	Δ^9-THC	Induction of apoptosis (apoptotic); decreased proliferation (antiproliferative)	Apoptosis mediated through CB1 receptors and BAD; CB1-mediated inhibition of both RAS-MAPK/ERK and PI3K-AKT survival signaling cascades; activation of the proapoptotic BCL-2 family member BAD	[62]
MDA-MB-231 (Breast cancer cells)	Δ^9-THC	Decreased proliferation (antiproliferative); decreased migration (antimigratory); reduced cell invasion (anti-invasive)	-	[81]
MDA-MB-436 (Breast cancer cells)	Δ^9-THC	Decreased proliferation (antiproliferative)	-	[81]
EVSA-T(Breast cancer cells)	Δ^9-THC	Decreased proliferation (antiproliferative)	Up-regulation of JunD gene expression; translocation of protein to the nuclear compartment	[46]
A549 and SW-1573 (Lung carcinoma)	Δ^9-THC	Inhibition of EGF-induced growth; chemotaxis and chemoinvasion	Inhibition the EGF-induced ERK1/2, JNK1/2 and Akt phosphorylation..	[49]

(Table 3) contd….

Cell line /cell type	Compound	Effect	Mechanism	Reference
MCF-7 (Breast cancer cells)	Δ^9-THC	Increased proliferation (proliferative)	Up regulation of human epithelial growth factor receptor type 2 (HER2) through modulation of HER2 transcription	[52]
MCF7-AR1(androgen receptor positive human mammary carcinoma cell line) and MCF-7	Δ^9-THC	Decreased proliferation (antiproliferative)	Activation of cannabinoid receptors	[78]
Cholangiocarcinoma cell lines (RMCCA1and HuCCA1)	Δ^9-THC	Decreased proliferation (antiproliferative); decreased migration (antimigratory); reduced invasion (antiinvasive); induction of apoptosis (apoptotic)	Decreased phosphorylation of MEK1/2 and Akt	[213]
HepG2 (human hepatocellular liver carcinoma cell line) and HuH-7 (hepatocellular carcinoma cells)	Δ^9-THC	Induction of apoptosis (apoptotic) and autophagy	Stimulation of CB2 receptor; telomere repeat binding factor 3 (TRB3) up regulation, inhibition of the serine-threonine kinase Akt.	[101]
Human umbilical vein endothelial cells (HUVEC)	Δ^9-THC	Decreased migration (antimigratory); inhibition of angiogenesis (antiangiogenic); inhibition of tube and sprout formation	CB1 and CB2 receptors or TRPV 1 dependent action	[68]
Michigan Cancer Foundation-7 (MCF-7)(Breast cancer cells)	CBD	Decreased proliferation (antiproliferative)	-	[214]
MCF-7 (Breast cancer cells)	CBD	Decreased proliferation (antiproliferative)	Blockade of cell cycle at the G_1/S phase transition; inhibition of anandamide metabolism	[5]
MDA-MB-231 (Breast cancer cells)	CBD	Decreased proliferation (antiproliferative); increased apoptosis (apoptotic)	Activation of CB2 and TRPV1 receptors; elevation of intracellular calcium; increased reactive oxygen species (ROS) production; inhibition of anandamide metabolism	[5]
MDA-MB-231 (Breast cancer cells)	CBD	Decreased proliferation (antiproliferative); decreased migration (antimigratory); reduced cell invasion (antiinvasive)	Down-regulation of helix-loop-helix protein Id-1; inhibition of Id-1 gene transcription	[81]
MDA-MB-436 (Breast cancer cells)	CBD	Decreased proliferation (antiproliferative); decreased migration (antimigratory)	Down-regulation of helix-loop-helix protein Id-1; inhibition of Id-1 gene transcription	[81]
MDA-MB-231 (Breast cancer cells)	CBD	Decreased migration (antimigratory)	G-protein-coupled receptor 55 (GPR55) receptor antagonism; inhibition of cancer cell migration and metastasis through blockade of G12 signaling	[195]
MDA-MB231 (Breast cancer cells)	CBD	Inhibition of human breast cancer cell proliferation (antiproliferative); decreased cell invasion (antiinvasive); decreased cancer cell aggressiveness	Up-regulation of ERK phosphorylation and Id-2 expression; induction of ROS	[139]
BV-2 microglial cells	CBD	Induction of cell death due to biphasic increase in intracellular calcium levels and changes in mitochondrial function; decreased VDAC1 channel conductance	Inhibition of voltage-dependent anion channel 1 (VDAC1) functioning	[215]

(Table 3) contd….

Cell line /cell type	Compound	Effect	Mechanism	Reference
Lung cancer cell lines (A549, H460) and metastatic cells derived from a lung cancer patient	CBD	Increased susceptibility of cancer cells to adhere and subsequent lysis by LAK cells; up regulation of intracellular adhesion molecule-1 (ICAM-1)	CB1 and CB2 receptors and transient receptor potential vanilloid 1 (TRPV1) mediated action	[134]
SUM159, 4T1.2, SCP2 cells (Breast cancer cells)	CBD	Inhibition of epidermal growth factor (EGF)-induced proliferation and chemotaxis of breast cancer cells; activation of EGFR, ERK, Akt and NF-kB pathway; decreased MMP2 and MMP9 secretion; inhibition of colony formation, migration and invasion (antimigratory and antiinvasive)	Inhibition of EGF/EGFR signaling	[147]
MCF-7 (Breast cancer cells)	CBG	Decreased proliferation (antiproliferative)	Activation of TRPV1 receptors; inhibition of anandamide metabolism	[5]
MDA-MB-231 (Breast cancer cells)	CBG	Decreased proliferation (antiproliferative)	Activation of TRPV1 receptors, inhibition of anandamide metabolism	[5]
MDA-MB-231 (Breast cancer cells)	CBG	Decreased proliferation (antiproliferative)	-	[81]
MDA-MB-436 (Breast cancer cells)	CBG	Decreased proliferation (antiproliferative)	-	[81]
Human colorectal carcinoma (Caco-2) cells	CBG	Increased apoptosis (apoptotic); increased ROS production, up regulation of CHOP mRNA and reduced cell growth.	Transient receptor potential (TRP) M8 (TRPM8) antagonism	[154]
MCF-7 (Breast cancer cells)	CBC	Decreased proliferation (antiproliferative)	Inhibition of endocannabinoid (anandamide) metabolism	[5]
MDA-MB-231 (Breast cancer cells)	CBC	Decreased proliferation (antiproliferative)	Inhibition of endocannabinoid (anandamide) metabolism	[5]
MCF-7 (Breast cancer cells)	CBDA	Decreased proliferation (antiproliferative)	Activation of TRPV1 receptors, inhibition of anandamide metabolism	[5]
MDA-MB-231 (Breast cancer cells)	CBDA	Decreased proliferation (antiproliferative)	Activation of TRPV1 receptors, inhibition of anandamide metabolism	[5]
MCF-7 (Breast cancer cells)	THCA	Decreased proliferation (antiproliferative)	Inhibition of endocannabinoid (anandamide) metabolism	[5]
MDA-MB-231 (Breast cancer cells)	THCA	Decreased proliferation (antiproliferative)	Inhibition of endocannabinoid (anandamide) metabolism	[5]
MDA-MB-231 (Breast cancer cells)	CBN	Decreased proliferation (antiproliferative)	-	[81]
MDA-MB-436 (Breast cancer cells)	CBN	Decreased proliferation (antiproliferative)	-	[81]
MCF-7 (Breast cancer cells)	Δ^9-THC, CBN, CBD	Increased proliferation (proliferative)	-	[84]
DLD-1 and HCT116 (Colorectal carcinoma cells)	*Cannabis* extract enrich in CBD	Selective anti-proliferative effect in tumoral cells	CB1 and CB2 receptors mediated action	[152]

Table 4. ***In vivo*** **actions of phytocannabinoids in various cell types.**

Compound	Animal (Species/strain)	Dose & Route	Effect	Reference
Δ^9-THC	B6D2F1 male mice	0, 25,200 mg/kg, p.o. for 10 days	Decreased tumor growth and tumor weight	[216]
Δ^9-THC	Wistar rat	50–3000 µg through cannula at the site of tumor inoculation for 7 days	Absence of tumoral mass; regression of malignant gliomas in rats	[205]
Δ^9-THC	Mice	500 µg/day, intratumorally for 7 days	Decreased tumor growth and tumor size	[205]
Δ^9-THC	Male C57BL/6 (H-2b) and BALB/c mice	5 mg/kg, four times/week i.p. for 4 weeks	Promoted tumorigenicity in immuno-competent mice whereas no effect on tumor growth in immuno-deficient mice	[54]
Δ^9-THC	Female C57BL/6 mice	0, 1, 3, or 5 mg/kg, i.p.	Reduced tumor load; increaseinduced tumor cell apoptosis	[82]
Δ^9-THC	Female BALB/c mice	12.5, 25 and 50 mg/kg/day, i.p. for 4 days	Increased in tumor growth; increased metastasis	[47]
Δ^9-THC	Immuno-deficient nude mice	15 mg/kg/day peritumorally for 15 days	Decreased tumor growth and volume; decreased metastasis; induction of apoptosis	[83]
Δ^9-THC	MMTV-neu mice	0.5 mg/animal/day, for three weeks	Decreased tumor number and growth; decreased proliferation; induction of apoptosis; impaired tumor angiogenesis	[50]
Δ^9-THC	Nude mice	15 mg/kg/day, peritumorally for 15 days	Decreased tumor volume and cell viability	[208]
CBD	Female BALB/c mice	1 and 5 mg/kg, i.p. for 15 days	Reduced breast cancer metastasis and tumor volume	[139]
CBD	Male ICR mice	1-40 mg/kg. i.p., three times/week for three months	Decreased aberrant crypt foci, polyps and tumor formation	[151]
CBD: BDS (CBD rich Cannabis extract)	Male MF-1 nude mice	1, 10 and 100 mg/kg/day, i.p.	Decreased tumor growth	[105]
CBD	Female Balb/C and FVB mouse cell line (MVT-1)	10 mg/kg, peritumorally on alternate days for 3 weeks	Decreased tumor growth, volume, weight and metastasis	[147]
CBG	Male ICR mice and athymic nude female mice	1-10 mg/kg/day, i.p.	Decreased tumor size, growth and progression of colon cancer	[154]
THC, CBD	Charles River 6-week-old male athymic mice	5 mg/kg i.p. every 72 h, for 21 days	Reduced tumor growth and inhibited metastasis	[5]
THC, CBD or THC and CBD combination (Sativex)	Female C57BL/6 mice	2 mg/kg i.p., 9, 13, and 16 days after tumor implantation	Reduced tumor growth and tumor size	[106]
THC and sativex like	Athymic nude male mice	THC (15/mg/kg, oral gavage) or Sativex (7.5 mg/kg THC-BDS + 7.5 mg/kg cannabidiol (CBD)-BDS, oral gavage) for 20 days	Reduced tumor growth, decreased proliferation and activates non-canonical autophagy-mediated apoptosis	[107]

(Table 4) contd….

Compound	Animal (Species/strain)	Dose & Route	Effect	Reference
THC, CBD or combination (Microparticles)	Athymic nude mice	THC-loaded MP (75 mg of MP comprising approximately 6.15 mg of THC per administration, one administration every 5 days), CBD-loaded MP (75 mg of MP comprising approximately 6.7 mg of CBD per administration, 1 administration every 5 days), a mixture (1:1; w:w) of THC and CBD-loaded MP (37.5 mg of THC-loaded MP and 37.5 mg of CBD-loaded MP per administration, one administration every 5 days)	Reduced tumor growth and tumor weight; inhibited cancer cell proliferation; enhanced apoptosis and inhibited tumor angiogenesis	[153]
THC, CBD or combination (solution form)	Athymic nude mice	THC (15 mg/kg/day equivalent to 0.5 mg THC per day), CBD (15 mg/kg/day equivalent to 0.5 mg CBD per day) or THC+CBD (7.5 mg/kg of THC and 7.5 mg/kg CBD equivalent to 0.25 mg of THC and 0.25 mg of CBD per day)	Reduced tumor weight and growth; reduced cancer cell proliferation; enhanced apoptosis and inhibited tumor angiogenesis	[153]
THC, CBD and THC+CBD	Nude mice	THC (7.5 mg/kg peritumorally); CBD (7.5 mg/kg peritumorally); THC+CBD (7.5 mg/kg+7.5 mg/kg peritumorally) for 14 days	Reduced tumor weight and growth; induced apoptosis	[104]

psychoactivity of Δ^9-THC, extends its therapeutic window and improves the patient tolerability and acceptability of Δ^9-THC [41]. The generalized mechanism of anticancer activity of pCBs including Δ^9-THC and CBD have been described in (Fig. **3**).

The appearance of resistance is a major problem in anticancer therapy. The P-glycoprotein (P-gp), an ATP-binding cassette (ABC) transporter overexpression is responsible for the efflux of several anticancer agents in chemotherapeutic-resistant tumors. CBD is reported to decreased the activity of P-gp in various types of drug resistance and non-drug resistant cell lines including drug resistant lymphoma cells [122]; human leukemia cell line [123]; experimental model of the blood-brain barrier [124]; BeWo cells, a human trophoblastic cell line [125] and in ovarian carcinoma cell lines [126, 127].

The increased tumor cell lysis following the infiltration of lymphocytes and decreased tumor growth are linked with the ICAM-1 over expression on tumor cells [128, 129]. In past few years, several experiments demonstrated that up-regulated ICAM-1 expression lead to higher susceptibility of tumor cells to lymphocyte adhesion and subsequent cell lysis [130, 131]. As the induction of ICAM-1 caused increased tumor cell killing, ICAM-1 expression is negatively linked with metastasis [132, 133]. Therefore, the modulation of ICAM-1 has role in cancer prevention and it is one of the targets of pCBs. Ramer and co-workers showed that in lung tumor cell lines and primary lung tumor cells the pCBs including CBD and Δ^9-THC induced the ICAM-1 expression [66, 127].

The anti-invasive and anti-metastatic actions of cannabinoids at least in part are known to be mediated through the promotion of ICAM-1 expression on the lung cancer cells. Haustein *et al.* [134] investigated the impact of cannabinoid-induced ICAM-1 on cancer cell adhesion to lymphokine-activated killer (LAK) cells subsequent LAK cell-mediated cytotoxicity using metastatic cells derived from a lung cancer patient and A549, H460 lung cancer cell lines. The CBD and Δ^9-THC increased the susceptibility of cancerous cells to adhere to LAK cells followed by lysis. The reversal of CBD effect after the pretreatment with cannabinoid receptors and TRPV1 antagonists suggest the role of these receptors in the effects of CBD. However, the effects of CBD and Δ^9-THC on ICAM-1 induced cell lysis were not observed in normal lung epithelial cells implying the restriction of this cytolytic activity to cancerous cells only [127, 134].

As described previously, cannabinoids have ability to either directly modulate the endothelial cells or decrease the formation of pro-angiogenic factors and acts as an anti-angiogenic factor [118, 135]. Few studies demonstrated the additional potential of CBD to act as an anti-angiogenic anticancer drug [127]. Soli-

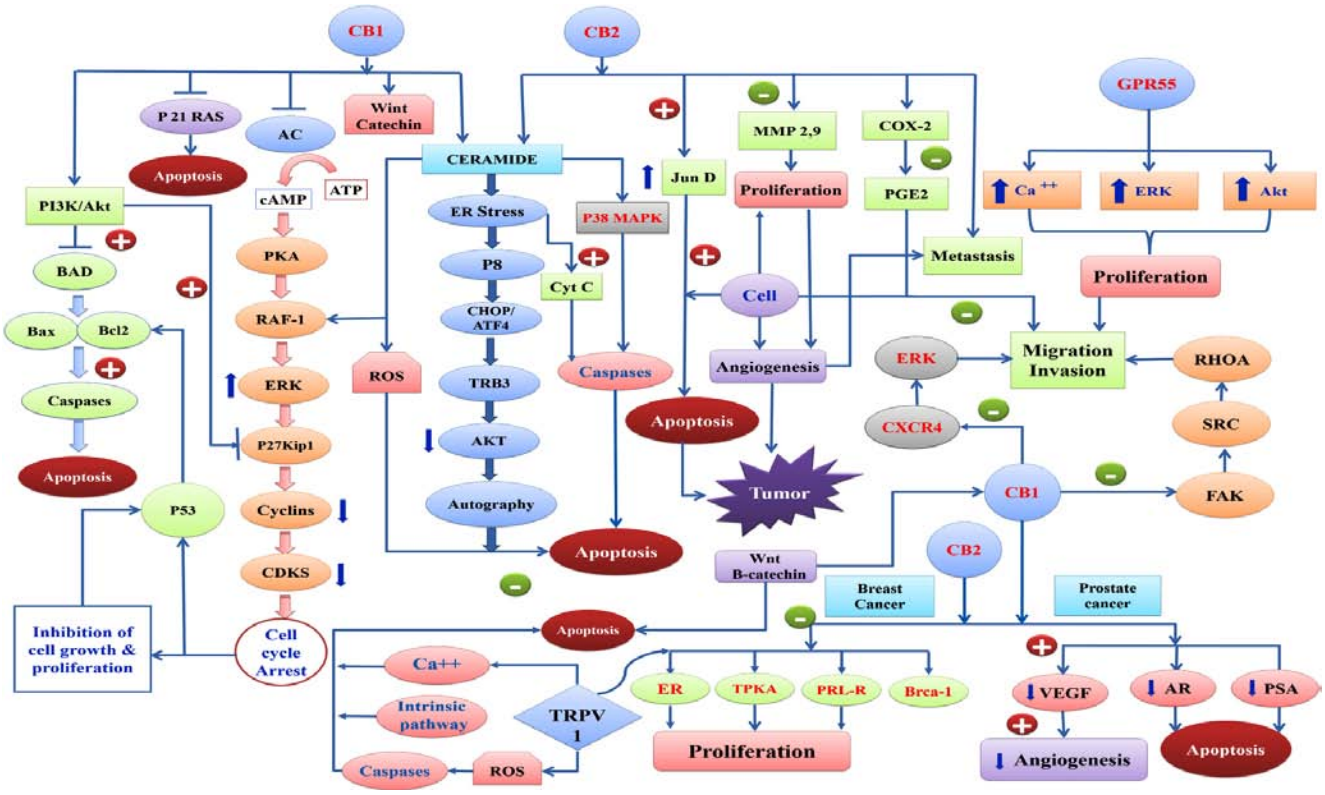

Fig. (3). Generalized mechanism of phytocannabinoid actions in modulation of cancer signaling pathway.

nas *et al*. [112] identified multiple molecular pathways responsible for antitumor actions of CBD. Authors observed *in vitro* decrease in cell proliferation and invasiveness of both U87-MG and T98G glioma cells by CBD. The hypoxia-inducible transcription factor (HIF-1α) is one of the most important stimuli for cell survival, motility and tumor angiogenesis. CBD treatment decreased the expressions of proteins involved in invasion, angiogenesis and cell growth along with of down regulated ERK and Akt prosurvival signaling pathways in both cell types whereas diminished hypoxia inducible factor HIF-1α expression in U87-MG cells [112, 118, 136].

Recently, a study by Solinas *et al.* [112] showed that CBD at concentrations >9 mM inhibited the *in vitro* proliferation, migration and invasion of HUVEC cells. Furthermore, the anti-angiogenic effect of CBD was demonstrated through *in vivo* experiment using matrigel sponge assay in C57/BL6 mice. Further, it also decreased several pro-angiogenic molecules like MMP2, MMP9, endothelin-1, PDGF-AA and CXCL16. These results suggested the anti-angiogenic potential of CBD in addition to its well-known anti-proliferative, anti-invasive and pro-apoptotic properties in cancer cells [112, 118, 127].

In contrast, recent work by Ramer and coworkers observed the increase in cell migration along with increased tube and sprout formation after treatment with CBD at 3 mM concentration [68]. The available literature on this issue suggested the dual role of pCBs. The pro-angiogenic effects of CBD were observed at lower concentrations (50 nM) whereas the anti-angiogenic effects were demonstrated at higher concentrations [112]. The proteolytic enzymes secreted by tumor cells and hem oxygenases-1 (HO-1) have known for their complex effects in angiogenesis. Among the proteolytic enzymes, in relation to angiogenesis, more important are the MMPs which represent an important targets for cannabinoids. In this direction Ramer *et al*. [14] in another study suggested the up-regulation of TIMP-1 by CBD, mediated by both cannabinoid and TRPV1 receptors. Recently, CBD was reported to up regulate the HO-1 in human umbilical vein endothelial cells (HUVEC) [127].

McAllister *et al*. reported that CBD can obstruct the cell invasion and metastasis in breast cancer. Inhibitor of DNA binding-1 (Id-1) an inhibitor of basic helix-loop-helix transcription factors responsible for the control of proliferation, migration and invasion. The inhibition of breast cancer cell invasion is associated with the down regulation of Id-1 [81]. Following a series of

studies, in continuation with this finding the same group of authors reconfirmed that the anti-invasive actions of CBD are mediated through modulation of Id-1 levels in glioblastoma cells [137]. In an orthotopic mouse model of intracranially injected U251 glioblastoma cells, the administration of CBD (15 mg/kg, i.p.) for 5 days/ week reduced tumor area upto 95% [137]. Additionally, no metastasis was observed in one among the five animals treated with CBD. There is also one recent report with similar findings on role of Id-1 down regulation in anti-invasive action of CB2 receptor agonist [138]. Murase *et al.* [138] recently reported the decrease of total breast metastasis by CBD at EC50 of 0.3 mg/kg. McAllister *et al.* [139] reported CBD to reduce the mean lung metastasis, in an orthotopic model induced by 4T1 mammary tumor cells were injected in nipple of Balb/c mice. CBD reduced the primary tumor growth at both the tested daily doses of 1 mg/kg and 5 mg/kg, i.p. Similar effect i.e. percentage decrease in the total number of metastases was obtained when breast cancer cells were injected into the tail vein [90, 139].

The correlation between cancer cell invasion and decreased TIMP-1 levels has been established by earlier studies and up-regulation of TIMP-1 is correlated with anticancer activities [127]. Through the series of studies by Ramer and co-worker [127] they established that the up-regulation of ICAM-1 by the pCBs including CBD is responsible for its anti-invasive activity. The A549 cell line represents the human lung carcinoma cells expressing both CB1 and CB2 receptors and TRPV1 receptors. The treatment of A549 cells with CBD at 5 mg/kg, i.p. every 72 h resulted in reduction (52-84%) of metastasis. In parallel, CBD treatment decreased the tumor cell invasiveness by up-regulation of the TIMP-1. The induction of P38 and ERK phosphorylation which leads to downstream activation of TIMP-1 was observed after CBD treatment. The authors also established that these effects of CBD were mediated through both the cannabinoid receptors and TRPV1 channel. The plasminogen activator inhibitor PAI-1 is additional factor involved in the regulation of cell spreading. The PAI-1 was found down-regulated in CBD treated A549 cell and accompanied the anti-invasive effect of CBD [14, 66, 140].

Very few studies demonstrated the effect of CBD on glioma cell proliferation and apoptosis. The effect of CBD upon C6 murine glioma cell proliferation was reported to be serum dependent [80]. CBD by inducing apoptosis effectively inhibited the U87-MG and U373 human glioma cell proliferation [1]. The anti-proliferative and apoptotic effects of CBD were observed at concentrations which are non-lethal to primary glial cells. Massi *et al.* [141] reported the involvement of both intrinsic and extrinsic apoptotic pathways in CBD mediated glioma cell death as evident by release of cytochrome C and activation of caspases. Similar observation was made by Gallily *et al.* [142] in human acute myeloid leukemia HL-60 cell line, in which CBD mediated apoptosis was linked to activation of caspase-3 [142]. Again, this effect was not demonstrated on human monocytes obtained from normal individuals [142]. CBD showed significant decrease in viable cell number and induced apoptosis in human Jurkat and Molt-4 leukemia cell lines as well as in murine EL-4 lymphoma cell line [143]. Additionally, the effect of CBD was found to mediate at least in part through modulation of phospho-p38 MAPK and reactive oxygen species (ROS) levels [143]. The selective inhibition of various tumor cell line (MCF7, MDA-MB-231) growth by CBD was probably demonstrated for the first time by Ligresti [5]. CBD also inhibited the growth of xenografts prompted by human MDA-MB-231 cells in athymic mice. Moreover, CBD decrease the infiltration of lung metastases derived from intra-paw injection of breast carcinoma cells. The proposed mechanisms behind the action of CBD are direct activation of TRPV1 and/or FAAH mediated indirect activation of CB2 receptors and induction of oxidative stress [5].

The mechanistic studies in the direction of autophagy and apoptosis revealed that CBD treatment leads to breakdown of Beclin1, a key signaling molecule in the autophagic process. The translocation of cleaved product to mitochondria stimulated the release of cytochrome C and induced apoptosis [118, 144, 145]. The detailed mechanism behind breast cell death by CBD was proposed by Shrivastava *et al.* [146]. CBD demonstrated concentration dependent effect on cell death of various breast cancer cells and observed effects are independent of CB receptor or TRPV1 receptor activation. The autophagy and apoptosis was proposed to be mediated by endoplasmic reticulum stress, inhibition of Akt/mTOR/4EBP1, increase in ROS production, translocation of the Beclin2 interacting protein (Bid), the release of cytochrome C and activation of apoptosis pathway [118, 146].

More recently, Elbaz *et al.* [147] highlighted the novel anti-tumor mechanisms of CBD in breast cancer. The authors reported that the inhibition of EGF/EGFR pathways and modulation of tumor microenvironment are responsible for the CBD mediated antitumor action. This was the first study demonstrated that CBD inhibit

epidermal growth factor (EGF)-induced proliferation and chemotaxis of breast cancer cells. CBD also inhibited EGF induced activation of various targets like epidermal growth factor receptor (EGFR), ERK, Akt, NF-kB, MMP2 and MMP9. Further, authors evaluated tumor growth inhibitory effect of CBD in different mouse models [147].

As the anti-proliferative effect of CBD was not completely reverted by CB2 receptor antagonist, SR144528, it directed the involvement of cannabinoid receptor independent mechanisms. Massi *et al.* [1] for the first time established that the induction of oxidative stress by the CBD is an additional mechanism for its antitumor effects. CBD induced the oxidative stress was confirmed by increased production of ROS and intracellular glutathione along with decreased GSH-associated enzymatic activity. The anti-proliferative effect of CBD was reverted by tocopherol, an anti-oxidant, confirming the involvement of ROS in CBD mediated anti-proliferative effects. However, CBD does not induced this oxidative stress in non-transformed primary glial cells [141]. *In vivo* experiment involving the treatment of CBD to nude mice revealed the decreased activity and content of 5-LOX and stimulation of FAAH and decreased AEA level in glioma tumor tissues excised from the animals [148]. Similar study [149], reported marked induction of apoptosis by CBD in both EL-4 thymoma cells and murine thymocytes. The cellular events triggered by CBD primarily involve generation of ROS and apoptosis [149]. McAllister *et al.* [139] demonstrated that production of ROS is another mechanism that contribute to CBD effects on the cellular proliferation. The effect of CBD was reversed by treatment with tocoferol suggests the importance of ROS production in CBD mediated anti-proliferative action [139].

The accumulation of a monoclonal plasma cell in the bone marrow is called as multiple myeloma. CBD has ability to modulate TRPV channel. The presence of CD1381TRPV21 and CD1381TRPV22 plasma cells were identified in multiple myeloma patients. CBD either individually or in combination with bortezomib, a commonly used drug in multiple myeloma, lead decreased growth of multiple myeloma cells, induced cell cycle arrest and cell death through the ERK, AKT and NF-κB pathways. This report suggested the beneficial role of CBD in multiple myeloma treatment as it potentiated the activity of bortezomib [150].

A study by Aviello *et al.* [151] evaluated effect of CBD in animal model of azoxymethane (AOM) induced colon cancer. The treatment of animals with azoxymethane leads to the development of aberrant crypt foci (ACF), polyps and tumor formation. The other changes associated with AOM treatment are changes in phospho-Akt, iNOS, COX-2 and caspase-3. The treatment with CBD showed significant decrease in all three parameter including ACF, polyps and tumors. CBD protected DNA from oxidative damage, increased endocannabinoid concentration and decreased cell proliferation in colorectal carcinoma cell lines. CBD effects were mediated by CB1, TRPV1 and PPAR-γ [151]. In a recent study, the same group [152] investigated the effect of standardized *Cannabis sativa* extract with high content of cannabidiol rich botanical drug substance (CBD-BDS), through *in vitro* and *in vivo* studies in colon cancer. The CBD and CBD-BDS decreased cell proliferation selectively in cancerous cells and not in healthy cells. *In vivo* results showed the CBD-BDS at the dose of 5 mg/kg, i.p. decrease the ACF (86% inhibition) and polyps (79% inhibition) and decreased tumor formation (40%) although statistically insignificant and inhibited tumor growth significantly in xenograft model of colon cancer. The effect of CBD-BDS on cell proliferation was proposed to be mediated through CB1 and CB2 receptor activation [152]. Macpherson *et al.* [113] recently reported that CaCo-2 cells which is a model of colon cancer and small intestinal epithelium cancer is modulated by physiological intestinal oxygen and leads to increased sensitivity of CBD.

De Petrocellis *et al.* measured the tumor volume in nude mice inoculated either with LNCaP (androgen-sensitive) or DU-145 (androgen-resistant) prostate cancer cells. The animals were treated with CBD-BDS alone or in combination with other drugs. The combination of CBD-BDS with bicalutamide showed a significantly better prognosis whereas in DU-145 cells CBD-BDS potentiated the effect of docetaxel [105]. A recent investigation proposed that human prostate cells over express the various receptors like cannabinoid receptors CB1 and CB2, vascular endothelial growth factor (VEGF), prostate specific antigen (PSA). When the effect of CBD was studied in human prostate cancer cell lines LNCaP, DU145, PC3 it was observed that the treatment of these cells with cannabis extract containing high CBD resulted into down regulation of CB1, CB2, VEGF, PSA along with pro-inflammatory cytokines (IL-6/IL-8). These results also confirmed that CBD is potent inhibitor of cancer cell growth with selectivity towards cancer cells and minimal toxicity towards normal cells [2]. This study suggested the use of CBD and other non-habit forming cannabinoids as a novel therapeutic agents in prostate cancer [2].

Recently, Ossa *et al.* [153] analyzed the effect of alternative long-term cannabinoid administration delivery system (CBD- and THC-loaded poly-e-caprolactone microparticles) in a murine xenograft model of glioma. The efficacy of prepared microparticles in reducing the tumor growth when administered locally every 5 days in xenografted mice was similar to daily administration of cannabinoids at the equal dose in solution. The treatment of mice with cannabinoid-loaded microparticles resulted in improved apoptosis along with reduced cell proliferation and angiogenesis in tumors [153].

1.3.3. Other Phytocannabinoids

The cannabis plant contains more than 100 pCBs including, Δ^9-THC and CBD. But, the major research has been focused on these two compounds which may be due to their abundance presence in the plant. The term pCBs in its broad sense covers the compounds having cannabinoid like actions derived from natural sources other than cannabis. Although, these cannabis or non-cannabis derived compounds have potential effects in the cancers, it is not possible to include all the compounds in single review. Therefore, we have made an attempt to summarize few of them.

CBG is non-psychoactive and safe cannabinoid derived from the cannabis plant. CBG has ability to interact with specific targets involved in carcinogenesis. CBG is a potent blocker of transient receptor potential (TRPM8) and it blocks 5-HT_{1A} whereas it stimulates TRPA1, TRPV1 and TRPV2 channels and inhibits the reuptake of endocannabinoids [154]. CBG at higher doses have cytotoxic effects on human oral epithelioid carcinoma cells. It is also effective against breast cancer and reduced the intestinal inflammation in experimental models which indicate the possible use of CBG in colorectal cancer. CBG is weak partial agonist of both CB1 and CB2 receptors. CBG can interact with TRP channel especially TRPM8 which is involved in tumoral cell growth [154]. Recently, Borelli *et al.* [154] demonstrated that CBG inhibit chemically induced carcinogenesis along with decreased growth of xenografted tumors. This study demonstrated the possible use of CBG as anticancer agent in colorectal cancer.

CBDA is another phytoconstituent in cannabis and is the precursor of CBD. Takeda *et al.* [155] investigated its effect in MDA-MB-231 human breast cancer cells. CBDA inhibited migration of highly invasive MDA-MB-231 cancer cells by inhibiting cAMP dependent protein kinase A (PKA) and activation of small GTPase, RhoA which are involved in inhibition of motility of various cells [155].

Recently, Liu *et al.* [156] reported that Betulinic acid, a pentacyclic triterpenoid exerted activity against breast cancer cell mediated through cannabinoid receptors. The BT474 and MDA-MB-453 breast cancer cells overexpressing ErbB2, upon treatment with 1 to 10 mmol/L betulinic acid inhibited cell growth, induced apoptosis, decrease expression of ErbB2 and down regulated specificity protein (Sp) transcription factors Sp1, Sp3, and Sp4. Treatment of ErbB2-overexpressing BT474 and MDA-MB-453 breast cancer cells with 1 to 10 mmol/L betulinic acid inhibited cell growth, induced apoptosis, down-regulated Sp transcription factors; Sp1, Sp3, and Sp4 and decreased expression of ErbB2. This study for the first time identified the CB1 and CB2 receptors as novel target of betulinic acid in cancer [156]. Bisdemethoxycurcumin (BDMC) is a natural derivative of curcumin is also reported to induce apoptosis through CB2 receptors in activated hepatic stellate cells. BDMC relatively induced a potent apoptosis in HSC-T6 cell line. The apoptosis was mediated by inhibition of proteins like Bcl-2 and heme oxygenase-1 and increased production of ROS [157].

1.3.4. Pharmacokinetics of Phytocannabinoids

Δ^9-THC is water insoluble and highly lipophilic molecule. Δ^9-THC is rapidly absorbed into the blood stream, being a lipophilic compound distributed throughout the body including brain and interacts with its molecular targets [39]. Although, Δ^9-THC is highly protein bound but steady state volume of distribution is large due to its lipophilicity [42]. After inhalation of marijuana, peak plasma levels are attained in less than 10 min which are directly related to Δ^9-THC content of marijuana [40]. Δ^9-THC is liable to undergo the extensive first pass effect upon oral administration. Therefore, the absorption of Δ^9-THC through the oral route is slow and variable [40, 158]. The half-life ($T_{1/2}$) of Δ^9-THC depends on the route of administration. Generally, Δ^9-THC has early $T_{1/2}$ of 3–4 h, followed by a terminal $T_{1/2}$ of 25–36 h [42]. Upon oral administration, peak plasma levels reach after 60-120 min with oral bioavaibility of about 6% whereas 45 min after the sublingual administration [40, 159]. The metabolism of Δ^9-THC by CYP2C subfamily occurs in liver and the predominant route of excretion are feces and urine. Δ^9-THC levels can be detected in urine even upto 12 days of administration, due to enterohepatic cyclization of Δ^9-THC metabolite, 11-hydroxy-delta 9-tetrahydrocannabinol (11-OH-THC).

Pharmacokinetics of CBD is complex and resembles to Δ^9-THC [114, 160]. The oral bioavaibility of CBD ranges between 13-19% whereas systemic bioavaibility upon inhalation of CBD was 31% [114, 160]. Oral-mucosal and sublingual delivery through sprays and/or lozenges has less variable and comparable bioavailability similar to the oral route [41]. CBD reported as low toxicity with minimal side effects and well tolerated even at high doses in humans and other species [160]. The intravenous administration of CBD in rhesus monkeys reported the LD_{50} of 212 mg/kg [114, 161]. The placebo-controlled and open trials in human reported the good tolerability of CBD at wide dose range. No obvious signs of toxicity have been observed after the acute and chronic administration at the oral doses of up to 1500 mg/day or intravenous dose of 30 mg [41, 162].

1.3.5. Clinical Studies on Phytocannabinoids

Despite the availability of vast preclinical reports on anticancer activity of various pCBs, there is the scarcity of clinical studies in this area. Guzman *et al.* [163] reported the phase I clinical study executed in the patients with refractory glioblastoma multiforme to assess the safety and efficacy of Δ^9-THC. In this study, Δ^9-THC was administered at the doses of 0.80 to 3.29 mg/day for 10-64 days to total nine patients. Except the occurrence of mild psychotropic effects, the Δ^9-THC was found safe and well tolerated [42, 163]. To evaluate the safety profile, assess the frequency and severity of adverse events a clinical study is underway in recurrent glioblastoma patients receiving the Sativex (THC and CBD) combination with dose-intense Temozolomide [164]. The pCBs mainly THC and CBD either individually or in combination (Sativex) are evaluated through clinical trials in relation to their analgesic effects in cancer patients. The administration of single oral dose of Δ^9-THC (10 mg) to patient with cancer pain produced mild analgesic effects with good tolerability. However, the use of Δ^9-THC (20 mg) at higher doses produces some adverse effects [44, 165]. Similarly, another study showed better pain relief with nitrogen analogue of Δ^9-THC as compared to placebo in cancer patients [44, 166]. In the later study, the nitrogen analogue of Δ^9-THC was not found effective analgesic as compared with placebo and moreover it was reported to augment pain perception in patients with malignancy associated pain [44, 167]. A recent study by Johnson *et al.* [168] reported that the administration of Sativex (THC:CBD in a 1:1 ratio) decreased pain as compared with placebo, although the effects of individual administration of Δ^9-THC on pain was not significant [44, 168].

2. CONCLUSION

Although, the therapeutic potential of cannabis has been known for centuries, the use of pCBs in clinical practice is still inadequate. The clinical trials data demonstrated safety of these compounds in several cancer patients. The pCBs especially the Δ^9-THC were found well tolerated except occurrence of mild to moderate side effects like dizziness and fatigue [48]. Recently, there are increased interests in evaluating the anticancer effects of pCBs in combination rather than their individual use. There is possibility that the combination of pCBs is a better therapeutic alternative as compared to individual use of Δ^9-THC or CBD [44]. The vast preclinical literature available on the anticancer activity of cannabinoid demonstrated multiple mechanisms for anti-tumor action of the pCBs. In many conditions, the pCBs show dual effects (e.g. proliferation and anti-proliferation) which are dependent on the concentrations of cannabinoids used and cellular context. Therefore, it is necessary to evaluate these effects through systemic clinical trials. On the basis of available literature, it is observed that in many cases, the pCBs exhibited selective cytotoxicity towards cancerous cells while sparing the normal cells, which is one of the most desirable properties of the anticancer drugs. The combination of pCBs with other anticancer drugs or therapies have shown promise in the treatment of drug resistant cancers. The combination of psychoactive pCBs (Δ^9-THC) with non-psychoactive pCBs (CBD), further decreased adversity of former and improve the pharmacological outcome. The pharmacokinetic properties of pCBs and the possibility of their administration through multiple routes suggest the likelihood of these compounds as potential candidates for drug development. The appropriate efforts of researchers, pharmaceutical industry and regulatory authorities may transfer this potential class of anticancer agents from laboratories to clinics.

LIST OF ABBREVIATIONS

AC	=	Adenylyl cyclase
AEA	=	Anandamide
2-AG	=	2-Arachidonoyl glycerol
BAD	=	B-cell lymphoma 2 (Bcl-2)-associated death promoter
BDMC	=	Bisdemethoxycurcumin
BDS	=	Botanical drug substance
CBD	=	Cannabidiol
CBG	=	Cannabigerol

CBC	=	Cannabichromene
CBN	=	Cannabinol
CBDV	=	Cannabidivarin
CBDA	=	Cannabidiolic acid
CBND	=	Cannabinodiol
CBT	=	Cannabitriol
CNS	=	Central nervous system
CBL	=	Cannabicyclol
CBE	=	Cannabielsoin
CBCA	=	Cannabichromentic acid
CB1	=	Cannabinoid receptors-1
CB2	=	Cannabinoid receptors-2
ERK1/2	=	Extracellular signal regulated kinase 1 and 2
JNK	=	c-Jun N terminal kinase
CXCR4	=	C-X-C chemokine receptor type 4
ERK	=	Extracellular-signal-regulated kinase
ECS	=	Endocannabinoid system
ENT	=	Equilibrative nucleoside transporter
eIF2α	=	Eukaryotic translation initiation factor 2alpha
FAAH	=	Fatty acid amide hydrolase
FA2H	=	Fatty acid 2 hydroxylase
FGF	=	Fibroblast growth factor
GOT	=	Geranyl olivatolate transferase
11-OH-THC	=	11-hydroxy-delta 9-tetrahydrocannabinol
ICAM-1	=	Intracellular adhesion molecule-1
MMP	=	Matrix metalloproteinases
MET	=	R-(+)-Methanandamide
MAGL	=	Monoacylglycerol lipase
MAPKs	=	Mitogen activated protein kinases
NGF	=	Nerve growth factor
NF-κB	=	Nuclear factor kappa beta
OSCC	=	Oral squamous cell carcinoma
PPARs	=	Peroxisome proliferator-activated receptors
pCBs	=	Phytocannabinoids
PSA	=	Prostate specific antigen
PKB	=	Protein kinase B
Sp	=	Specificity protein
Δ^9-THC	=	Δ^9-Tetrahydrocannabinol
Δ^9-THCA	=	Δ^9-Tetrahydrocannabinolic acid
Δ^9-THCV	=	Δ^9-Tetrahydrocannabivarin
THC-C4	=	Tetrahydrocannabinol-C4
TRPV1	=	Transient receptor potential cation channel subfamily V member 1
TIMPs	=	Tissue inhibitors of matrix metalloproteinases
TRB3-dependent	=	Tribbles homolog 3-dependent
TRPV2	=	Transient receptor potential of vanilloid type 2
TRPM8	=	Transient receptor potential of melastatin type 8
VEGF	=	Vascular endothelial growth factor

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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