A Review on Potential Role of Cannabinoids as Therapeutic Agents in Different Types of Cancer

A Project submitted

by

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Dedicated to my beloved parents who always supported me and teachers who guided and assisted me to achieve my goals.

Certification Statement

This is to certify that the project titled "A Review on Potential Role of Cannabinoids as Therapeutic Agents in Different Types of Cancer" is submitted for partial fulfillment of the requirements for degree of Bachelor of Pharmacy from Department of Pharmacy, BRAC University comprises my own work under supervision of Dr. Mesbah Talukdar, Associate Professor of Department of Pharmacy, BRAC University and that appropriate credit is given where I have used the language, ideas or writings of another.

Signed

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Countersigned by the supervisor

_____

Acknowledgement

First of all, I am very grateful to Almighty god for his blessings on me to accomplish my work. Then, I want to convey my gratitude towards Dr. Eva Rahman Kabir, respected chairperson of the Department of Pharmacy, for her advice and support during my project work. Next, I am really thankful to my honorable supervisor Dr. Mesbah Talukdar, for his consistent assistance, inspiration and encouragement. Without his direction and guidelines, it would have been impossible for me to complete my project successfully.

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Abstract

Cannabinoids are the main chemical component of marijuana plant, which is also known as psychotropic and habit forming substance. There is a long history of using cannabinoids in therapeutic purpose. According to Baker et al.(2003), even Queen Victoria was recommended cannabis for alleviation of dysmenorrhea. Currently in the area of cancer research role of cannabinoid's has been created a great interest. Medicinal marijuana has been approved for therapeutic purpose in various nations, for example, Canada, Australia, Israel, and Germany. In USA synthetic cannabinoid based medicine Nabilone and Darbinol is approved by U.S. food and Drug Administration (FDA) for treatment of nausea and vomiting that is side effect of chemotherapy. In addition, researchers found that endocannabinoid system has important function as pro-tumorigenic. Many preclinical *in-vitro* examinations recommend that, Δ9-THC (tetrahydrocannabinol) or other phaytocannabinoid, synthetic and endocannabinoids have anti-proliferative, anti-metastatic, anti-angiogenic, pro-apoptotic effects to lung carcinoma, lymphoma, gliomas, skin carcinoma, prostate carcinoma, pancreatic growth and breast carcinoma. Recent studies suggest that cannabinoids have ability to regulate cellular signaling pathways which create critical environment for cell growth and survival. This review is a summary of function of different cannabinoids in different types of cancer and lastly correlates the situation in Bangladesh perspective.

List of abbreviations

CB1: Cannabinoid receptor type 1

CB2: Cannabinoid receptor type 2

CINV: Chemotherapy induced nausea and vomiting

THC: Tetrahydrocannabinol

CBC: Cannabichromene

CBD: Cannabidiol

CBN: Cannabinol

CBG: Cannabigerol

THCV: Tetrahydrocannabivarin

CBGV: Cannabigerovarin

AEA: Anandamide

2-AG: 2 Arachidonoyl-glycerol

PEA: Palmitoyl-ethanolamide

Ach: Acetylcholine

GPCR: G protein couple receptor

HCC: Hepato cellular carcinoma

FAAH: Fatty acid amid hydrolase

STP: Serine palmitoyltransferase

AKT: Protein kinase B

TRIB3: Tribbles-homologue-3

mTORC2: Mammalian focus of rapamycin complex 2

ERK: Extracellular receptor kinase

ATF-4: Activating transcription factor 4

MAPK: Mitogen-activated protein kinase

Chapter 1: Introduction

1. Introduction:

In spite of the ongoing surge of interest for the potential therapeutic utilization of cannabis, it deserves recollecting that cannabis is anything but another medication. It has a long history of therapeutic and in addition nonmedical use in numerous parts of the world. At the point when cannabis was acquainted with Western prescription, its medical applications were quickly perceived and its utilization spread quickly. (Hand, Blake, Kerrigan, & Samuel, 2016). Hundreds of years back, the Chinese prescription recommends cannabis plant to treat discomfort and mind flight. The earliest evidence of cannabis development originates from China as pollen deposit found in the town site of Pan-p'o dated to 4000 BCE. Around then cannabis were respected among the 'five grains and was cultivated as a noteworthy food crop and its significant part in the generation of materials, rope, paper, and oil. The main record of its utilization in pharmaceutical originates from the Pen-ts'ao ching, the world's most established Pharmacopeia. In spite of the fact that gathered between 0-100 AD, the Pen-ts'ao has been attributed to Emperor Shen-nung, who ruled in 2700 BCE. It perceives cannabis as being valuable for in excess of 100 illnesses, including rheumatic arthritis, gout and jungle fever (Baker, Pryce, Giovannoni, & Thompson, 2003). A British doctor and specialist William O'Shaughnessy found the pain relieving, appetite stimulant, antiemetic, muscle relaxant and anticonvulsant properties of cannabis when he was in India in 1839. The publication of his perceptions immediately prompted the development of the therapeutic utilization of cannabis (O'Shaughnessy, 1843). According to Baker et al.(2003), even Queen Victoria was recommended cannabis for alleviation of dysmenorrhea .In the glorious Egyptian history of 2nd century it was first consider as anticancer medicine by Fayyum Medical Papyrus. In the mid nineteenth century, Europe was among the last civic establishments to experience the plant, with veering purposes behind utilizing cannabis. In France, the psychoactive impacts of cannabis were sought after, while in England the utilization of cannabis concentrated on medicinal purposes.13 kinds of Cannabis removes were recorded in the British, and later in the US Pharmacopeia (1850), for narcotic and anticonvulsant impacts (Madras, 2015). Around the turn of century, the medicinal utilization of cannabis declined, in light of the fact that reproducible clinical impacts could not be acquired. During the time, dynamic constituent in cannabis had not been separated in pure form. Work on medicinal utilization of cannabis was continued in 1964 when pure form of the major and fundamental compound of cannabis, Δ-9-tetrahydrocannabinol (THC) was isolated (Mechoulam & Hanuš, 2001).

There are different types of cannabis. The varieties of cannbis with greatest significant effects are *Cannabis sativa*, *Cannabis indica* and *Cannabis ruderalis*. The biggest assortment is the *Cannabis sativa*, develops in both tropical and pacific atmospheres. There are two fundamental classifications got from cannabis, which are marijuana and hashish. Cannabis is a Mexican expression at first credited to low cost tobacco however today it is recommended as the dried leaves and flowers of the hemp plant. On the other hand, Hashish is the Arabic name for Indian hemp that is the resin of the plant. (Ben Amar, 2006). Both therapeutic and recreational purpose *Cannabis sativa* plant has been utilized for a many years. The major 3 classes of bioactive compound that it contains are flavonoids, terpenoids and in excess of 60 sorts of cannabinoids.(Chakravarti, Ravi, & Ganju, 2014). The main chemical component of this marijuana plant is cannabinoids, which is referred as psychotropic and habit forming agent. The discovery of most active constituent of this plant is Δ9-tetrahydrocannabinol (Δ9-THC) opens the better way to recognizable evidence of the molecular activity of different cannabinoids and the cannabinoid receptors. Cannabinoid is nothing but a group of complex synthetic substances (terpenophenolic aggravates) that shows the vast majority of their activities when bind with specific cannabinoid receptor individually (Sarfaraz, Adhami, Syed, Afaq, & Mukhtar, 2008). First CB1 and CB2 receptors was discovered and identified in mammalian tissues, which showed the difference in their expression pattern (Bosier, Muccioli, Hermans, Lambert, 2010). CB1 receptors are situated all over the body, but mostly elevated found in the different parts of brain where these receptors exert psychoactive impacts. Expression of CB2 receptor is generally restricted specifically in the immune system improved territory of B lymphocyte (Madras, 2015). Endocannabinoid system has significant role in different disorder like Alzheimer's disease, Parkinson's disease,glaucoma and cancer which is composed by cannabinoid receptors and their ligands.

Currently in the area of cancer research role of cannabinoid's has been created a great interest. Cancer is a disease occurred due to uncontrolled multiplication of cells and their capacity to attack towards different tissues and spread.To treat cancer anti cancer substances used that induce apoptosis, impair cell cycle or DNA damage operators. The main discovery in carcinoma disease in cannabinoid use is its capacity in focused murdering of tumors. Many preclinical in vitro examinations recommend that, Δ9-THC or other phaytocannabinoid, synthetic and endocannabinoids have anticancer effect to lung carcinoma, lymphoma, gliomas, skin carcinoma,

prostate carcinoma, pancreatic growth and breast carcinoma (Sarfaraz et al., 2008). These discoveries were also appreciated by in vivo considers and the major part of impacts of cannabinoids are intervened by means of CB1 and CB2. For few cannabinoid an extra receptor has been identified called transient receptor potential vanilloid compose 1 (TRPV1).. In a few countries Marinol, Dronabinol that is synthetic form of THC and its subordinate nabilone (Cesamet), and Sativex, have been approved to treat sickness and growth related complications in tumor patients experiencing chemotherapy (Velasco, Sánchez, & Guzmán, 2012).

1.1 Legislation for medical and recreational cannabis:

Cannabis has been utilized for therapeutic purposes for a thousand of years yet to a great extent dropped out of support in Europe and North America in the twentieth century as better solutions were designed and disallowance confined access (O'Shaughnessy, 1843). The 1961 Single Convention on Narcotic Drugs enables signatories to deliver cannabis for medicinal purposes as long as development is controlled by a governmental organization. There is a developing assemblage of research on the remedial advantages of entire plant material and separates and the exploration base is relied upon to grow as limitations on cannabis are relaxed (Kilmer, 2017). The first state that legalizes medicinal cannabis in the United States in 1996 was California. At the time of that initial copy, Washington D.C. with other 29 states, have authorized medicinal marijuana under different usage conditions and courses of organization. Washington, D.C. and other eight states of United States have endorsed recreational cannabis. The dynamic scene can be observed in the United States for both therapeutic and recreational marijuana (Jett, Stone, Warren, & Cummings, 2018). Medicinal marijuana has been approved in various nations, for example, Canada, Australia, Israel, Germany, and others, and it administrative condition is quickly evolving. In 2013, the Czech Republic passed a medical law, and locally created cannabis for the program was first conveyed to the State Agency for Medical Cannabis in 2016 (preceding then it had been foreign made). In 2017, Germany passed a law to extend access to therapeutic cannabis and to permit residential generation (already, it must be transported in) (Kilmer, 2017). In Uruguay marijuana that used as recreational purpose was endorsed in 2013 and later in 2017, Canada is probably going to be affirmed (Jett et al., 2018). Australia, in 2017 passed a law at the Commonwealth level to make an administrative system for commercial cannabis generation for therapeutic purposes (Kilmer, 2017).Cannabis has ordered as schedule-I

substance according to the Controlled Substance Act of 1970, involving the maximum prohibitive substances that "have no therapeutic utilize". The administration related to Drug Enforcement dismissed a request of to rename cannabis as Schedule II tranquilizes in 2016. Pharmaceutical review cannabinoids are arranged in an unexpected way, functioning to Schedule III from Schedule I and incorporate a few U.S. food and Drug Administration (FDA)- endorsed cannabinoid-based medications(Jett et al., 2018). Dronabinol capsule (tetrahydrocannabinol) is endorsed in USA for use in CINV (chemotherapy induced nausea vomiting) and has been approved in patients with analyzed human immunodeficiency infection who have anorexia for stimulation of appetite. Synthetic medicine darbinol have higher affinity CB1 though bind with both CB1 and CB2 receptors. Nabilone is another synthetic cannabinoid approved by FDA for CINV. Nabiximol is prescribed as oral spray for pain relieving impacts and spasticity because of multiple sclerosis. It is actually nothing but a blend of THC and CBD in a 1:1 proportion. Nabiximol is approved Canada, Germany and 15 other unique nations however, in United nations it is as of now under FDA audit (Śledziński, Zeyland, Słomski, & Nowak, 2018). Another synthetic cannabinoid is levonantradol gotten from dronabinol, yet it is 100 times more powerful. Created in the 1980s by Pfizer (New York, NY), the medication isn't approved for use by patients however has been utilized as a part of preclinical investigations to assess agonist impacts of CB1 receptors(Jett et al., 2018).

1.2 Cannabinoids:

There are three primary sources for cannabinoids. They are: (i) Phytocannabinoids are combination of different types of cannabinoid that extracted from plant sources; (ii) Endocannabinoids are one kind of neurotransmitters delivered in the brain and carry out on cannabinoid receptors; (iii) Synthetic cannabinoids, synthesize in the lab, are basically closely resembling phytocannabinoids or endocannabinoids and act by comparative organic system (Madras, 2015). At first cannabinoids were defined as a structure of C21 terpenophenolic compound particularly formed by cannabis (Mechoulam and Gaoni 1967). However, consistent improvement cannabinoids by producing synthetic analogue (e.g., HU-210) has obscured this definition. Additionally, the discovery of endogenous cannabinoids (e.g.,anandamide), characterized as "endocannabinoids" by DiMarzo and Fontana (1995) has changed the traditional concept . In this manner, Pate (1999) suggested the expression of "phytocannabinoids" to assign

the C21 compounds delivered by cannabis. In compare with synthetic cannabinoid, phytocannabinoids show low mammalian toxicity, and combination of cannabinoids are less dangerous than pure THC (Thompson, Rosenkrantz, Schaeppi, & Braude, 1973).

After THC, Cannabidiol (CBD) is the going with best-considered phytocannabinoid as medicinal cannabis has higher concentration of this. CBD has narcotic characteristic which also diminishes the pure THC induced anxiety and other unpleasant mental responses demonstrated by a clinical trial. It also gives antipsychotic effects (Waldo Zuardi et al., 2012). It increases dopamine movement, completely inhibits serotonin take-up, and improves norepinephrine action. CBD has ability to protect neurons from glutamate toxicity and works as a cancer prevention agent, more powerfully than ascorbate and α-tocopherol. In the brain CBD cannot reduce acetylcholine circulation (McPartland & Russo, 2001). On the other side, THC diminishes hippocampal ACh discharge in rats and these associates with short-term memory loss. Additionally, THC in the hippocampus restrains N-methyl-D-aspartate (NMDA) receptor action(Shimizu, Tang, Rampon, & Tsien, 2000). An experiment shows that CBD in combination with THC exhibited a synergetic effect in reduce of intestinal motility in mice. This might be an assertive part of observed advantages of cannabis in inflammatory bowel disease (Waldo Zuardi et al., 2012). CBD gives a better analgesic activity than THC; it has ability to represses erythema significantly more than THC, it also inhibits COX- movement with a greater maximum inhibition in compare to THC, and it further inhibits lipoxygenase (asthma-inciting leukotrienes producing chemical), more appropriately than THC. CBD really eliminates microbes and organisms, with a more prominent intensity than THC. (McPartland & Russo, 2001).

Cannabinol (CBN) is the phytocannabinoid that is reduction result of THC and found frequently in matured cannabis items. In human CBN intensify the impacts of THC though it shows just opposite impacts of THC in mice. CBN expands plasma concentration of follicle-stimulating hormone, and improves the creation of testicular testosterone. CBN contributes a few qualities to CBD; such as, it has anti-convulsant activity and mitigating action. CBN has partiality for CB1 receptors and acts as an agonist (Turner et al. 1980).

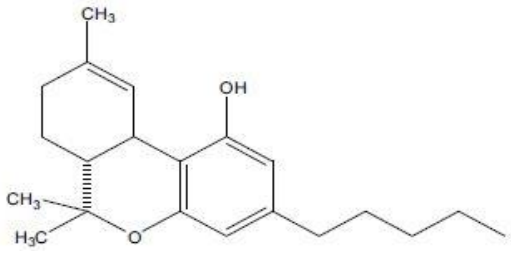
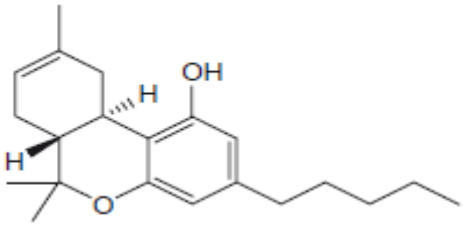
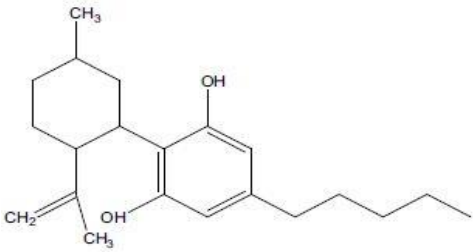
Another identified phytocannabinoid is cannabigerol (CBG) that is considered as the biosynthetic precursor of other cannabinoids like CBC, CBD, and THC, and is available just in lower amount. In compare with THC,CBG is the “inactive" phytocannabinoid, yet CBG shows

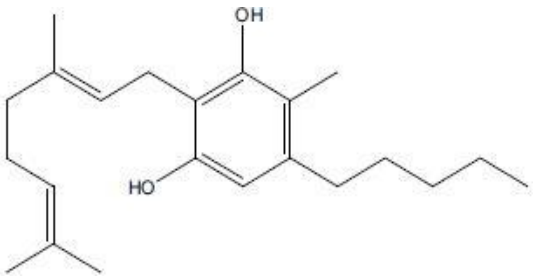
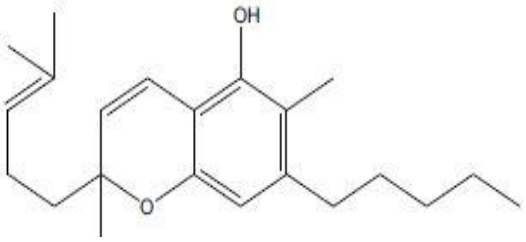
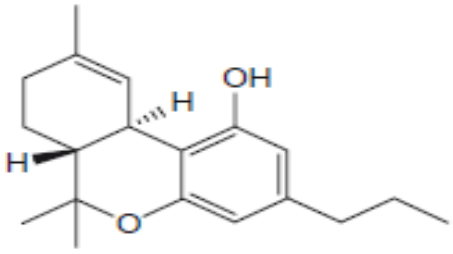
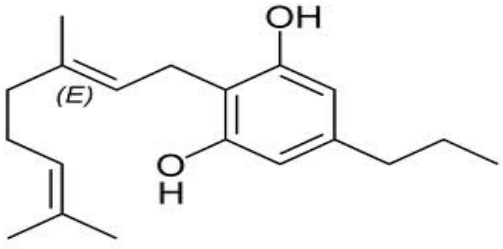
affinity for CB1 receptors which is insignificant like CBD (Mcpartland, 2000). CBG has antibacterial properties. It has better action against gram-positive microscopic organisms, mycobacteria, and growth in contrast with THC, CBD, and CBC. CBG plays an important role in oral epitheloid carcinoma by inhibiting cancer cell (McPartland & Russo, 2001).

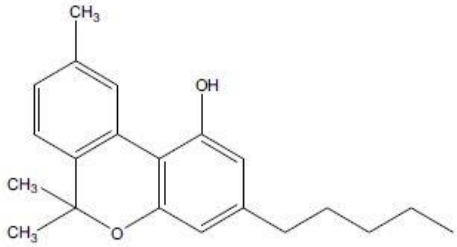
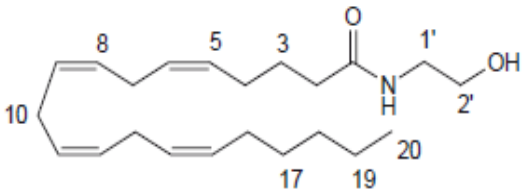
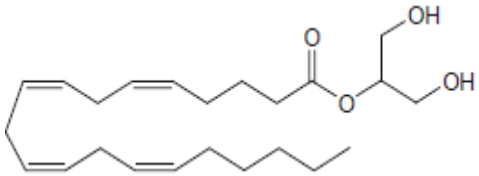
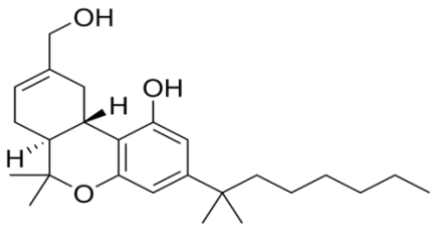
The fourth major cannabinoid is cannabichromene (CBC) that found overwhelmingly in tropical Cannabis spp. strains. CBC was usually misidentified as CBD before the mid-1970s, because CBC and CBD have almost a similar maintenance time in gas chromatography. Like CBD, CBC reduces irritation and gives analgesic impacts. CBC inhibits synthesis of prostaglandin in vitro; however, CBD or THC shows stronger action. CBC displays solid antibacterial action and mellow antifungal movement, better than THC and CBD in many examples(Davis & Hatoum, 1983) .

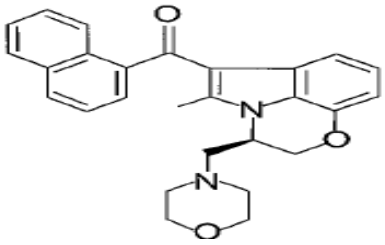
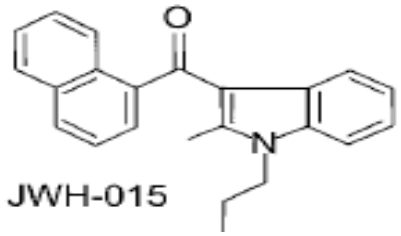
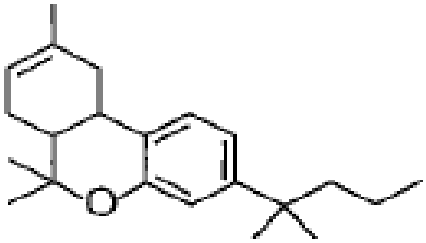
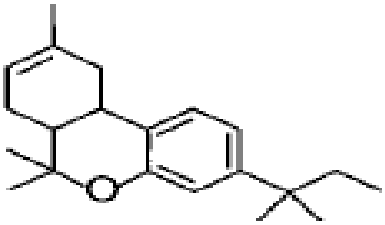
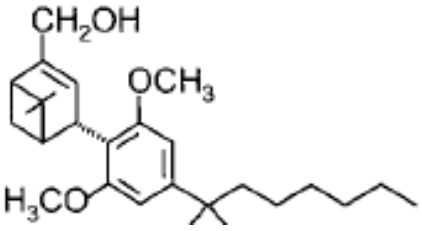
The principle psychoactive constituent found in cannabis that is Δ -9-THC and Δ8-THC is an isomer of it. These two molecules are differentiates just by the region of the double bond present in the cyclohexal "C" ring. Δ9-THC exhibits more psychoactive properties than Δ8-THC. The chemical stability and relative simplicity of Δ8-THC synthesis contrasted with Δ9-THC, have produced Δ8-THC the layout for the advancement of two essential synthetic subordinates, one of them possess a strong psychoactive CB1 agonist, HU-210, and the other one is non-psychoactive antiemetic and neuroprotectant, HU-211which is also known as dexanabinol (Mechoulam & Hanuš, 2001). Δ8-THC was utilized clinically in a vital examination in which hematological malignancy effected children were treated with the medication to treat chemotherapy-related sickness and retching (McPartland & Russo, 2001). Tetrahydrocannabivarin (THCV) is a homolog of Δ9-THC having propyl side chain, primarily found in indica and afghanica type of cannabis, for example, hashish from Nepal, India, Pakistan, Afghanistan, Zambia, Thailand, dagga from Southern and western Africa. Compare to Δ9-THC, THCV possess only 20-25% as psychoactive property (Hollister 1974). It exhibits a quicker onset of action than Δ9-THC, is of short duration, and synergizes THC action. THCV uses more as anti-diabetic, appetite stimulant and anxiolytic substance rather than as anti-cancer drug (McPartland & Russo, 2001).

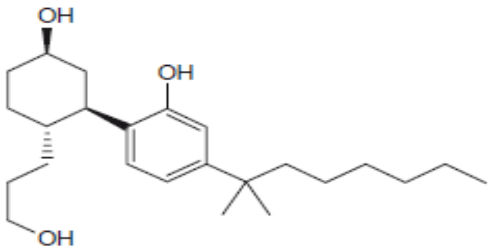
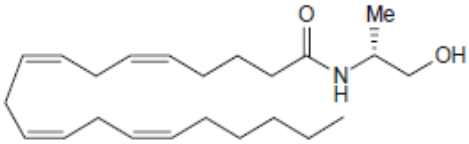
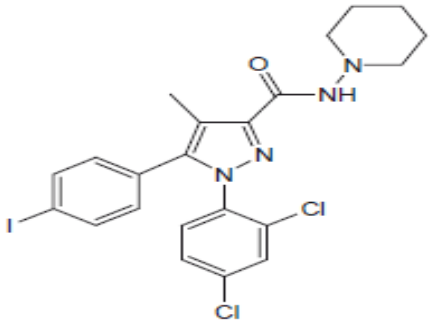
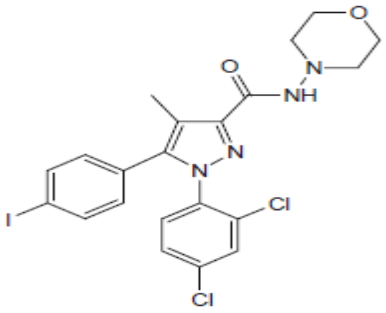
Table 1.1: Structure of different cannabinoids and their role in different physiological processes

Cannabinoids	Structure	Role
Δ-9-tetrahydrocannabinol (THC)		Antiemetic, Appetite stimulant, Tumor growth inhibitor, Pain reliever(analgesic) (Chakravarti et al., 2014)
Δ-8-tetrahydrocannabinol (Δ8-THC)		Anti-tumor agent, In human sperm it inhibits mitochondrial O_2 consumption, Antiemetic, Appetite stimulant (Chakravarti et al., 2014)
Cannabidiol (CBD)		Anxiolytic, Analgesic Antipsychotic, Anti-inflammatory, Antioxidant, Antispasmodic (McPartland & Russo, 2001)

Cannabigerol (CBG)		Multiple sclerosis, Neurological disorder treatment ,Anti-nausea medication , Anti-inflammatory agent, (Chakravarti et al., 2014)
Cannabichromene (CBC)		Anti-inflammatory agent, Anti-fungal, Anti-depressant, Anti-nociceptive (Chakravarti et al., 2014)
Tetrahydrocannabivarin (THCV)		Hepatic ischemia, Anti-inflammatory (Chakravarti et al., 2014)
Cannabigerovarin (CBGV)		Anti-inflammatory (McPartland & Russo, 2001)

Cannabinol (CBN)		Oxidation breakdown product, Sedative, Antibiotic (McPartland & Russo, 2001)
Anandamide (AEA)		Antiemetic, Stimulates appetite, Inhibits tumor growth (Chakravarti et al., 2014)
2-arachidonoyl-glycerol (2-AG)		Antiemetic, Pain reliever Stimulates appetite , Inhibits tumor growth
Palmitoyl-ethanolamide (PEA)		Neuromodulatory , Immunomodulatory
HU-210		Analgesic, Multiple sclerosis, Neuroprotective

R-(+)-WIN 55,212-2		Antiemetic, Pain reliever, Stimulates appetite, Inhibits growth of tumor, Multiple sclerosis
JWH-015		Anti-tumor, Anti-inflammatory, Anti-nausea medication
JWH-133		Neurological disorders, Anti-cancer
JWH-139		Antiemetic, Stimulates appetite, Inhibits growth of tumor, Pain reliever
HU-308		Inhibits tumor in glioma, Skin carcinoma, Lymphoma

CP-55940		Appetite-stimulant,Pain reliever,Antiemetic, Tumor growth inhibitor, Multiple sclerosis
R-(+)-methanandamide		Stimulates appetite, Pain reliever, Inhibits growth of tumor, Antiemetic
AM251		Metabolic syndrome
AM281		Shows numerous pharmacological activities, In morphine withdrawal mice helps to Improve recognition loss induced by naloxone

1.3 Cannabinoid receptor and its distribution:

It was at first thought that cannabinoids apply different natural impacts by rapturing the cell membrane nonspecifically because of the lipophilic characteristics. The discovery of THC that resulted in the uprising of a few synthetically produced cannabinoids. It also shows the effective signaling and the pharmacological classification about binding sites of cannabinoid in the brain, the presence of an assumed cannabinoid receptor and its affinity towards GPCR (G-protein couple receptor) nature. There are two sub type of known cannabinoid receptors discovered termed as cannabinoid receptor type-1 (CB1) and cannabinoid receptor type-2 (CB2).The receptor which shows similar properties of a GPCR, is currently identified as CB1 (Gaoni & Mechoulam, 1964). In human CB1, receptor is translated by the CNR1 gene and comprises of 472 amino acids. A few varieties of CNR1 have been related with Cannabis reliance (Whellan et al., 2013). A research by Zou and Kumar (2018) illustrated, the basic structural variations of CB1 receptor on agonist binding, developed the composition of the various recognized structures and signaling affinity of CB1 receptor agonists. Despite the long type of the CB1R receptor established, additionally two isoforms have been characterized with shorter N-end, both coming about because of alternative splicing. Lately, at the mRNA of human brain, pancreatic islet, liver, and skeletal muscle the distinctive expression have been characterized of examples of these three isoforms. The full-length of CB1 receptor regulates mainly in the skeletal muscle and brain, while the isoform CB1Rb (in which at the N-end 33 amino acid removed) present in liver and pancreatic islet cells demonstrates a higher expression level where it involves in the process of digestion. The physiological and pharmacological characteristics of the two splice variations still can't seem to be investigated, as present examinations achieved in non-human models disclosed discrepancies (Zou & Kumar, 2018). CB1R was initially found in the brain. Following on, CB1R was appeared as most generally communicated receptor protein from the GPCR group in the brain by utilizing autoradiography, immunohistochemistry and in situ hybridization. The highest expression of CB1 receptor shown in the brain incorporates olfactory lobe, limbic system, basal ganglia, and cerebellum. In the outer layer of the cerebrum, amygdala, hypothalamus, parts of the brainstem and the dorsal root of spinal cord moderate expression of CB1 receptor is found. Low expression of CB1 receptor is found while it is localized in the thalamus and the ventral horn of spinal line. Despite neurons, the CB1R is communicated, regardless of the way that to a very lower degree, in different types of neurons like where it has been appeared to intervene

synaptic transmission (Mackie, 2005). Additionally in the peripheral nervous system, (PNS) CB1R is highly expressed and in the peripheral tissues in particular way. In PNS, the CB1R is

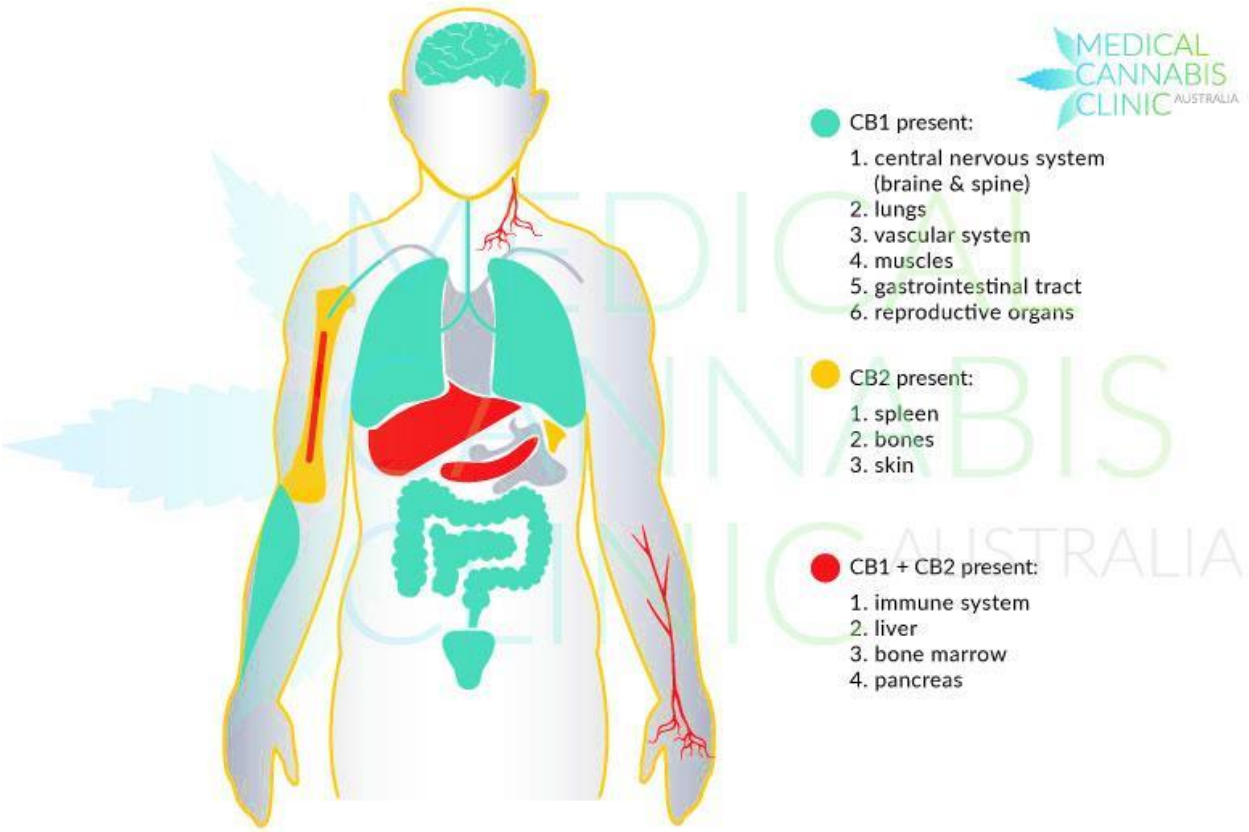


Figure1.1: Illustrates the distribution of cannabinoid receptor throughout our body.CB1 receptors are found in the brain, skeletal muscle, lung, gastrointestinal tract, reproductive tissues and in cardiovascular system. In contrast, CB2 receptors are present in the immune derived cells, extensively in the spleen. Moreover, both CB1 and CB2 receptors are present in the central nervous system, liver, pancreas and bone marrow.
Adapted from: www.medicalcannabisclinic.com.au

for the most part communicated in thoughtful nerve terminals. In addition, the CB1 receptor is found in trigeminal ganglion, dorsal root ganglion, and dermic nerve endings of primary sensory neurons, where it controls nociceptive pain from afferent nerve filaments. The CB1 is also present in the gastrointestinal (GI) tract where it balances the flexibility of GI tract, the discharge of gastric acids, liquids, neurochemical, hormones through neuronal and non-neuronal routes, and additionally the penetrability of the intestinal epithelium. As a result, CB1 might control

hunger from the hypothalamus within the CNS and manage the vitality regulate and sustenance admission from the GI tract also. Interestingly, hepatic CB1R as well takes an interest in the management of vitality adjust and digestion, yet in associate with irregular means. Normally, the CB1R outflow in the liver is low(Izzo & Sharkey, 2010). On the opposite side, Montecucco & Di Marzo (2012) stated that, the CB1R expression in a few varieties of hepatic cells is surprisingly enhanced under pathological conditions, wherever the CB1R effectively adds to hepatic insulin obstruction, fibrosis, and lipogenesis. Additionally, in the pathological condition the CB1 is controlled the cardiovascular system, therefore advances disease progression and cardiac dysfunction. CB1 initiation in myocardiocytes, vascular endothelial cells, and smooth muscle cells causes oxidative pressure, inflammation and fibrosis (Montecucco & Di Marzo, 2012). Moreover, the CB1 expression has been found in fat tissue, striated muscle, bone, skin, leydig cells, ovaries and several kinds of cancer cells (Zou & Kumar, 2018).

After three years of the discovery of CB1, another receptor CB2R distinguished in macrophage of spleen. In human CB2 comprises of 360 amino acid and encoded by the CNR2 gene. It expresses just 44% sequential homology to CB1 only in transmembrane region but receptors present in other parts of the body possess 68% amino acid similarity with CB1. The 2-arachidonoylglycerol (2-AG) is considered as the principal endogenous ligand for the CB2 receptor. In human, two isoforms of the CB2 have been recognized, with one overwhelmingly present in testis and at lower levels in brain remunerate areas, though the other is predominantly found in the spleen and at lower levels in the brain (Liu et al., 2009). Follow up research uncovered that in immune cells CB2 shows dominating expression and in the cardiovascular system, GI tract, liver, fat tissue, bone and reproductive system average expression is observed. According to Howlett (2002), CB2 was not present in the CNS, in this way called to as ' the fringe CBR'. However, this idea has been tested recently by a few examinations showing the CB2 expression in the brain, which is very inferior compare to the immune system or the presence of CB1. In spite of the fact that, in the CNS and PNS the expression of the CB2 is comparatively limited, it is obvious that the CB2 considered as functioning part in neurological activities, for example, nociception, tranquilize habit and neuroinflammation (Dhopeshwarkar & Mackie, 2014). Primary research on CB2 has concentrate on the functioning effects of this particular receptor on the leukocytes immunological activity. Specifically, different functions like immune suppression, initiation of apoptosis, and inhibition of cell migration has been

modulated by the involvement of CB2 receptor. Later studies revealed that CB2 receptors agonist JWH-015changes in cAMP levels as a result phosphorylation of leukocyte receptor tyrosine kinase at Tyr-505 occurs, which ultimately inhibits the signal of T-cell receptor. Therefore, CB2 agonists may also be beneficial in inflammation and pain treatment (Cheng & Hitchcock, 2007).

1.4 Function of endocannabinoid system in tumor formation and development:

The endocannabinoid system is considered as a biological system which is composed of endocannabinoids that are primarily lipid based neurotransmitter, receptors and proteins related to the synthesis, transport and degradation of endocannabinoids which has various activities within the body (Katona & Freund, 2008). Endocannabinoids principally works like retrograde messengers that influence the discharge of various neurotransmitters in the neural and peripheral tissues. In addition, endocannabinoids has important function in inflammation, insulin sensitivity, and fat and energy metabolism. So, frequency of metabolic disorder can be reduced by blocking endocannabinoids (Chakravarti, Ravi, & Ganju, 2014). N-arachidonoylethanolamine (AEA-anandamide) and 2-arachidonoylglycerol (2-AG) are considered as the best endocannabinoids ligand that has impact on our mood, inflammation reaction, pain sensation, appetite stimulation, and memory. Both endocannabinoids are produced on request (in spite of controversy exists on account of 2-AG), in response to expanded intracellular Ca2+ focus (Zou & Kumar, 2018). A comparatively huge number of information has collected in the most recent years related to the tumor formation and function of endocannabinoid system, which showed in table-2. Most of the time, these reports demonstrate that endocannabinoids level and their receptors are increased in malignancy, a circumstance that as often as possible connects with tumor development (Malfitano et al., 2011). Therefore, anandamide and 2-AG are looked as if it would be over-expressed in a very few kinds of tumors together with pituitary adenoma, glioblastoma multiforme (GBM), prostate cancer, colon cancer and endometrial sarcoma. Additionally, regulating endocannabinoid levels are related with expanded disease movement in a mouse model with metastatic malignant melanoma and in human examples of this same disease (Sailler et al., 2014). A research related to this issue has proposed a circumstance for cannabinoid receptors condition and degrading endocannabinoid enzymes. Hence, CB1 level was observed to be up regulated in Hodgkin lymphoma cells and in

the patient with hepatoma both type of receptor expression are associated with proper diagnosis (Mukhopadhyay et al., 2015). In addition levels of CB1 receptor are increased with severity of disease in human ovarian tumor and have been proposed to be a factor of poor anticipation following surgery in stage IV colorectal malignancy (Guillermo Velasco, Hernández-Tiedra, Dávila, & Lorente, 2016). Research found a connection between CB2 receptor and breast cancer in respect to its expression, histologic review and prognosis (Caffarel, Sarrió, Palacios, Guzmán, & Sánchez, 2006) and glioma (Sanchez, Ceballos, Gomez, Sa, & Guzma, 2001). In the glioma, it has been showed that increase of both CB1 and CB2 receptors along with a decreasing the amount of the enzyme engaged with endocannabinoid degradation contrasted with healthy controls. So in mantle cell cancer CB1 and CB2 receptor expression is improved, whereas fatty acid amide hydrolase (FAAH) expression is diminished contrasted with non-malignant B-cells(Sarfaraz, Adhami, Syed, Afaq, & Mukhtar, 2008). Recently, the role of GPR55 a non-standard cannabinoid receptor in cancer advancement has been characterized. Higher microscopic evaluations of human glioblastomas, breast, pancreatic and skin malignancies have been accounted for in association with expanded GPR55 expression. (Sanchez et al. 2001).

Table 1.2: variation in the CB receptors expression or endocannabinoids (ECB)-degrading enzymes in human cancer (Guillermo Velasco et al., 2016)

Type of tumor	Condition of CB receptors or ECB enzymes
Hodgkin lymphoma	Increased levels of CB1
Non-Hodgkin lymphoma	Increased levels of CB1
Chemically-induced cellular hepatic-carcinoma	Increased CB1 level

Liver cancer (HCC)	Expression of CB1 and CB2 correlates with improved prognosis in the patients with hepatoma
Ovarian cancer	Increased level of CB1, correlates with severity of disease
Stage IV colorectal cancer	CB1 levels reduced, growth of colon carcinomas increase due to CB1 genetic ablation
Pancreatic cancer	Increased level of CB1 and CB2, monoacylglycerol lipase (MAGL) and FAAH levels reduced which is associated with poor prognosis
Prostate cancer	In severe stage of disease CB1 levels enhanced
Prostate cancer	FAAH levels changes with the severity of the disease but not the CB1 level
Breast cancer	Increased CB2 level related to severity of disease

Glioma	CB2 levels enhanced with intensity of gliomas
Mantle cell lymphoma	Level of FAAH decreased but Both CB1 and CB2 levels increased
Skin cancer	Ultra violet light induced skin carcinoma is decreased due to genetic ablation of CB1, CB2 receptor
Leukemia	After viral infection, overexpression of CB2 enhances the susceptibility to leukemia
Glioma, breast cancer, skin carcinoma	In higher histological tumor grade, higher level of GPR55 is found

Altogether, this information recommends that the endocannabinoid has important function as s a pro-tumorigenic component.In addition, CB2 receptor distinctive perceptions appreciate that the endocannabinoid system considered as a tumor controller part in various type of cancer. In this manner, intestinal tumor may developed due to genetic inactivation of CB1 receptor (Guillermo Velasco et al., 2016).

1.5 Anticancer activity of cannabinoid:

Tumor development can be impaired by cannabinoids at different levels. The most general impact of cannabinoid is that the initiation of cancer cell death by apoptosis or programmed cell death and the cancer cell growth inhibition (G. Velasco, Sánchez, & Guzmán, 2016). No less

than one of those activities has been exhibited in all malignancy cell type tested. In the past fifteen years, a wide range of researches has trying to demonstrate the mechanism pattern of cannabinoid. Different kind of cannabinoid obtained from plant origin like THC, endogenous cannabinoid such as 2-AG (2-arachidonoylglycerol) or synthetic cannabinoid like JWH-133, WIN 55,2121-2 that are CB1 or CB2 agonist, shows antitumor impacts in laboratory experiment. Due to antitumrigenic characteristic of CB receptor, treatment with cannabinoid progress the death of cancer cell, inhibit tumor development , block invasion and progression (Guillermo Velasco, Sánchez, & Guzmán, 2012).

1.5.1 Cancer cell death induced by cannabinoids:

The mechanism of anticancer activity of cannabinoid largely depends on the capability of those factors that helps to activate autophagy-mediated apoptotic death of cancer cell (Guillermo Velasco et al., 2012). Early researches demonstrated that, in the glioma cell apoptosis is initiated by THC and other cannabinoids which is dependent on the stimulation of CB1and CB2 receptors (Gómez del Pulgar, Velasco, Sánchez, Haro, & Guzmán, 2002). Additional researches on glioma cell that is specially THC sensitive and resistant provided advance understanding of the particular signaling events produced by cannabinoids in cancer cell that is downstream of ceramide. In this manner, it was identified that, THC treatment has ability to increase expression of the P8 that is a kind of stress-regulated protein. This is also a transcriptional controller that has been concerned within the control of tumor formation and tumor advancement, along with many of its downstream targets, to illustrate, the endoplasmic reticulum (ER) stress related translation factors ATF 4, CHOP and therefore pseudokinase tribbles homologue 3 (TRIB 3) (Carracedo et al., 2006). This THC activated stimulation of the P8-managed pathway (Figure 2) improves the inhibitory communication of TRIB3, AKT that prompts restraint of the mammalian focus of rapamycin complex 1 and the resulting incitement of autophagy-intervened cell death (Salazar et al., 2009). Autophagy is a fundamental cell process taking an interest in various physiologic functions inside the cell. During autophagy, organelles and other cytoplasmic segments are immersed inside twofold layer vesicles known as "autophagosomes." The autophagosomes are developed with combination of vesicles and lysosomes. Enzymes present in lysosome causes the degradation of the autophagosome. Autophagy mechanism is principally a

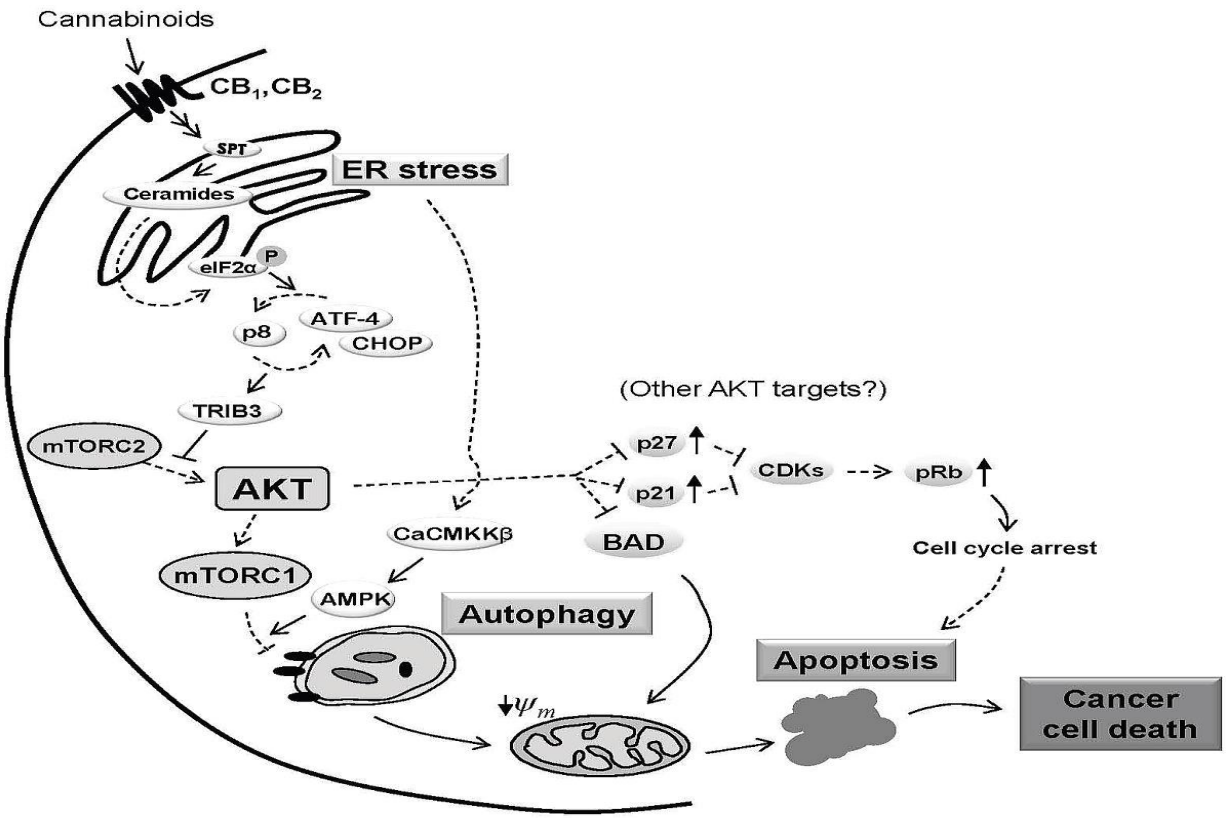


Figure 1.2: Illustrates the pathway of apoptosis induced by cannabinoids that is depends on the ER-stress stimulation and autophagy. This signaling pathway of apoptosis can be found in different types of cancer cell induced by cannabinoid. Here, SPT = serine palmitoyltransferase; ATF-4 = enacting translation factor-4; elF2α = eukaryotic interpretation commencement factor-2α; CHOP = C/EBP homologous protein; AKT = protein kinase B; TRIB3 = tribbles-homologue-3; mTORC2 = mammalian focus of rapamycin complex 2; CDKs = cyclin-subordinate kinases; pRb = retinoblastoma protein; CaCMKKβ = calcium/calmodulin–subordinate protein kinase 2β; AMPK = AMP-actuated protein kinase and P stands for protein phosphorylation on THC therapy.
Adapted from: https://www.semanticscholar.org

cytoprotective system, in spite of the fact that its actuation can- likewise prompt cell death (Verfaillie, Salazar, Velasco, & Agostinis, 2010). The immediate interest of the autophagy as antineoplastic mechanism of cannabinoids can be clearly found in various sorts of cancer in particular, glioma, pancreatic and hepatic malignancy cells (Armstrong et al., 2015). All these researches have supported that, autophagy is the general mechanism responsible for CB receptor induced cancer cell death. According to G. Velasco et al.,(2016), there could be another mechanism for some specific cell collaborate with this autophagy pathway to initiate cancer cell

death. Research conducted in the most recent couple of years has revealed insight into the intracellular signaling systems underlying cannabinoid anticancer activity. In any case, various essential perceptions—specifically, those identified with the pretended by cannabinoid receptors in the activating of the signs stay to be cleared up (G. Velasco et al., 2016)

1.5.2 Cannabinoids inhibit tumor formation, development and progression:

Cannabinoids acts as antitumor substances by blocking the initiation of the vascular endothelial growth factor (VEGF) that is, an activator of angiogenesis in cancer cells. Particularly, completely different elements of the cascade, for instance, the principle substance VEGF and the dynamic varieties of its basic receptors (VEGFR1 and VEGFR2), are down regulated with cannabinoid treatment of skin cancer (Casanova, 2003),gliomas(Sanchez et al., 2001), and thyroid carcinomas(Portella et al., 2003). Activation of cannabinoid receptor present in vascular endothelial cells, represses proliferation, migration, and initiates apoptosis. May be alternative cannabinoid-induced activities causes a standardized tumor vasculature which are more separated smaller and fewer vessels with limited leak (G. Velasco et al., 2016). Researches on different types of cancer in animal model found that, agonist of cannabinoid receptor helps to reduce the tumor formation, inhibit attachment, migration, and invasiveness of glioma (Sanchez et al., 2001),breast(Pisanti, 2007), lung(Jett, Stone, Warren, & Cummings, 2018), and cervical cancer cells in culture. Those impacts depend, in any event to some degree, on the balance of, matrix metalloproteinase-2 (MMP-2) which is a kind of extracellular proteases and their specific tissue inhibitors (Ramer & Hinz, 2008). Another research shows that, particularly in the glioma xenograft inhibition of ceramide biosynthesis nullifies the antitumor and antiangiogenic impact of cannabinoid receptor agonists (CB1or CB2) and reductions VEGF generation in vitro and in vivo (G. Velasco et al., 2016). Additionally, inhibition of MMP-2 that plays an important role in different cancers expression and invasion is counteracted by preventing biosynthesis of ceramide and by reducing expression of P8. Further examine is as yet important to definitely characterize the atomic components in charge of those activities of cannabinoids(Ramer & Hinz, 2008). Sailler et al., (2014) found that, in animal model CBD shows strong effect on CB1 and CB2 receptors as well as delivers an amazing antitumor effect including reduction of invasiveness and metastasis (Sailler et al., 2014).

Chapter 2: Research methodology

2. Research methodology:

To collect information for this review paper a systemic search was carried out in online database resources like PubMed, PMC, Elsevier and Science direct. The information was also obtained from books, online scholarly and different peer reviewed journals found in Oncotarget. Here, a list of some of many journals that were thoroughly searched for this study

- History of medical cannabis
- Cannabis and Cannabis Extracts
- Pharmacological review of cannabinoid
- New developments in cannabis regulation by European Monitoring center of drugs and drug addiction
- Guidance for the use of medicinal cannabis by Australian Government
- Proposed approach to regulation of cannabis in Canada
- Anticancer mechanism of cannabinoids
- Current status and future cannabis research
- Antitumor activity of plant cannabis in breast cancer
- Regulation of endocannabinoids associated with cancer

Recently, cannabinoids has become a center of interest in cancer research. Many researches and clinical application try to establishing cannabinoids as anticancer substance. Also cannabinoids shows the palliative impacts in chemotherapy related nausea, vomiting, appetite stimulation, help with discomfort, state of mood disorder and relief from insomnia in tumor patients. The purpose of this review paper is to collect nearly all the information discovered by scientist related to the anticancer activity of cannabinoid all over the world in past years also including recent researches.

Chapter 3: Cannabinoid in cancer regulation

3. Cannabinoids in cancer regulation:

An effective anti-neoplastic drug is considered when it inhibits mitosis of cancer cell Cancer cells multiply rapidly in uncontrolled way. In addition, cancer cells do not experience normal cell death mechanism, like a healthy cell experiences. Apoptosis is more controlled and regulated mechanism also known as programmed cell death (PCD), requires initiation of caspase (protease enzyme) dependent or independent signaling (Hanahan & Weinberg, 2000). Cannabinoids are signified as anti-proliferative and apoptotic drugs in many researches. In this section the elaborate function of cannabinoids in tumor proliferation, cell cycle and apoptosis in numerous types of cancer is thoroughly described.

3.1Glioma and cannabinoids:

A glioma is a tumor in the glial cells of the brain or the spine and the most aggressive forms of cancer that is often highly resistance to conventional chemotherapies. The presence of cannabinoid receptors in glioblastoma endothelial cells was analyzed by immunohistochemistry, which shows 38% of CB1 and 54% of CB2 receptors. In glioblastoma tissues, CB2 receptor shows higher expression levels in contrast with CB1. For the treatment of glioma selective CB2 agonists could become vital targets. In *in vivo* model of glioma MMP-2 (matrix metalloproteinase-2) that is an extracellular matrix degrading enzyme is inhibited by administration of Δ9-THC and JWH- 133 (Blázquez, Casanova, Planas, & et al., 2003). According to Carracedo, Lorente, et al., (2006), cannabinoid has growth inhibitory effect on cancer cells due to presence of ceramide and P8. If biosynthesis of ceramide and expression of P8 is blocked than, it prevents the growth inhibitory impact of cannabinoids. Another study showed that, activation of ERK1/2 and inhibition of AKT signaling occurs due to administration of Δ9-THC and WIN-55,212-2 (Ellert-Miklaszewska, Kaminska, & Konarska, 2005). Furthermore, stimulation of phosphorylation in eIF2alpha and consequently initiation of ER stress response that advanced autophagy by means of inhibition of the AKT or mammalian target of rapamycin complex-1 (mTORC1) axis can be observed through Δ9-THC administration. For the *in vivo* anticancer activity of cannabinoids the activation of this pathway was necessary (Salazar et al., 2009). Besides, in vitro study found that in glioma cell apoptosis is induced and tumor formation is suppressed by CBD through initiation of caspases signaling pathway and reactive oxygen species by means of receptor-independent process. Moreover, research showed

that apoptosis in glioma cell in somehow dependent on Ca2+ concentration and TRPV2-dependent Ca2+ influx is activated by CBD to triggers cytotoxic agents to induce apoptosis (Nabissi, Morelli, Santoni, & Santoni, 2013).Marcu et al., (2011) reviewed that, CBD do not significantly associate with cannabinoid receptors, able to regulate the control pattern of Δ9-THC. They established the growth inhibitory impact of CBD in combination with Δ9-THC in the glioblastoma cell lines on that basis. At the meantime, another study showed that, in glioma xenografts a strong antitumor activity exerted by the combination treatment of Δ9-THC and temozolomide (TMZ) that initiates autophagy. In the TMZ-sensitive and TMZ-resistant tumors combination treatment of Δ9-THC and CBD with TMZ delivered a strong antitumoral action (Torres et al., 2011). Research on glioma cell line observed that, synthetic cannabinoid ligand KM-233, modulates in the phosphorylation profiles of, ERK1/2, AKT, STAT3, MEK and p70S6K. It has been found that, KM-133 therapy reduce tumor size by 80% at the amount of 12mg/kg daily dose for 20 days in the orthotopic model (Gurley et al., 2012). Though cannabinoid treatment shows significant effect on glioma, regulation of Amphiregulin (EGFR family ligand) and the growth factor midkine (Mdk) causes resistance to it (M. Lorente et al., 2009). Expression of Amphiregulin was associated with increased level of ERK activation and Mdk with ALK mediated its protective effect that interferes with autophagic cell death(M. Lorente et al., 2011). The drug resistance of glioma to cannabinoid treatment could be reduced by supressing amphiregulin and Mdk or ALK . Furthermore, microencapsulation methods was used to improve the efficacy of cannabinoids action, which facilitates a sustained release of the two cannabinoids for several days(Hernán Pérez de la Ossa et al., 2013).

3.2 Breast cancer and cannabinoids:

Breast cancer is considered as most common type of cancer in women that is the second leading cause of cancer-related deaths in women and its incidence in the developing world is rising(Ocaña & Pandiella, 2008). Each year around 30% of recently diagnosed cancers represents breast cancer. According to molecular profile generally breast cancer can be characterized into three main subtypes: hormone receptor-positive, HER2-positive (ErbB2-positive, a member of EGFR family) and triple-negative tumors(Baselga & Swain, 2009). Research on breast cancer shows that cannabinoid based medicines have significant effect in the treatment of all those three subtypes of breast cancer.

Western blot, immunohistochemistry and real time PCR methods were used to identified the cannabinoid receptor expression in breast cancer cell line which provides effective result mentioning CB1 receptor expression was detected in 14% of human HER-2 positive breast cancer tissue. However, there was no connection found in between CB1 receptor and ERBB2 expression. In contrast, immunoreactive CB2 expression was recognized in 91% of ERBB2-positive tumor tissue, saying a correlation within CB2 and ERBB2- expression(Caffarel et al., 2010).

It has been demonstrated by Ligresti(2006) that, anandamide a potential endocannabinoid which specifically inhibits the proliferation of breast cancer cells. This anti-proliferative action of anandamide was characterized by blocking cell cycle in the S-phase and suppression of prolactin receptor. Research demonstrated that Met-F-AEA a synthetic analog of anandamide, decreases

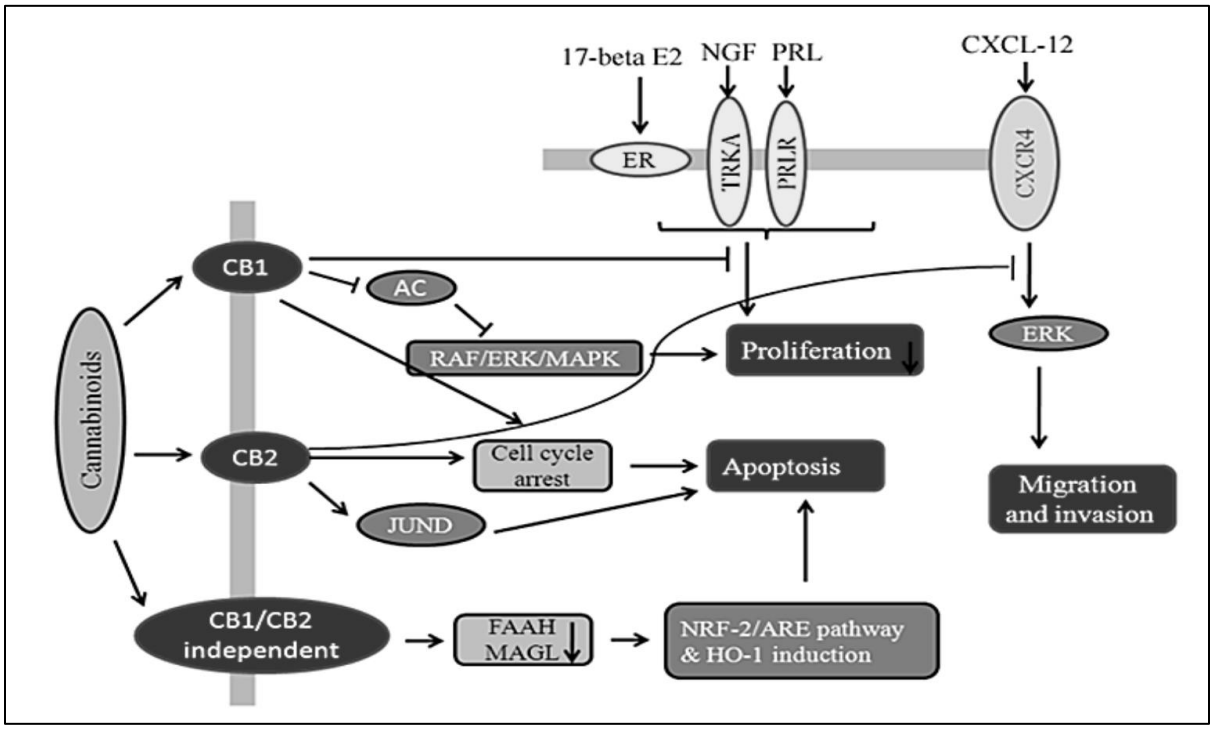


Figure 3.1: Showing modulatory impact of cannabinoids on breast cancer cells that is hormone sensitive. Cannabinoids affected in breast cancer cell formation, migration and invasion through receptor dependent/independent manner shown in this figure. Synthetic cannabinoid like JWH-O15 shows inhibitory effect on hormone sensitive breast cancer progression by modulating CXCL12 signaling axis.

Adapted from: https://www.researchgate.net/figure

MDA-MB-231 cell line proliferation by interrupting the S-phase of the cell cycle(Laezza, Simona Pisanti, Crescenzi, & Bifulco, 2006). Various experiment has been done to find out the mechanism of anandamide which shows that it inhibits adenylyl cyclase (AC) of cancer cell as a result activates the MAP ,Raf-1 or ERK, pathway in ER+(cancer cells grows in response to estrogen hormone)/ PR+(cancer cells grows in response to progesterone hormone) type of breast cancer. At the same time, transcription factor JunD in ER-/PR+ breast cancer cells is activated by THC to finally accomplish action of apoptosis (De Petrocellis et al., 1998). Study demonstrates that anandamide modulates Wnt/β-catenin signaling path to inhibit demonstrates proliferation of MDA-MB31 cells. Due to suppression of the cyclin-dependent kinase CDK2 this effect is occurred (Laezza et al., 2012). In MCF-7 cell line anti-estrogenic property of cannabinoid can be found through JWH series synthetic cannabinoid compounds like JWH-210, JWH-018, JWH-122 and JWH-073 and one benzoylindole AM-694 (Koller, Zlabinger, Auwärter, Fuchs, & Knasmueller, 2013). Another synthetic cannabinoid WIN 55,212-2 and

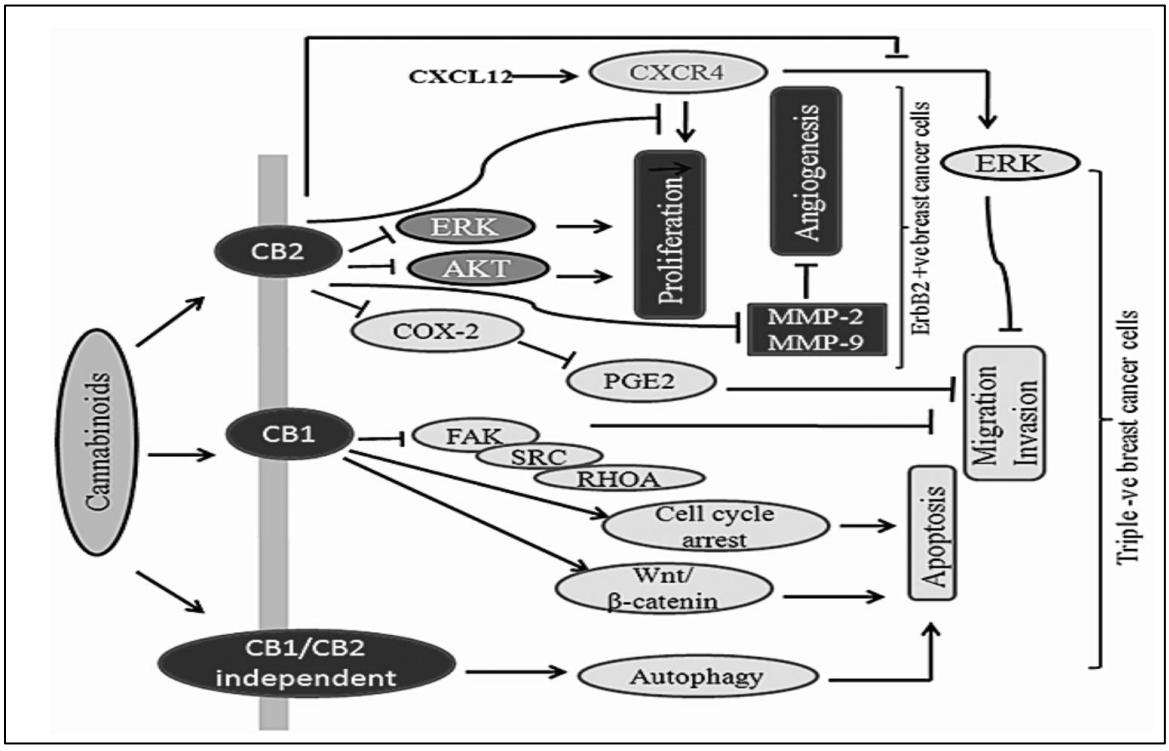


Figure 3.2: Cannabinoids have modulatory impact on HER-2 positive and triple negative breast cancer cells.

Adapted from: https://www.researchgate.net/figure

JWH-133 also causes an inhibitory effect on MDA-MB-231 cell line proliferation by irrupting the cell cycle at G1 to S phase progression and induced apoptosis. The triple-negative breast cancer model created by xenograft and PyMT proved the anti-proliferative effect of WIN 55,212-2 and JWH-133 compound, which is modulate by the COX-2/PGE2 signaling path. Additionally, synthetic cannabinoid JWH-015 not only exert anticancer effect by means of cytokine/chemokine suppression but also reduces bone pain and bone loss induced by breast cancer (Qamri et al., 2009). Another research on breast cancer found that, AKT and mTOR signaling is inhibited and mTOR, cyclin D1 phosphorylation is decreased by CBD(Shrivastava, Kuzontkoski, Groopman, & Prasad, 2011). Moreover, CBD blocks LPI which has proliferative impact on breast cancer (Ford et al., 2010). In this manner, cannabinoids in combination with other selective NSAIDs or chemotherapeutic agents may consider as novel chemotherapeutic agent in breast cancer therapy.

3.3 Prostate cancer and cannabinoids:

Prostate cancer is considered as most common type of cancer in men that arises due to abnormal cell division of prostate gland. Research found that in the prostate cancer tissues, CB1 and CB2 receptor shows higher expression level. Several type of cell lines such as LNCaP, CA-HPV-10, DU-145, PC-3, CWR22Rv1 found in prostate cancer tissue shows higher receptor expression in contrast with normal prostate epithelial cells(Sarfaraz, Afaq, Adhami, Malik, & Mukhtar, 2006).Ruiz, Miguel, & Díaz-Laviada, (1999), found in their study that Δ9-tetrahydrocannabinol exert a significant effect on PC-3 cells by inducing apoptosis through receptor-independent manner. Additionally activation of cannabinoid receptors are causing the stimulation of AKT or PI3K signaling on PC-3 cells where ERK1/2, Raf-1 signaling is also involved. (Nithipatikom et al., 2004). In the aspect of higher-level expression of cannabinoid receptor in prostate cancer, a synthetic cannabinoid WIN-55,212-2 was administered to treat LNCaP cells. This medication provides an effective result by inhibiting cell growth, inducing apoptosis and interrupting cell cycle at the G0-G1 phase. According to Sarfaraz et al., (2006),cell cycle was interrupted by WIN-55,212-2 through stimulating ERK1/2 pathway. To determine in-vivo consequence with in-vitro findings, Sarfaraz, Adhami, Syed, Afaq, & Mukhtar, (2008), has been showed that, in mice model, tumor growth is inhibited by administration of WIN-55,212. Nithipatikom et al. (2004) showed that, prostate cancer cell DU-145 and PC-3 invasion is also inhibited by stable

analogue endogenous 2-arachidonoylglycerol and noladin. Anandamide also exert apoptotic and antiproliferative impacts in prostate cancer cell lines PC3, DU145 and LNCaP which is mediated through down-regulation of EGFR and ceramide accumulation(Mimeault, Pommery, Wattez, Bailly, & Hénichart, 2003). Interestingly, anandamide analogue (R)-methanandamide at teribbly low doses shown to possess a mitogenic impact on LNCaP cells (Sánchez, Sánchez, Ruiz-Llorente, & Díaz-Laviada, 2003).

3.4 Lung cancer and cannabinoids:

The abnormal and uncontrolled growth of lung tissue causes lung cancer, which has the lowest survival rates among cancer-suffering patients. Lung cancer is classified in to two groups: small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC). Guzman(2003), recorded for the first time that, oral administration of Δ9-tetrahydrocannabinol inhibited the development of Lewis lung carcinoma. In vitro experiment identified, suppression of DNA synthesis was responsible for these effect. Another research by Preet, Ganju, & Groopman, (2008) reported that, a concentration of Δ9-tetrahydrocannabinol has significant effect on human NSCLC cells. Treatment of NSCLC cell line such as A549 and SW-1573 with Δ9-tetrahydrocannabinol inhibits the epidermal growth factor (EGF) induced cell migration in dose dependent manner. That inhibition occurs because of EGFR-mediated activation of ERK1/2 and PKB or AKT signaling. This research also showed that, Δ9-tetrahydrocannabinol treatment reduce EGF–induced phosphorylation of MAP kinase ERK1/2,FAK, AKT in lung cancer cell line as well as suppress metastatic advancement and subcutaneous tumor growth in severe combined immunodeficient (SCID) mice (Preet, Ganju, & Groopman, 2008). Recently, a different study demonstrated that, in NSCLC cells the FAAH has direct impacts on endocannabinoid anandamide (AEA).It has been showed that, AEA level increases when FAAH is blocked, which is responsible for inhibiting EGFR signaling pathway, as a result cell cycle is arrested and apoptosis occurred (Ravi, Sneh, Shilo, Nasser, & Ganju, 2014).

3.5 Pancreatic cancer and cannabinoids:

Pancreatic cancer is a consequence of uncontrolled and rapid growth of pancreatic cells. It is considered as one of the most threating human malignancies for which survival rate has not improved. Both in normal as well as pancreatic cancer tissues CB1and CB2 receptor expression

was determined by RT-PCR. In pancreatic ductal adenocarcinoma (PDAC) patients expression of cannabinoid receptors may influence the prognosis (Son et al., 2007). Apoptotic cell death of pancreatic tumor induced by administration of cannabinoid as a drug because the presence of CB2 receptor, modulation of ceramide-dependent p8 , ATF-4 and TRB3 stress–related genes(Carracedo, et al., 2006). Another research demonstrated that, in pancreatic cells administration of CB1 receptor antagonist AM251–induced apoptosis which is occurred by means of receptor-autonomous way(Fogli et al., 2006). Although researches mentioned above, illustrate different type of anticancer mechanism of cannabinoids, both emphasize the implication of cannabinoids in pancreatic cancer. Therefore, to establish precise mechanism of action and specific dosage form more research is needed.

3.6 Lymphoma and cannabinoids:

Lymphoma is a cancer that developed from lymphocyte a type of infection fighting cell. The two types of lymphomas are identified as Hodgkin lymphoma and non-Hodgkin lymphoma (most common). Researches established that over expression of cannabinoid receptors were found in mantle cell lymphoma (MCL) and non-Hodgkin lymphoma (Flygare, Gustafsson, Kimby, Christensson, & Sander, 2005). In EL4 tumor bearing mice, cell viability is inhibited and apoptosis increased in-vitro by Δ9-THC both in EL4 and MCL cells. Another experiment in leukemia cell shows that, different cytotoxic drugs in combination with Δ9-THC activated apoptosis through MAPK/ERK pathway (Liu, Scott, Shamash, Joel, & Powles, 2008). Furthermore in MCL cells, administration of R(+)-methanandamide or WIN-55,212- 2 initiate apoptosis, which was also related to accumulation of ceramide and p38, mitochondrial membrane depolarization, activation of caspase(Manuscript & Structures, 2009). Additionally, in CLL cells R(+)-methanandamide initiate apoptosis mechanism (Gustafsson, Christensson, Sander, & Flygare, 2006). In contrast, according to Wasik et al., (2011), cell viability is decreased by cannabinoids when assessed through metabolic activity. Research disclose that, cannabinoids also induce paraptosis a type of programmed cell death mechanism (Wasik et al., 2011).

3.7 Skin cancer and cannabinoids:

Skin cancer is the type of cancer originated from skin cell as a result of abnormal cell growth which can spread rapidly to other parts of the body. There are three types of skin cancer classified as basal skin cancer (BCC), squamous cell skin cancer (SCC) and melanoma. In worldwide maximum number of skin cancer related deaths occurs due to melanoma, as it is most aggressive type of cancer. In normal skin and skin tumor of both human and mice model, presence of cannabinoid receptors (CB1 and CB2) are elucidated by scientists. It is found in in-vitro experiment that activated cannabinoid receptors have ability to initiate the apoptosis in tumorigenic epidermal cells; interestingly, the non-transformed epidermal cells are not affected. Experiment on malignant tumor in nude mice shows that, synthetic cannabinoid WIN-55,212-2 or JWH-133 which is selective CB2 agonist administration exhibit growth inhibitory effect (Carracedo et al., 2006). Another research by Blázquez et al.,(2006), revealed that, activated cannabinoid receptors in mice helps to decrease growth of tumor and development, metastasis of melanomas and by blocking hypo-phosphorylation or AKT pathway prohibits proliferation of melanoma cells. These studies related to cannabinoid and melanoma offer an exciting opportunity to further investigate about the mechanism of action of cannabinoid as anticancer in the management of skin cancer.

Chapter 4: Discussion

4. Discussion:

Research shows that cannabinoids and its natural or synthetic analogues are potential component to reduce CINV impact and stimulate appetite. Additionally, cannabinoids shows anticancer effect on cancer tissues of different origin by preventing proliferation, migration and cancer cell invasion. In the laboratory experiment on natural and synthetic cannabinoid has been proved that, it possess effects in the initiating apoptosis, blocking cell division, preventing new blood vessels forming into tumors, reducing metastasis so that cancer cells cannot have the chance of spreading through the body or neighboring tissue. In addition, cannabinoids regulate other major processes like energy metabolism, inflammation etc. The information has given in this review paper are not only collected from tissue culture test but also taken from relevant animal model. Data presented in this review paper suggested that cannabinoid extracted from both natural and synthetic sources modulate different tumor cell types, regulate differently signaling pathway and host physiological system. After evaluating all the information it is noticed that, combination therapy of cannabidiol (CBD) with purified THC, a phytocannabinoid that counteract the psychoactive effects of THC provides the best results in the lab or animal models. However, scientists working with synthetic cannabinoids molecule like JWH-133, WIN55, 212-2, JWH-015 have also showed positive results. Cannabinoids are establishing to be a classic drug because of their mode of action and unique potentiality to retain normal cells from damaging. Therefore, to ensure effective use of cannabinoid as anticancer at first the expression pattern and activation of cannabinoid receptors need to identify in different type of tumors since cannabinoids produces receptor dependent signaling mechanism. Different cancer cell lines and tumor model exhibits different effects of cannabinoids because affinity of cannabinoid receptors varies depending on the strength of cannabinoid. Therefore, overexpression of cannabinoid receptors is beneficial in execution of tumors. On the other side, if these receptors do not expressed on cannabinoid therapy may causes cell proliferation and metastasis because of the suppression of the antitumor immune response.

Furthermore, in different type of cancer tumor growth and metastasis is enhanced when endocannabinoids- AEA and 2-AG produces prostaglandin (PGE2) and epoxyeicosatetraenoic acid (EE) which is known as secondary metabolites. It is also observed that administration of cannabinoid in low dose contribute to cancer progression as it cannot induced apoptosis. On the

other side, higher dosage could be a reason of toxicity. Long-term use of cannabinoids may cause drug tolerance. Though researchers are still working on, there is not sufficient information about the precise anticancer mechanism and proper dose of cannabinoids. Additionally, the tumor condition is another reason for different cellular response to cannabinoids because environment of inflammatory cells, fibroblasts, macrophages, etc. varies. Therefore, centralize understanding is needed on the function of cannabinoids in connection with the environment of tumor. The variety of affecting different signaling pathways might establish developing cannabinoids that selectively inhibit a particular process, in this manner opening new pathway for specific targeted treatment.

Moreover, studies showed that it is beneficial to use of combinational anticancer therapies compare to single substance medication, as it support for the synchronous focusing on growth and development of tumor at various levels. In addition, cannabinoids shows more specific action on cancer cells than normal cells. The single cannabinoids administration may able to produce drug resistance related problems or show no effect compared to combinational therapy with other anticancer drug. In this way, cannabinoids may provide an effective clinical outcome, increase specificity, reduce toxicity and overcome drug resistance related complication when administered in combination with other chemotherapeutic drugs.

Nowadays, on practical grounds, interpretation of empirical records on medical cannabis use, combined with a rational application of our current understanding of the mechanism of cannabinoid action, as well as some “trial and error” may be the only way to delineate which cannabis preparations may adjust best (in terms of efficacy and tolerability) to the specific needs of each patient at each disease stage. Before using cannabinoids as medical purpose it could be considered from three complementary level to overcome any kind of problem: (1) pre-clinical studies at evaluating interaction between different cannabinoid and non-cannabinoid compounds, investigate more information on anti-tumorigenic and anti-metastatic mechanisms and identify more susceptible patient based on cannabinoid therapies. (2) Controlled clinical trial with most appropriate selection of that kind of combinations of compound, which could provide precise data on safety and efficacy (e.g. dosage and treatment duration, pharmacokinetic parameters). (3) Observational studies with different chemotypes, preparations and delivery procedure. Though many researches has been conducted based on effect of cannabinoid in different cancer there is

not sufficient information about specific activity of synthetic or natural cannabinoid which will work effectively in human body , particular dosage form and stage of cancer at which cannabinoids are more effective. Maximum research has been done based on THC, which is widely found phytocannabinoid, but other natural and synthetic cannabinoids are also seen to be effective on different types of cancer cells. THC shows promising outcome in laboratory experiments on glioma and prostate cancer cells, while breast cancer cells are more responsive to CBD. After all, many patients may think of the psychoactive impact of cannabinoids that is mainly developed by THC at high dose. Research shows that, this psychotic effect can be overcome by combination therapy with another cannabinoid CBD. There is also a big concern is the chemical makeup of cannabinoid which is not dissolve in water. This characteristic makes it hard to penetrate drug into a tumor therefore, proper dosage form need to identify.

Chapter 5: Conclusion and future direction

5. Conclusion and future direction:

There is no confusion that the "Mother Nature" is the hidden treasure of biologically advantageous substances. However, different types of plants contain various complex chemicals, which may not be medicinally advantageous. There are some plants contains very low amount of chemical substance that has a significant impact on our body. In the same way, active substances of marijuana that possess anticancer effect on numerous cancer types are called cannabinoids. Though cannabinoids have possibilities to use extensively as recreational drug, their medicinal properties cannot be ignored. Many potent anticancer drugs are obtained from plant sources like vincristine, vinblastine known as vinca alkaloids. Similarly, therapeutic advantages of cannabinoid as anticancer substance are widespread. However, it should remember that only highly purified active constituents of cannabis plant is used as a drug, not the entire plant. So, use of cannabinoid as addictive purpose cannot treat any disease except producing side effects. Therefore, in future cannabinoid based medication need to overcome all prejudice, gain trust from both patient and doctors, highly controlled by regulatory authority for safe and effective use. More researches are needed based on clinical trial to gain trust from patient. Recently Cancer Research UK created fund for cannabinoid research. In USA, FDA takes responsibilities and approves two cannabinoid-based drugs for therapeutic purpose. In compare to all these developed countries Bangladesh is still lag behind from such an opportunity to research on cannabinoid due to legal complaints. Therefore, all pharmaceutical companies in Bangladesh should come forward to collaborate with government regarding legal issue and create a pathway for cannabinoid research in near future.

Chapter 6: References

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