Pharmacologic Effects of Cannabidiol and Its Molecular Mechanism

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Abstract Cannabidiol (CBD) is the active constituent of *Cannabis sativa* and exhibits a diverse range of pharmacologic effects, including anticancer, antibacterial, anti-inflammatory, antioxidant, and antiepileptic properties. The pharmacologic effects of CBD and its molecular mechanisms are reviewed with the objective of proposing novel approaches for basic research and clinical applications of CBD and related pharmaceuticals.

Key words Cannabidiol; Anticancer; Antibacterial; Anti-inflammatory; Antioxidant; Antiepileptic

1 Introduction

Cannabis sativa L., also known as white hemp, hemp, wild hemp, *etc.*, is a species that is widely distributed, with the majority of its cultivation occurring in Kyrgyzstan, Nepal, Afghanistan, and part regions of China. *C. sativa* is an annual herbaceous plant with documented efficacy in the management of pain, pus, and internal lesion caused by overexertion. CBD is a white crystalline powder extracted from industrial *C. sativa*, with the molecular formula $C_{21}H_{30}O_2$. It is soluble in ethanol, ether, and other organic solvents[1]. CBD exhibits a diverse range of pharmacologic effects, including anticancer, antibacterial, anti-inflammatory, antioxidant, and antiepileptic properties. The research progress on the pharmacologic effects of CBD and its molecular mechanisms is now organized in a systematic manner to provide a reference for further research and clinical applications of medicinal CBD.

2 Pharmacologic effects of CBD

2.1 Anticancer effect Cancer is a disease in which normal cells undergo a mutation process that results in the formation of cancer cells. This process is caused by the excessive secretion of oncogenic factors, which are influenced by various internal and external factors over an extended period of time. Cancer poses a significant threat to human health and life. CBD has been demonstrated to be an effective inhibitor of cancer cell proliferation and inducer of apoptosis in a range of cancer cell types[2].

2.1.1 Inhibiting the proliferation of cancer cells. Cancer cells are distinguished by their capacity for uncontrolled proliferation. The inactivation of oncogenes results in the malignant growth of cancer cells becoming unregulated by the human body, which in turn causes erosion of the human body and significantly increases the mortality rate of patients. The inhibition of cancer cell proliferation and subsequent elimination represents a crucial strategy for the more effective control of cancer cell spread and further development. A number of studies have demonstrated that CBD exerts an inhibitory effect on the proliferation of a range of cancer cells, including those of the lung, colorectum, and breast.

Inhibitor of DNA binding (Id-1) has been demonstrated to play an important role in the proliferation of cancer cells by regulating cyclins and matrix metalloproteinases. In a study by Soroceanu *et al.*[3], the antiproliferative effect of CBD on glioblastoma multiforme (GBM) was examined by protein immunoblotting. The findings demonstrated that CBD could reduce the expression level of Id-1 protein in a concentration-dependent manner, thereby effectively inhibiting the proliferation of GBM. In a study conducted by Milian *et al.*[4], the inhibitory effect of CBD on the proliferation of human lung cancer cell lines A549, H460, and H1792 was evaluated using the MTT assay. The results demonstrated that the survival rate of lung cancer cell lines A549, H460, and H1792 gradually decreased with the increase in CBD administration concentration and the extension of administration time. The findings demonstrate that CBD exerts a significant inhibitory effect on the proliferation of GBM, A549, H460, and H1792 cancer cells.

Lukhele *et al.*[5] investigated the inhibitory effect of CBD on the proliferation of human cervical cancer HeLa and SiHa cells using the MTT assay. The results demonstrated that CBD exhibited a significant inhibitory effect on the proliferation of human cervical cancer HeLa and SiHa cells following 24 h of treatment with varying concentrations of CBD (0, 50, 100, and 150 μg/mL). Further evidence of the cycle-blocking effects of CBD on three cervical cancer cell lines (HeLa, SiHa and ME-180) was obtained through flow cytometry and protein immunoblotting. Following treatment with varying concentrations of CBD (0, 50, 100 and 150 μg/mL) for 24 h, it was observed that the expression levels of pro-apoptotic proteins p53 and Bax increased, while the number of cells at the G_0 phase gradually increased. In contrast, the number of cells at the G_0/G_1, S, and G_2/M phases exhibited a gradual decline. The aforementioned outcomes indicate that CBD may exert an inhibitory effect on the proliferation of human cervical cancer cells. This is achieved by blocking the G_0 phase of the cancer cell cycle and subsequently inducing apoptosis in the cancer cells,

Received: March 5, 2024 Accepted: June 2, 2023

Supported by Central Government Supports Local College Reform and Development Fund Talent Training Projects (2020GSP16); Heilongjiang Provincial Key Research and Development Plan Guidance Project (GZ20220039).

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ultimately leading to a reduction in the proliferation of human cervical cancer cells.

2.1.2 Inducing apoptosis of cancer cells. Apoptosis is a stimulatory signal sent by normal human cells in response to abnormal changes in external environmental conditions. It is a complex molecular biological mechanism. One of the most effective and commonly used approaches to treat cancer is the induction of apoptosis in cancer cells. CBD has been demonstrated to have a beneficial effect on the induction of apoptosis in cancer cells.

Mohamad *et al.* [6] conducted a study on the apoptotic effect of CBD on triple-negative breast cancer (TNBC) SUM159 and MDA-MB231 cells in a nude mice tumor model using flow cytometry. The results demonstrated that following regular intraperitoneal injections of 5 mg/kg of doxorubicin and 5 mg/kg of CBD for one month, the apoptotic rate of TNBC SUM159 and MDA-MB231 cells in the nude mice tumor model was significantly elevated. Noxa is a member of the pro-apoptotic protein family Bcl-2, which plays a pivotal role in the process of cell apoptosis and represents an efficacious target for the treatment of cancer. In a study by Soyeon *et al.* [7], the pro-apoptotic effect of CBD on colorectal cancer (CRC) HCT116 and DLD-1 cell lines was investigated using flow cytometry. The results demonstrated that the expression level of Noxa protein was significantly increased in the human CRC HCT116 and DLD-1 cell lines following treatment with 0 – 8 μM CBD for 24 h. CBD was found to exhibit a concentration-dependent decrease in viability and promotion of apoptosis in CRC cells, as observed by colony formation assay. Robert *et al.* [8] employed real-time fluorescence quantitative PCR and protein immunoblotting to investigate the pro-apoptotic effects of CBD on lung cancer A549 and H460 cell lines. Their findings indicated that CBD up-regulated the expression levels of cyclooxygenase 2 (COX-2) and peroxisome proliferators-activated receptors (PPARs), which in turn induced apoptosis in lung cancer cells. Zhang Lihong *et al.* [9] evaluated the pro-apoptotic effect of CBD on endometrial carcinoma Ishikawa cells using Hoechst 33258 staining and Annexin-V FITC/PI double staining. Their findings indicated that as the concentration of CBD increased (0, 5, 10, and 20 μmol/L) and the treatment duration extended, the number of apoptotic endometrial carcinoma Ishikawa cells increased significantly. Further studies on the expression of apoptosis-related proteins in endometrial cancer Ishikawa cells by CBD, as detected by protein immunoblotting, revealed that the expression level of the pro-apoptotic protein Bax was elevated. The aforementioned outcomes demonstrate that CBD exerts a favorable apoptotic effect on triple-negative breast, colorectal, lung, and endometrial cancer cells.

2.2 Antibacterial effect Bacteria represent one of the most significant groups of organisms that reproduce through both asexual and genetic recombination. Bacterial infections are contagious diseases that predispose the body to organ failure, respiratory infections, and impaired lung function. CBD has been demonstrated to exhibit potent antibacterial activity. The phenolic hydroxyl groups present in CBD have been demonstrated to inhibit bacterial growth by disrupting cellular morphology.

In a study conducted by Wu Qi *et al.* [10], the antibacterial activity of CBD was tested against a range of bacterial strains, including gram-positive bacteria (*Staphylococcus aureus*, *Enterococcus faecalis*, and *Bacillus cereus*) and gram-negative bacteria (*Escherichia coli*). The double dilution method was employed to assess the minimum inhibitory concentration (MIC) of CBD against these bacterial strains. The findings revealed that at a concentration of 100 μmol/L, the MIC for gram-positive and gram-negative bacteria was below 100 μg/mL. Martinenghi *et al.* [11] investigated the inhibitory activity of CBD against gram-positive *S. aureus* and *S. epidermidis* using high performance liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MSMS). Their findings revealed that the MIC of gram-positive bacteria was significantly elevated when gram-positive bacteria were treated for 24 h with 120 μmol/L CBD. Liu Juan *et al.* [12] conducted a study to assess the inhibitory effect of CBD on the anaerobic bacterium *Propionibacterium acnes* using the shock flask method. The results demonstrated that CBD exhibited a high inhibitory effect on *P. acnes*, with an inhibition rate of 99% observed at a CBD concentration of 80 μmol/L. Wassmann *et al.* [13] conducted a study to assess the antimicrobial efficacy of CBD against *E. faecalis*, *S. aureus*, *Listeria monocytogenes*, and methicillin-resistant *Staphylococcus* epidermidis (MRSE) using a combinatorial therapeutic assay. Their findings revealed that the MIC of CBD was 4 μg/mL for *S. aureus*, *L. monocytogenes*, and MRSE strains, and 8 μg/mL for *E. faecalis*. Further studies on the antibacterial activity of CBD against *S. aureus* as detected by transmission electron microscopy (TEM) demonstrated a significant reduction in the bacterial autolysis rate of *S. aureus*. The aforementioned outcomes demonstrate that CBD exerts a considerable inhibitory effect on both gram-positive and gram-negative bacteria.

2.3 Anti-inflammatory effect Inflammation is the body's response to changes within the body, primarily caused by pathogens infecting the body. Inflammation is often defined by the presence of redness, swelling, heat, pain, and dysfunction. It is well established that inflammatory reactions not only lead to the development of numerous diseases but also cause significant damage to the respiratory, digestive, and neurological systems of the body. This damage can have a profoundly negative impact on human health and life. CBD has been demonstrated to be an effective inhibitor of the expression levels of numerous inflammatory factors.

In a study conducted by Jastrzb *et al.* [14], the effect of CBD on the expression level of pro-inflammatory cytokines was investigated through the ultraviolet irradiation method. The findings revealed that the administration of CBD at a dosage concentration of 30 μg/mL led to a notable reduction in the expression level of the

pro-inflammatory factor TNF-α. Liu Juan *et al.* [12] examined the inhibitory effect of CBD on the inflammatory factors secreted by human keratinocyte HaCat cells and mouse macrophage RAW264.7 cells. This was done by means of an LPS (lipopolysaccharide) induction assay, and the results demonstrated that CBD could effectively inhibit the protein expression level of the pro-inflammatory factor TNF-α. Zeng Liang *et al.* [15] employed a protein immunoblotting assay to investigate the inhibitory effects of CBD on inflammatory bowel disease (IBD). Their findings indicated that CBD effectively inhibited the expression levels of inflammatory cytokines IL-4 and IL-10. Wu Zhongbao *et al.* [16] conducted an enzyme-linked immunosorbent assay (ELISA) to investigate the effect of CBD on the expression of inflammatory cytokines in rats. The results demonstrated that the inhibition of TNF-α protein expression level increased significantly with the increase in the dosage of CBD injections (0, 15, and 30 mg/kg) and the prolongation of the administration time. Furthermore, the expression levels of inflammatory infiltration-regulating proteins in rats in each group were quantified by protein immunoblotting. The results demonstrated that the expression levels of inflammatory infiltration-regulating proteins Semaphorin 7α, AHSG, and Uteroglobin were significantly elevated in the CBD high-dose group (30 mg/kg). The aforementioned outcomes demonstrate that CBD exerts a favorable inhibitory effect on the expression levels of pro-inflammatory factors, including TNF-α, IL-4, and IL-10.

2.4 Antioxidant effect Oxidation is a process whereby the body produces excessive free radicals, which can lead to the peroxidation of lipids, the oxidation of proteins and the damage of DNA. Excessive oxidized free radicals can cause the body to suffer from a variety of chronic diseases, including rheumatoid arthritis, cancer, and AIDS. These diseases can seriously affect human health, aging, and life. Antioxidants have the capacity to effectively inhibit the oxidative reactions of free radicals or to directly scavenge free radicals within the body. CBD has been demonstrated to possess antioxidant properties. CBD contains polyphenols that scavenge free radicals in the body. These polyphenols can be broadly categorized by structure into flavonoids, astragals, phenolic acids, and lignans.

In a study conducted by Wu Qi *et al.* [10], the scavenging ability of CBD for 1,1-diphenyl-2-trinitrophenylhydrazine (DPPH) radicals was examined through the use of the Friedel-crafts alkylation as well as O-alkylation of organic bromides. The findings revealed that the EC_{50} for scavenging of DPPH and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals was 23.8% and 25.1% of CBD, respectively The antioxidant effects of polyphenolic compounds were more pronounced with increasing concentrations of CBD. Further studies on the scavenging ability of DPPH radicals by *in vitro* antioxidant assays demonstrated that the scavenging rate of DPPH radicals increased progressively with increasing concentrations of CBD treatments and prolonged duration of action. Ma Run *et al.* [17] examined the antioxidant effect of CBD on a carbon tetrachloride (CCl_4) constructed mouse liver fibrosis model using the Western Blot assay. The results indicated that the gp91 (oxidase subunit) protein level was significantly reduced, while the Nrf2 (nuclear transcription-related factor) protein level was significantly increased. Jastrzb *et al.* [14] conducted a spectrophotometric assay to investigate the conversion of DADPH free radical reductase amount by CBD. Their findings indicated that the conversion of DADPH free radical reductase amount exhibited a gradual increase with increasing concentrations of CBD.

2.5 Antiepileptic effect Epilepsy is a neurological disorder characterized by recurrent abnormalities of neurons, which result in temporary disorders of brain function. Epilepsy is classified into two categories: primary and secondary epilepsy. The underlying pathologic and physiologic mechanisms of these categories are complex and diverse. The clinical manifestations of epilepsy encompass convulsions, foaming at the mouth, and loss of consciousness[18]. The majority of drugs currently available for the treatment of epilepsy have significant limitations, including suboptimal efficacy, high rates of adverse effects, and high costs. Long-term use of these drugs can result in significant physical and psychological harm and burden to epileptic patients.

In a study by Rosenberg *et al.* [19], the effect of CBD on seizure frequency in patients with epilepsy was examined through gastric tube administration. The results indicated that, after 12 weeks of CBD use, the median overall seizure frequency of the patients was 27.5 ($P<0.001$). Additionally, the antiepileptic effect was found to be more pronounced as the dose of CBD medication increased. Xu Wen *et al.* [20] employed an inhibitory calcium ion channel method to assess the impact of CBD on intracellular calcium ion concentration in epileptic tissue. Their findings indicated that CBD effectively reduced intracellular calcium ion concentration in epileptic tissue, inhibited glutamate release, and suppressed abnormal neuron excitation. Further studies on the inhibitory effects of CBD on epileptic seizures, conducted by inhibiting G-protein coupling, demonstrated that CBD modulated seizure frequency and duration, and suppressed epileptic seizures.

In a study by Devinsky *et al.* [21], the efficacy of CBD in reducing seizure frequency in epileptic patients was evaluated. The Mann-Whitney U test was employed to assess the impact of CBD administration at a dosage of 2 mg/kg orally per day. The results demonstrated a reduction in the rate of motor seizures per month, with an average of 36% observed. The aforementioned results indicate that CBD is efficacious in reducing seizure frequency.

3 Prospects

CBD, a natural extract of *C. sativa*, exhibits antitumor, antibacterial, anti-inflammatory, antioxidant, and antiepileptic activities. In particular, its potential for anti-tumor development is considera-

ble, yet the specific pharmacological molecular mechanism of action and clinical effects remain unclear. Further research is required at the molecular, cellular, and animal levels, in conjunction with relevant basic medical theories, to evaluate the safety, effectiveness, and objectivity of clinical experiments. This will provide a theoretical basis for the study of the pharmacologic effects of CBD and its further exploitation.

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