

Cannabinoids and their medicinal potential

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ABSTRACT

Cannabis sativa L preparations have been used therapeutically since many years. In spite of their medicinal value, the danger of its abusive potential led to the ban on its use in clinical practice in many countries. The recent research and in depth knowledge about the cannabinoid system which throw a light on their disease management potential has paved way for the cannabinoids to become a new therapeutic focus of attention. Cannabinoids are a group of compounds that mediate their effects through cannabinoid receptors which include CB₁, predominantly expressed in the brain and CB₂ which is primarily found in the cells of the immune system. Despite the addictive properties of cannabis, the therapeutic value of cannabinoids is too high to be put aside. Numerous diseases such as anorexia, pain, inflammation, obesity, cardiovascular disorders, neurodegenerative diseases, cancer, gastrointestinal diseases, hepatic disorders, skin related diseases, respiratory disorders like asthma and eye diseases like glaucoma have suggested cannabinoid agonists/antagonists/ cannabinoid related compounds as potential treatment options. Developments of new specific ligands for the cannabinoid receptors are now underway and it needs to be seen, if in future, they can prove to be a boon for the medical world. The paper reviews the current understanding of the cannabinoid receptors, their ligands and their possible role in various diseases supported by preclinical and clinical studies.

Keywords: Cannabinoids, cannabinoid receptors, clinical practice

INTRODUCTION

Cannabinoids are the active compounds from the plant *Cannabis sativa* and the first major psychoactive component of cannabis discovered was Δ^9 -tetrahydrocannabinol (THC) in 1964. Further research in this field eventually led to the discovery of endogenous cannabinoids (endocannabinoids) which were the naturally occurring substances within the body and they mimicked the action of THC.¹ The first successful endocannabinoid ligand discovered in the process was arachidonylethanolamide named as Anandamide, which in Sanskrit means 'bliss'(a-nanda) + amide. Later, a second endocannabinoid, 2-arachidonoyl glycerol (2-AG) was identified. Other less well established endocannabinoid candidates are virhodamine, nolandin and *N*-Arachidonoyl dopamine.²

The serendipitous identification of the heptahelical G-protein-coupled receptors CB₁ and CB₂ in 1990s fuelled further interest in the cannabinoid galaxy. CB₁ receptors,

which activate G-proteins mainly of the *Pertussis* toxin (PTX)-sensitive Gi/Go family are expressed in both central nervous system (mainly) and in the periphery. In the central nervous system, they are present in the hippocampus, cerebellum, hypothalamus, substantia nigra, mesolimbic dopamine pathways and in association areas of cerebral cortex.^{3,4} Activation of CB₁ receptors, which are present pre-synaptically leads to inhibition of adenylate cyclase and calcium channels while causing activation of potassium channels which results in inhibition of synaptic transmission. In the periphery, CB₁ receptors are present in endothelial cells and adipocytes where they promote lipogenesis and regulate arterial tone and vasorelaxation.^{2,5}

CB₂ receptors are mainly expressed in the immune system (spleen, tonsils, thymus, circulating lymphocytes, monocytes and mast cells of the tissues) with much lower distribution in the CNS.⁶ Activation of cannabinoid receptors can produce other physiological effects like antinociception, hypothermia, sedation, hypotension, inhibition of intestinal motility and motor depression.²

Cannabinol, the precursor of THC and cannabidiol, a breakdown product of THC which is devoid of psychoactive effects, are the most abundant plant cannabinoids obtained from the plant *Cannabis sativa*. Δ^9 -tetrahydrocannabinol, which is the active principle when given to humans results in a lot of central and peripheral effects. The behavioral effects include physical relaxation, feeling of well being, mild euphoria, impaired motor coordination, stimulation of appetite, antiemetic action and analgesia. Chronic use results in decreased attention, verbal learning and memory and increases the chances of developing psychiatric disorders.⁷ The peripheral effects include tachycardia, vasodilatation, decreased intraocular pressure and bronchodilatation.²

Endogenous cannabinoids, anandamide and 2-AG are synthesized and released when 'demanded' and not stored in the 'resting' cells. They are synthesized and released in response to various physiological and pathological stimuli like neuronal depolarization and bacterial lipopolysaccharides.⁴ Anandamide is synthesized from N-acyl-phosphatidylethanolamines (NAPEs) via enzymatic conversion by transacylase and phospholipase D.⁴ It is an agonist at CB₁ receptors mainly with very low affinity for CB₂ receptors.^{8,9} Endocannabinoids have also been seen to act on certain G-protein coupled receptors (GPCRs) like GPR55 and ion channels like vanilloid receptor-type 1 (TRPV1 receptors).⁷

Anandamide from the extracellular space is rapidly taken up by neurons and other cells by selective, saturable, temperature dependant and Na⁺ independent facilitated transport mechanisms called as anandamide membrane transporters (AMT). It then gets metabolized by fatty acid amide hydrolase (FAAH) to arachidonic acid and ethanolamine.⁴

2-arachidonoyl glycerol is synthesized by hydrolysis from phosphatidyl inositol by action of the enzymes phospholipase G and diacylglycerol lipases (DAGL- α and DAGL- β). It binds to both CB receptors but somewhat has a higher affinity for CB₂ receptors.¹ Although 2-AG is also hydrolyzed by FAAH to arachidonic acid and glycerol *in vitro* but *in vivo* it is hydrolyzed by a monoacylglyceride lipase (MGL) which is co-expressed with CB₁ receptors in hippocampus.¹⁰⁻¹³

The knowledge about cannabinoid system due to the availability of many preclinical and clinical studies during the past few years has paved way for them to become a new therapeutic focus of attention. Novel ligands for the CB₁ and CB₂ receptors are being synthesized and developed and their possible role as a future treatment modality in various diseases has been discussed below.

For this review article, resources which were relevant to the topic were searched in the months of August and September from online papers (pubmed) using the key words cannabinoids, cannabinoid receptors, rimonabant, endocannabinoids, therapeutic potential, novel therapeutic

targets. Apart from text selection from text book (Rang and Dale's Pharmacology 6th edition), more than 100 articles from pubmed were retrieved (including abstracts) and 94 out of them were used for preparation of the review which have been mentioned in the reference list. The articles were studied and the ones (especially the full length available articles) in which the information regarding the outline of endocannabinoid system including the cannabinoid receptors, novel therapeutic potential of cannabinoids in various diseases with supporting evidence was presented and discussed in details, were considered. All the data gathered was then used to draft a review article about cannabinoids, highlighting their therapeutic implications, by the authors, with an aim to cover all the important aspects.

THERAPEUTIC POTENTIAL OF CANNABINOIDS

Appetite, Emesis and Obesity

The endocannabinoid system via CB₁ receptors regulates central appetite control, peripheral metabolism and body weight management. Anorexia remains a grave problem in patients suffering from cancer or AIDS. In United States (US) and many European countries, a synthetic delta-9-THC preparation (dronabinol, marinol) and its synthetic analog (nabilone, Cesamet), which are primarily approved for treatment of nausea and vomiting in cancer and HIV infected patients, caused a consistent improvement in appetite along with improvement in mood in this patient population. Dronabinol was efficacious and safe in HIV wasting syndrome and advanced cancer and it showed greater efficacy than conventional antiemetics like chlorpromazine and similar drugs in chemotherapy induced emesis and patients preferred them.^{14,15} But their side effects like dizziness, dysphoria, hallucinations may limit their widespread use while sedation and euphoria seemed to be preferred by the patients.¹⁶ Therefore it becomes important to identify selective/ specific group of patients who will benefit from the antiemetic effect of cannabinoids and also have less neuropsychiatric adverse effects.

The other side of the coin is obesity, and cannabis has been known to increase appetite and food consumption. One of the first generation CB₁ receptor blocker, rimonabant was seen to cause short term reduction in food intake by its central action. Various studies investigating the effect of rimonabant in obese patients with hyperlipidemia demonstrated that rimonabant caused a significant decrease in hunger, body weight along with improvement in insulin resistance, increase in HDL cholesterol levels and decrease in triglyceride levels as compared to placebo group.^{14,15} But adverse effects like depression and suicidal ideations have halted its usage in many European countries and it did not get the approval of Food and Drug Administration (FDA). This alongside also led to the termination of development of other CB₁ receptor blockers like taranabant and otanabant.^{17, 18}

Still the interest in the potential of CB₁ antagonists called the peripherally restricted CB₁ receptor antagonists is retained and several such compounds are being synthesized and developed. These compounds will attain lower brain concentrations and will be free of the aversive side effects seen with rimonabant.¹⁷⁻¹⁹ A novel peripherally active cannabinoid receptor antagonist, AM6545, which is more selective for CB₁ than CB₂ was tested in rodents to evaluate its effect on food intake and body weight. This agent showed limited brain permeability and dose dependently reduced food intake and inhibited body weight gain in rodents.¹⁷ Hence CB₁ receptor antagonists designed to have limited brain permeability hold a promising future in weight management.

Neuro-inflammatory and neurodegenerative disorders

Multiple Sclerosis

Multiple Sclerosis (MS) is a chronic inflammatory, autoimmune and demyelinating disease of the brain which results in muscle weakness, spasticity, tremors, ataxia, cognitive impairment, difficulty in coordination and balance, loss of bladder control and constipation. Degeneration of myelin sheath is mediated by T cells and this inflammatory process causes breakdown of blood brain barrier (BBB) which further activates macrophages & produces cytokines.¹⁹

Cannabis has been used in the past to relieve muscle cramps/ spasms and pain.²⁰ Current knowledge about the endocannabinoid system changes in MS patients has revealed that both CB₁ and CB₂ expressing cells collect around plaques in human MS oligodendrocytes, along with their precursors and macrophages while perivascular T cells expressing CB₁ receptors have been identified to be present in multiple sclerosis. Microglia and macrophages forming plaques, nearby astrocytes and T cells express CB₂ receptors.²¹ In blood samples of primary progressive MS patients, an up-regulation of CB₁ and CB₂ levels has been detected which shows that immune regulation of the endocannabinoid system occurred both in brain as well as periphery.²²

Various animal and human studies of MS, demonstrating the role of endocannabinoids have been done. In an experimental autoimmune encephalomyelitis (EAE) rat model, parenteral THC demonstrated inhibition of neural inflammation and improved survival.²⁰ Dexabinol, a non-psychoactive synthetic cannabinoid also caused a reduction in the inflammatory response in the brain and spinal cord of EAE rats. Cannabinoids cause immunosuppression by induction of apoptosis via partial activation of CB₂ receptors and also decreased production of IL-2, IFN- γ and TNF- α production by activated T cells which is a CB₁ receptor mediated effect.¹⁹ Other mechanism by which cannabinoids offer immunoprotective action is by action on astrocytes which during disease progression are activated to release cytokines, chemokines and nitric oxide (NO).

Cannabinoids in various studies have been found to trigger production of IL-6 (which inhibits TNF- α), inhibit NO production and decrease various other inflammatory products.^{15,23}

In another model of MS in mice called as chronic relapsing experimental allergic encephalomyelitis (CREAE) which reproduces many pathological features of MS found in humans, decrease in CB₁ receptor density in caudate-putamen, globus pallidus and cerebellum was seen. The cannabinoids improve motor symptoms like spasticity, tremors and ataxia.^{19,20} These mice showed an increase in AEA, 2-AG and palmitoylethanolamide (PEA) levels in areas of nerve damage and selective inhibitors of endocannabinoid transport or hydrolysis also relieved spasticity in EAE.^{24,25} In CB₁ deficient mice, substantial neurodegeneration developed on inducing EAE.²⁰

In a survey in 112 humans with MS in USA and UK, who took cannabis as self medication, it was seen that cannabis improved spasticity, pain, tremors, depression in majority of the patients.^{20,26} Another large multicentric study conducted in MS patients who received either synthetic THC or a Cannabis or placebo, recorded significant improvements in spasticity, pain and sleep quality of the patients. The 12 month follow up also showed a remarkable decrease in spasticity.^{20,27} In contrast one of the studies reported impairment in both balance and posture after a single dose of smoked cannabis in MS patients and normal subjects.²⁸

All these studies have shown mixed results and it remains to be seen whether cannabinoids with targeted drug delivery, optimized for modifying neuroinflammation will be useful in MS treatment. Future large scale trials are needed to establish them as efficacious therapeutic modality in management of multiple sclerosis.

Amyotrophic lateral sclerosis (ALS)

It is a neurodegenerative disease characterized by progressive loss of cortical motor neurons in the spinal cord, brain stem and motor cortex, leading to muscle wasting, weakness and spasticity, which can progress to complete paralysis.²²

In SOD1G93A mice, a model of ALS, cannabinoids was seen to delay onset of symptoms and prolong survival by non CB₁ receptor mechanism.¹⁵ Genetic ablation of FAAH, the enzyme responsible for metabolizing endocannabinoids, prevented the onset of disease signs in these mice. CB₁ receptor induced mechanism did not seem to play a role in this. A safety study of THC done on ALS patients showed beneficial effects in spasticity, insomnia and appetite.²² Very few clinical studies evaluating the benefits of cannabinoids are available in ALS patients and there seems to be hope for them as preclinical studies have shown the neuroprotective effect of cannabinoids.

Parkinson's disease (PD)

Another chronic and progressive neurodegenerative disease, PD is characterized by degeneration of dopamine containing neurons in substantia nigra which causes bradykinesia, tremor and rigidity in the patients. The progression of neurodegeneration and appearance of major motor symptoms are related to increased endocannabinoids. Various studies have found an increase in the CB₁ receptor levels in the striatum of monkeys and humans with parkinsonism. Activation of CB₁ receptor signaling reduced the glutamate release from the cortex of striatum (beneficial effect) and enhanced GABA transmission causing inhibition of globus pallidus (non beneficial effect).^{20,29} Therefore use of CB₁ antagonists can have pro or antiparkinsonian actions depending upon the target area. CB₁ receptor agonists can cause a decrease in the tremors due to overactivity of subthalamic nucleus and improve motor symptoms while CB₁ antagonists can be used to alleviate bradykinesia.²⁰ Administration of CB₁ antagonists in animals showed an improvement in parkinsonian symptoms and levodopa induced dyskinesias.³⁰

We have many preclinical studies demonstrating the therapeutic potential of cannabinoids in PD but clinical trials in this area are still limited and in the coming years it is expected that the number of studies examining this potential will increase and provide us with a clear picture of its role in PD management.

Alzheimer's disease (AD)

The pathogenesis of AD involves loss of cholinergic neurons in hippocampus and frontal cortex which leads to cognitive deficit and loss of short term memory in patients. There occurs formation of extracellular, amyloid plaques consisting of β amyloid proteins and intraneuronal neurofibrillary tangles containing hyperphosphorylated microtubule associated tau proteins. There occurs acceleration of the formation of above protein aggregates, neuronal inflammation and hence loss of neurons with disease progression.² THC competitively inhibits acetylcholinesterase and thus prevents acetylcholinesterase induced β amyloid protein aggregation.³¹ CB₂ receptor stimulation is also involved in suppression of inflammatory mediators like NO, chemokines and cytokines involved in neuronal damage.³² Hence if these findings are further confirmed in future, there can be a ray of light for the patients of Alzheimer's disease.

Huntington's disease (HD)

It is an inherited autosomal dominant disorder resulting in progressive brain degeneration and is associated with choreiform movements, motor disturbance and cognitive deficits. There occurs reduction in the activity of glutamic acid decarboxylase (GAD), an enzyme responsible for synthesis of gamma amino butyric acid (GABA) and this loss of GABA mediated inhibition in the basal ganglia causes hyperactivity of dopaminergic synapses.⁷ Also, there occurs loss of CB receptors in substantia nigra in

HD patients. A hybrid endocannabinoid and vanilloid compound (arvanil) exhibited antihyperkinetic action in rat HD model.¹⁵ Administration of CB receptor agonist have been shown to have neuroprotective effects by preventing glutamate induced excitotoxicity. A small trial with Nabilone and cannabidiol showed no efficacy in HD patients and increased choreatic movements in them.²⁰ Therefore, these results do not support the results of preclinical studies but, very few clinical studies have been done so far and more are needed to establish the usefulness of cannabinoids in HD. Also it is important to evaluate whether cannabinoids are important in early phase of the disease or even in advanced stages.

Psychiatric disorders**Schizophrenia**

Schizophrenia is a functional disorder characterized by positive symptoms like delusions, illusions, hallucinations, thought disorders, distortion of perception and aggressive behaviour at times. The psychotic episodes are separated by periods with negative symptoms like introvert behaviour, poor socialization, emotional blunting, lack of motivation and attention deficit along with loss of memory.³³

Hypoglutamatergic and hypodopaminergic transmission in the prefrontal cortex may be causing negative symptoms while increased dopaminergic transmission in the mesencephalic projections to nucleus accumbens could lead to positive symptoms.²⁰ It has been seen that overactivity of the endocannabinoid system may lead to an increased dopaminergic and decreased glutamatergic levels, which may cause some of the symptoms of schizophrenia.^{20,34}

Various theories supporting the endocannabinoid hypothesis in schizophrenia have been presented. It has been reported that cannabis has worsened the positive symptoms of schizophrenia in patients even when they were on antipsychotics.^{34,35} Also, the use of large amounts of cannabis and THC are likely to produce psychotic symptoms in normal individuals, including delusions, hallucinations, and impairment of cognitive functions just like schizophrenia.^{8,36,37} Heavy use of cannabis can be an independent risk factor for development of schizophrenia in susceptible patients.³⁸ The levels of anandamide are found to be elevated in cerebrospinal fluid or blood taken from patients of schizophrenic and treatment with neuroleptics corrects this endocannabinoid imbalance.^{39,40} Two independent postmortem studies showed an increase of CB₁ receptors in subregions of the prefrontal cortex, dorsolateral and anterior cingulate regions in schizophrenia. As it is known that cannabinoids impair cognition, attention, and motivation, it can be postulated that the increase in CB₁ receptors in the prefrontal cortex may be involved in schizophrenia especially the negative symptoms.³⁴

Hence, utilization of CB₁ receptor antagonists may be used for treatment of negative symptoms of schizophrenia, but if its other neurological side effects can be prevented is a question of prime importance as such undesirable effects will not be acceptable and can limit its usefulness.

Mood disorders

Evidences have suggested possible use of cannabinoids in treating mood disorders like generalized anxiety and depression. CB₁ receptors are widely distributed in brain areas associated with emotional regulation and stress responsiveness such as prefrontal cortex, hippocampus, amygdala, and hypothalamus, suggesting its role in anxiety-related disorders.⁴¹

CB₁R knock-out mice display increased anxiety levels compared to control animals (wild-type) in a variety of behavioral paradigms like the light-dark box, the elevated plus-maze test and the social interaction test. Adult rodents treated with low dose nabilone and THC exerted anxiolytic effects in elevated plus maze model.^{41,42} Similarly, rimonabant, a cannabinoid antagonist produced anxiogenic effects in rats in elevated plus-maze and defensive withdrawal test. In contrast, rimonabant was found to be anxiolytic in mice in another elevated plus-maze study.⁴¹ But clinical data supports the anxiogenic effect of rimonabant seen in rats because it was withdrawn from the market as an anti obesity drug for causing increased anxiety and depressive mood.^{41,43} Facilitation of anxiolytic endocannabinoid tone by inhibitors of FAAH or anandamide transport also elicited anti anxiety and antidepressant effects in animal studies.¹⁴

The results suggest that amplification of the endocannabinoid signaling by mechanisms like inhibition of their transporters or FAAH can be an innovative target for treatment of anxiety disorders as this will not be associated with psychoactive adverse effects.

Insomnia

Insomnia is defined as difficulty in initiation, maintenance, duration or quality of sleep. Earlier studies have shown the effects of marijuana and THC on sleep patterns in humans and animals. A recent study of cannabis extracts in eight healthy volunteers on sleep quality, early morning performance and memory showed that 15 mg of THC was sedative while 15 mg of CBD caused alertness and counteracted the residual sedative effect of THC.²⁰ Another study showed that anandamide induced sleep and also increased the level of adenosine (sleep inducing substance) in the basal forebrain and these effects were blocked by rimonabant. The role of FAAH inhibitors which lack the addictive properties like psychotropic cannabinoids has also been seen.²⁰

As psychoactive cannabinoid ligands may not be desirable for use in insomnia, hence use of active FAAH inhibitors can be further evaluated for therapeutic gain as they lack addictive action.

Pain

Preclinical studies demonstrating the analgesic property of cannabinoids against chemical, mechanical and thermal pain stimuli are available.^{20,44} There is considerable evidence suggesting that cannabinoid signaling system functions parallelly but distinctly from the opioids and their activation leads to antinociception along with other similar behavioural effects.⁴² In experimental animals with neuropathic pain, activation of opioid and cannabinoid receptors by their respective agonists leads to antinociceptive action.¹⁴ Data from studies on intact animal models show that acute administration of THC or other CB receptor agonists led to opioid peptide release while chronic administration of THC increased the gene expression of endogenous opioid precursors (preproenkephalin, prodynorphin and proopioidmelanocortin).⁴⁵

Human studies with cannabinoids have shown mixed results. A buccal spray, Sativex (CBD +THC) was found to be effective in management of neuropathic pain in patients suffering from traumatic nerve injury or multiple sclerosis.⁴⁶ Dronabinol has also been reported to provide relief from pain in patients with multiple sclerosis.⁴⁷ In contrast, in a clinical study, Sativex did not provide any relief from central neuropathic pain due to brachial plexus avulsion. Dronabinol did not show analgesic effect in neuropathic pain and post-operative pain in a clinical trial.²⁰ A clinical study with a synthetically modified compound derived from THC metabolite, THC-11-oic acid (ajulemic acid) has shown antinociceptive activity without causing neurological side effects of cannabinoids. The mechanism of action here might involve inhibition of cyclooxygenase-2 as ajulemic acid does not bind strongly to cannabinoid receptors.¹⁴

Currently there is not sufficient evidence in humans regarding the analgesic potential of cannabinoids and the mechanism of action also remains unclear. But animal data have predicted that cannabinoids have antinociceptive activity and it therefore needs to be further determined in future human studies and a better understanding of the underlying mechanism would also be useful.

Cardiovascular diseases

The most consistent effects of marijuana smoking and intravenous administration of THC are peripheral vasodilation and tachycardia.¹⁴ CB₁ receptors are more important in cardiovascular regulation while CB₂ receptors are involved in ischemic preconditioning and ischemia/reperfusion injury of the myocardium.²⁰ CB₁ receptors have been detected in human, rat and mouse

myocardium where they have a negative inotropic effect and in vascular tissues where they cause vasodilation. These effects lead to hypotensive action of anandamide seen in anaesthetized rodents.^{20,48,49,50} In anaesthetized spontaneously hypertensive rats (SHR), THC and anandamide caused a long lasting hypotension than in control group and administration of CB₁ receptor antagonists markedly increase the blood pressure and cardiac contractility further.^{20,48,51}

A clinical study in male cannabis smokers, reported that 22% of them experienced symptomatic hypotension which could be attenuated by giving 30-90 mg of rimonabant indicating that CB₁ receptors mediate the hypotensive effect.⁵² These findings suggested the effect of endocannabinoids in hypertension is primarily mediated by tonic activation of cardiac and vascular CB₁ receptors which are upregulated or due to increase in its signaling system.⁴⁸

In myocardial reperfusion injury, role of CB₂ receptors has been implicated and synthetic CB₂ receptor agonists by action on endothelial cells from human coronary artery disease reduced the activation by TNF- α and production of other chemokines.⁵³ In anesthetized rats, where ischemia/reperfusion injury was induced by coronary occlusion/ reocclusion, anandamide decreased both the incidence of ventricular arrhythmias and infarct size by activation of CB₂ receptors and not CB₁ receptors. A moderately selective CB₂ receptor agonist reduced the myocardial damage in an animal model of ischemia/reperfusion which was abolished by selective CB₂ receptor antagonist, indicating that the protective effect of endocannabinoids against myocardial ischemic injury mainly occurs via CB₂ receptors.^{20,54}

Another role postulated for endocannabinoids is their effect on progression of atherosclerosis. Cannabinoids exert potent anti inflammatory and antioxidant effects. Stimulation of CB₂ receptors leads to decrease in the inflammatory activity of TNF- α , generation of high glucose induced mitochondrial superoxide, NF-kappa B activation and other inflammatory mediators and this can be helpful in atherosclerosis.⁵⁵

Hence the role of cannabinoid receptors suggested in the treatment of cardiovascular disease seems beneficial. They appear to be a useful therapeutic target as CB₁ receptors have shown promise in hypertension and CB₂ receptors play an important role in myocardial perfusion injury and atherosclerosis by their anti-inflammatory and anti oxidant effects.

Cancer

The palliative effects of cannabinoids in cancer patients include appetite stimulation, inhibition of chemotherapy/radiotherapy induced nausea and vomiting, analgesia, mood elevation and relief from insomnia. Many other potential benefits of cannabinoids have been and are still

being evaluated in various tumors and the proposed mechanisms by which they show anti tumor action are anti proliferative action, inhibition of angiogenesis and tumor cell migration.²⁰ The anti-tumor action of cannabinoids on glioma may be exerted either via CB₁ or CB₂ receptors. Intratumour administration of THC and CB₁ agonist induced regression of glioma in wistar rats and in RAG-2-deficient mice.⁵⁶ Selective CB₂ receptor agonists showed good efficacy in vivo by regressing human astrocytoma Grade IV.⁵⁷ Cannabinoid receptors could have a protective role against programmed cell death, as reported in human neuroblastoma and C6 cells, where AEA induced apoptosis by action on vanilloid receptors, activation of COX, increase in intracellular calcium concentration, release of cytochrome c and activation of caspase 3.⁵⁸ Therefore the mechanism of AEA induced apoptosis in cells expressing both cannabinoid and vanilloid receptors is still controversial and might depend on the experiment conducted.

Studies investigating the role of AEA in breast tumor cells showed that, submicromolar concentration of AEA caused inhibition of mitogen induced activation of G0/G1-S phase in epithelial human breast cell lines (HBCC) EFM-19 and MCF-7⁵⁹, while HBCC lines MCF-7 and MDA-MB-231, mouse breast cancer 4T1 were found to be resistant to the cytotoxic action of THC and caused tumor proliferation.⁶⁰ The difference in the response could be attributed to the level of cannabinoid receptor expression where low density of receptors could result in tumor progression on exposure to THC.⁶¹ Also it was said that 4T1 cells expressed a good number of vanilloid receptors which is a target for AEA and hence these cells may be more responsive to action by AEA rather than THC.⁶² In colorectal cancer, apoptotic and antiproliferative effects are mediated by CB₁ receptors and AEA inhibited migration of cancer cells and cell death in apoptosis resistant cancer cells via CB₁, depending on the presence of cyclo-oxygenase 2, which is abundantly expressed in colon tumor cells.⁶³ Role of FAAH in colon tumor by increasing endocannabinoid levels has also been postulated and both these targets maybe helpful in clinical management of colorectal carcinoma.

Cannabinoids also have a role in other cancers like androgen dependent prostate cancer (apoptosis of human prostate cancer cells by G1 arrest) and pancreatic cancer (apoptosis of cancer cells via stress protein p8 and other genes).^{64,65}

The difference in activity of cannabinoids in different tumor models can be attributed to the variability in expression of CB₁ and CB₂ receptors. Tumors like glioma and human astrocytoma grade IV have shown good response while in breast cancer cell lines, low density of cannabinoid receptors resulted in proliferation of tumor on exposure to THC. The battle is not lost yet, as many of the findings are encouraging and propose the beneficial role of cannabinoids in inhibiting tumor proliferation.

Still a lot of extensive studies are warranted which can give a clear picture of the 'good' and the 'bad' effects of cannabinoids on different tumors and it needs to be seen if the same can be translated to clinical practice.

Liver diseases

The hepatic endocannabinoid system comprises of both CB₁ and CB₂ receptors which have a faint distribution, where CB₁ receptors are present in endothelial cells and hepatocytes while CB₂ receptors are distributed in Kupffer cells. Any liver injury leads to increase in the hepato endocannabinoid tone.⁶⁶

The role of CB₁ receptors in non-alcoholic fatty liver disease (NAFLD) was investigated in some experimental animals and the data showed that there occurred marked induction of CB₁ receptors in NAFLD which promoted obesity leading to fatty liver and insulin resistance. Therefore selectively targeting CB₁ receptors with agents lacking brain penetrance may prove to be an effective treatment option in such patients.^{67,68} The role of CB₂ receptors on adipose tissue inflammation was also evaluated. It was seen that treatment with CB₂ selective agonist in obese mouse model, led to increase in insulin resistance and liver steatogenesis associated with obesity.⁶⁹

Cannabinoids are also said to be involved in the pathogenesis of alcoholic liver disease. Rimonabant by antagonizing CB₁ receptors prevented the development of fatty liver in mice fed with an ethanol diet. This was because of paracrine activation of CB₁ receptors in surrounding liver cells and activation of lipogenesis along with inhibition of fatty acid oxidation.⁷⁰

Patients with chronic liver diseases may eventually develop liver cirrhosis, portal hypertension and liver failure. Search for effective liver specific anti fibrotic treatments is still continuing and the role of cannabinoid receptors has been explored for this. CB₂ receptors show antifibrogenic properties as administration of its agonist in cirrhotic rats showed an improvement in liver fibrosis, decrease in the inflammation and increased apoptosis of hepatic myofibroblasts.^{70,71} As CB₁ receptors are profibrogenic, so treatment with rimonabant or in CB₁ knock-out mice, reduction in TGF- β 1 (profibrogenic) and fibrogenic cell levels were seen. It therefore, seems likely that selective CB₂ receptor agonists and peripherally restricted CB₁ receptor antagonists could provide a breakthrough in management of liver fibrosis.

As outlined above, growing evidences suggest that endocannabinoids are involved in various pathophysiological processes of liver diseases like NAFLD, alcoholic liver disease, chronic liver disease and its complications. CB₁ receptors have an important role in NAFLD and alcoholic liver disease while the anti-inflammatory action CB₂ receptors can be useful in liver inflammatory conditions. As of now, more preclinical

studies are required to accurately delineate the conditions where we can make use of agonists/ antagonists of CB receptors and then proceed to clinical studies to further substantiate their beneficial effects in these potential therapeutic indications.

Gastrointestinal diseases

Historically, marijuana was prescribed for the treatment of diarrhea and inflammatory bowel disease.⁷² But recent evidences indicate that cannabinoids can be used for a variety of gastrointestinal diseases like nausea and vomiting, gastric ulcers, irritable bowel syndrome, inflammatory bowel diseases and gastrointestinal reflux disease.^{14,73,74,75}

Gastrointestinal reflux disease (GERD)

Δ^9 - tetrahydrocannabinol has been shown to decrease meal induced transient lower esophageal sphincter relaxation and lower esophageal sphincter pressure in humans.⁷⁶

Irritable Bowel Syndrome (IBS)

Cannabinoids are the major relaxants of gastrointestinal motility and CB₁ controls peristalsis.⁷⁷ Pharmacological studies have demonstrated that anandamide and various CB₁ agonists inhibit gastrointestinal motility in rodents *in vivo* and in isolated ileum and colon from both animals and humans.^{20,80,81} In clinical trials with rimonabant for nicotine cessation or obesity, it was seen that incidence of diarrhea was higher in patients receiving rimonabant than with placebo. Therefore this suggests that blocking CB₁ receptors accelerated gastrointestinal transit and secretions²⁰, so CB₁ agonists may prove useful in diarrhea-predominant IBS while CB₁ antagonists can help in constipation- predominant IBS.⁸⁰

Inflammatory Bowel Disease (IBD)

It comprises of two clinical manifestations, Crohn's disease and ulcerative colitis and is associated with uncontrolled immune response to microbial antigens. CB₁ receptors were seen to be involved in compensatory protective mechanisms in mice with chemically (croton oil, 2,4-dinitro benzene sulfonic acid and dextran sulfate) induced colitis. There occurs up regulation of CB₁ receptors and CB₁ agonists inhibited intestinal motility in these mice as compared to controls.^{72,81} In contrast, role of endocannabinoids in pathophysiology of complicated diverticulitis, a frequent complication of colitis, was also suggested as anandamide levels were raised and there was desensitization of presynaptic neural CB₁ receptors in colonic longitudinal muscle strips from patients undergoing surgery for this complication.⁸²

Gastric ulcer

In isolated stomach preparations, inhibition of histamine induced gastric and intestinal secretion by THC was demonstrated.²⁰ Also CB₁ agonists inhibited pentagastrin induced gastric acid secretion and this action could be prevented by CB₁ antagonist.^{83,84} Anti ulcer activity of

CB₁ agonists was demonstrated in rat model of aspirin and cold resistant stress induced gastric ulcers.²⁰

Cannabinoids offers a novel opportunity for future drug targeting by either cannabinoid receptor modulation via specific agonists/ antagonists or inhibition of FAAH and its transporters which increase the cannabinoid levels. Agonists of CB₁ receptors can be useful in diarrhea predominant IBS, IBD, gastric ulcers while CB₂ agonists have an important role in constipation predominant IBS. CB₁ receptors are predominantly present in brain, hence its ligands may be associated with neurological side effects which may be a cause of concern. The need is to introduce more peripherally acting CB₁ ligands with limited brain penetration or explore CB₂ receptors more as they have low expression in the brain. Developing agents that are more efficacious and lacking the psychoactive side effects would be a better treatment option.

Dermatological diseases

Cutaneous endocannabinoid system (ECS) has been shown to be important in the regulation of skin cell proliferation, survival and differentiation which regulates cutaneous homeostasis. By proper modulation of ECS in the skin, various skin diseases may find a new therapeutic target for action. Hyperproliferative skin diseases like psoriasis in which there occurs increased turnover of epidermal keratinocyte proliferation and skin cancers can benefit from the fact that ECS inhibits cell growth, angiogenesis and induces apoptosis in many types of skin cells. Cannabinoid receptor 1 agonism can be used in hirsutism as CB₁ receptor negatively regulates hair growth while by antagonizing CB₁ receptor, conditions like alopecia areata may be treated.⁸⁵

In sebaceous glands, CB₂ receptor activation can increase lipid synthesis. Antagonizing these receptors in diseased sebaceous glands conditions (acne, seborrhea), can be a new promising drug target⁸⁵ while sebaceous gland CB₂ receptor agonists can be exploited in dry skin diseases and itching. Topical formulations containing cannabinoids which enhance ECS tone in skin can be of use in skin inflammations and a recent study has stated about the use of CB₂ receptor agonists for management of early inflammatory stages of systemic sclerosis.⁸⁶

The above data suggests an important role of cutaneous ECS in normal physiological processes of skin and focus should be on proper targeting of the cannabinoid system in various pathological skin diseases. The studies available till now have shown positive results but still, there is a long way to go and the in depth knowledge and exploration of the complete potential of cutaneous ECS requires further evaluation.

Asthma

Marijuana smoking and ingestion of THC caused an increase in the airway conductance in normal healthy

subjects and, in subjects with mild to moderate asthma, it caused a relief in bronchospasm.²⁰ In animal studies, rimonabant enhanced capsaicin induced bronchospasm and cough in guinea pig while anandamide inhibited this effect of rimonabant suggesting the presence of CB₁ receptors on axon terminals innervating airway smooth muscles.²⁰ A recent study of capsaicin induced cough in mice showed that inhibition of anandamide transporter suppressed cough and this may be a new target for cough management.⁸⁷ Role of cannabinoids in allergic asthma due to its anti inflammatory effect of decreasing serum IgE levels, interleukin expression and mucous overproduction can be of medicinal potential.^{20,88}

The effects of cannabinoids on respiratory system are complex and psychoactive effects can limit the use of CB₁ receptor ligands, therefore non psychoactive cannabinoid compounds can be a good therapeutic option and targeting cannabinoids for their anti inflammatory property in allergic asthma seems effective.

Eye diseases

Glaucoma and Diabetic retinopathy

In healthy marijuana smokers, a decrease in intra ocular pressure (IOP) was an accidental finding which was then confirmed in various studies where natural marijuana with THC or synthetic THC (orally, topically or intravenously) showed a decrease in IOP. The side effects reported were hypotension, tachycardia, euphoria, changes in pupil size, decreased tear production and conjunctival hyperemia.²⁰ Also in animal studies, topical administration of AEA caused a significant decrease in intra ocular pressure (IOP) in normotensive albino and pigmented rabbits.⁸⁹ Evidence available suggest the role of CB₁ receptors in regulation of IOP as they are present in rat ciliary body, human ciliary epithelium, ciliary muscle, ciliary body vessels, trabecular meshwork, Schlemm's canal and retina.^{20,90,91} Topical application of CB₁ receptor agonists in animals as well as humans reduced IOP and CB₂ receptor agonist did not effect IOP suggesting that IOP regulation is not majorly effected by CB₂ receptors.^{92,93} Mechanisms by which cannabinoids lower IOP is still not clear but they may have an effect on ciliary processes by causing vasodilatation, reduction of capillary pressure and secretion. They can also cause a decrease in aqueous humor production by inhibiting calcium influx in presynaptic ion channels thus decreasing the release of norepinephrine in ciliary body and may improve uveoscleral outflow by dilatation of anterior uveal vessels.²⁰ All these factors further substantiate the beneficial role of cannabinoids in glaucoma and required is a preparation which can acting locally and have least side effects.

The antioxidative and anti-inflammatory effect of cannabinoids represents a novel therapeutic approach for the diabetic complications of the eye. Adenosine and its agonists have a protective role in inflammation in various animal models and cannabinoids have been shown to

inhibit its reuptake in murine microglia by a non cannabinoid receptor mechanism, thus inhibiting diabetes-induced retinal inflammation.⁹⁴ Therefore the neuroprotective role of cannabinoids in diabetic retinopathy seems to have a good potential and needs further evaluation.

CONCLUSION

Marijuana was used for centuries as a medicinal plant but during the last century, because of its addictive potential, it was taken out of clinical practice. The discovery of the endocannabinoid signaling system opened new possibilities for research into understanding the mechanism of action of cannabinoids and their therapeutic potential in various diseases. Cannabinoid receptor agonists/ antagonists have been found to be useful in management of plethora of diseases. Now we believe that cannabinoid and its constituents along with its related compounds need to be brought back into clinical use and the reasons are their medicinal potential as described in the present review. The therapeutic benefits of cannabinoids outweigh the negative aspects and the potential clinical use of such a system should not be neglected and instead a target oriented approach to study and characterize specific or more selective agonists/antagonists with selective distribution patterns is the need of the hour, so that the desirable clinical effects are retained while the undesirable effects are minimized.

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