RYKUCKA, Aleksandra, ZOZULA, Natalia, WĄS, Marcin, KIEŁBASA, Justyna, KOWALCZYK, Agata, BIL, Katarzyna, ŚLESICKA, Iga, LATAŁA, Aleksandra, TOMCZEWSKA, Zuzanna and PRZESTRZELSKA, Magda. Exploring the efficacy of cannabinoids in the management of multiple sclerosis. Journal of Education, Health and Sport. 2024;74:52563. eISSN 2391-8306.
https://dx.doi.org/10.12775/JEHS.2024.74.52563
https://apcz.umk.pl/JEHS/article/view/52563

The journal has had 40 points in Minister of Science and Higher Education of Poland parametric evaluation. Annex to the announcement of the Minister of Education and Science of 05.01.2024 No. 32318. Has a Journal's Unique Identifier: 201159. Scientific disciplines assigned: Physical culture sciences (Field of medical and health sciences); Health Sciences (Field of medical and health sciences).
Punkty Ministerialne 40 punktów. Załącznik do komunikatu Ministra Nauki i Szkolnictwa Wyższego z dnia 05.01.2024 Lp. 32318. Posiada Unikatowy Identyfikator Czasopisma: 201159. Przypisane dyscypliny naukowe: Nauki o kulturze fizycznej (Dziedzina nauk medycznych i nauk o zdrowiu); Nauki o zdrowiu (Dziedzina nauk medycznych i nauk o zdrowiu).
The authors declare that there is no conflict of interests regarding the publication of this paper.
Received: 25.04.2024. Revised: 10.05.2024. Accepted: 22.05.2024. Published: 21.06.2024.

Exploring the efficacy of cannabinoids in the management of multiple sclerosis

Aleksandra Rykucka
Rear Admiral Professor Wiesław Łasiński 7th Military Navy Hospital with Outpatient Clinic named after in Gdańsk, Polanki 117 street, 80-305 Gdańsk, Poland
aleksandra.rykucka@wp.pl
https://orcid.org/0009-0003-0171-8647

Natalia Zozula
District Medical Center in Grójec, Piotra Skargi 10 street, 05-600 Grójec, Poland
nataliazozula0@gmail.com
https://orcid.org/0009-0009-9741-5061

Marcin Wąs
District Medical Center in Grójec, Piotra Skargi 10 street, 05-600 Grójec, Poland
mos1@vp.pl
https://orcid.org/0009-0004-8645-0121

Justyna Kiełbasa
Independent Public Healthcare Complex in Pruszków, 2/4 Armii Krajowej Avenue, 05-800 Pruszków, Poland
justynakielbasa1b@gmail.com
https://orcid.org/0009-0002-0616-7402

Agata Kowalczyk
Mazovian Specialized Hospital in Ostrołęka of Józef Psarski, Jana Pawła II 120A, 07-410 Ostrołęka, Poland
agata.kowalczyk95@gmail.com
https://orcid.org/0009-0007-9497-0970

Katarzyna Bil
Independent Public Specialist Western Hospital named after St John Paul II,
Daleka 11, 05-825 Grodzisk Mazowiecki, Poland
katarzynabil2503@gmail.com
https://orcid.org/0009-0003-6518-1711

Iga Ślesicka
Mazovian Specialized Hospital in Ostrołęka of Józef Psarski, Jana Pawła II 120A, 07-410 Ostrołęka, Poland
igaslesicka@gmail.com
https://orcid.org/0000-0001-7282-4875

Aleksandra Latała
District Medical Center in Grójec Piotra Skargi 10 street, 05-600 Grójec, Poland
aleksandra.latala10@gmail.com
https://orcid.org/0009-0007-1701-2728

Zuzanna Tomczewska
Provincial Specialist Hospital named after Blessed Father Jerzy Popiełuszko in Włocławek, Wieniecka 49, 87-800 Włocławek, Poland
ztomczewska@gmail.com
https://orcid.org/0000-0002-4327-3311

Magda Przestrzelska
Faculty of Medicine Collegium Medicum Uniwersytet Kardynała Stefana Wyszyńskiego w Warszawie, Wóycickiego 1/3, 01-938 Warszawa, Poland
magda.przestrzelska123@gmail.com
https://orcid.org/0009-0009-8688-7047

Abstract

Introduction: Multiple Sclerosis (MS) is a chronic autoimmune disease of the central nervous system, marked by inflammation, demyelination, and neurodegeneration. It severely impacts quality of life with symptoms like spasticity, pain, and cognitive impairment. Conventional treatments often fail, necessitating alternative therapies.

Purpose: This review evaluates the efficacy and safety of cannabis extracts in treating MS, enhancing understanding of their benefits and limitations.

State of knowledge: Studies suggest that cannabinoids may alleviate MS symptoms, particularly spasticity and pain. Clinical trials have shown significant reductions in muscle stiffness and pain, along with improved sleep quality. Meta-analyses, by Whiting et al. and Cochrane, support these findings but highlight the risk of side effects, which may limit long-term use. Additionally, research on animal models and in vitro studies indicate that cannabinoids may have neuroprotective and immunomodulatory properties, potentially reducing neuroinflammation and demyelination.

Conclusion: Cannabinoids exhibit potential as a complementary therapy for managing MS symptoms, especially spasticity and pain. However, evidence is mixed, with some studies showing limited efficacy and considerable risk of side effects. More long-term, high-quality studies are needed to fully understand the therapeutic potential and safety profile of cannabinoids in MS treatment. Despite the challenges, the growing availability and changing

legal status of medical cannabis suggest it could be a viable option for some patients, provided its use is carefully monitored and adapted to individual needs.

Keywords: multiple sclerosis; Cannabis; cannabinoids; neuropathic pain; spasticity; therapeutic strategies

Introduction and purpose

Multiple sclerosis (MS) is an autoimmune condition that occurs in the central nervous system (CNS). MS impacts approximately 2.3 million individuals globally, especially young adults [1]. It involves chronic inflammation with demyelination and multifocal damage particularly in white matter but also in gray matter. This process leads to neurodegeneration and disability as the disease progresses. Several researches state that permanent axonal deterioration appears even in the early stages of MS, before the very first symptoms could be visible to the patient and physicians [2 3].

Etiopathogenesis

The etiopathogenesis of multiple sclerosis is still unknown. Like other autoimmune disorders, in MS, autoreactive myelin-specific T-lymphocytes play a crucial role. These T cells activate an inflammatory cascade that damages surrounding structures, resulting in the formation of the typical plaques seen in MRI images [4 5]. Certain scientific research shows with the strongest indications that Epstein-Barr virus, infectious mononucleosis and smoking are associated with MS [6]. Significant genome-wide association research (GWAS) submits information about the genetic ground of this disease. With great possibility, HLA-DRB1 in the Class II region of the histocompatibility complex (MHC, 6p21.3) could be important in the etiology developing MS, particularly in early adulthood [7].

Characteristic symptoms and diagnosis

In MS, several characteristic symptoms occur. They include spasticity and muscular contractions, tremor, ataxia, persistent neuropathic pain, bladder issues or cognitive impairment [8]. These manifestations (especially spasticity) frequently cause significant discomfort due to pain, decreased mobility, and disruption of daily activities, including the quality of sleep [9].
Making a definitive diagnosis presents a challenge even for very experienced clinicians and requires a series of additional tests. Magnetic resonance imaging (MRI) and 2010 McDonald criteria serve as a crucial clinical instrument. The identification of MS can be additionally supported by test results such as prolonged latency of visual evoked potentials and unmatched oligoclonal IgG bands in the cerebrospinal fluid (CSF).

Phenotypes of the disease

Determining the primary phenotypes demands analysis of the disease activity. It is determined by clinical relapses or characteristic lesions identified in MRI scans, along with the evaluation of continuous disability progression. The most frequent occurring type of MS is clinically isolated syndrome (CIS**)**- shows up in 80% of MS patients. This form of the disease can proceed to relapsing remitting MS **(**RRMS**)**. Subsequently, in the spectrum of the MS, the following type will be Progressive Disease. Depending on the time of gradual increase in disability, we can divide it to two groups: starting from the beginning- primary progressive MS (PPMS) or after receiving a diagnosis of RRMS- secondary progressive MS (SPMS) [4] [10].
Both kinds can be active or not active.

Expanded Disability Status Scale (EDSS)

Among the methods used to evaluate various clinical parameters in patients with multiple sclerosis is the Expanded Disability Status Scale **(**EDSS**)**. It is commonly utilized to assess the grade of disability in MS patients. The following scale evaluates the functioning of the central nervous system, sensory and vision disturbances, gastrointestinal and bladder dysfunctions, and cognitive functionality. Scores scope from 0 (no abnormalities in the neurological examination) to 10 (death due to MS) [11] [12].

Current treatments and their limitations

Treatment methods in MS can be separated into three groups including acute relapse management, disease-modifying treatments (DMTs) and symptomatic treatments. Their effectiveness is constrained by their toxicity and coexistent side effects.

In the first group of agents, the most popular are corticosteroids. To manage acute MS relapses, high doses of methylprednisolone are recommended. The main challenge to take care of a relapse is to estimate if it is a genuine relapse or possibly, a worsening or fluctuation caused by an already existing demyelinating lesion [13]. Corticosteroids can help reduce inflammation and shorten the duration of relapses. If the episode of relapse is especially severe or progressive rapid plasma exchange is a helpful tool. Non-medical interventions in the form of physiotherapy could be additionally used.

Another group represents disease-modifying treatments (DMTs). These therapies are crucial for managing MS long-term and include a range of options like beta-interferons, glatiramer acetate, mitoxantrone, teriflunomide, fingolimod, dimethyl fumarate and monoclonal antibodies such as natalizumab or alemtuzumab. The choice of DMT often depends on the severity, the specific characteristics of the clinical type of disease and patient preferences. They are typically prescribed based on NICE criteria, which consider factors like frequency of clinical relapses, MRI findings and the level of disability. DMTs alter the disease's progression by modulating the immune system. Their anti-inflammatory effect decreasing the relapse frequency, slowing the accumulation of lesions seen in MRI scans and sometimes slightly improving disability [14]. They could be considered disease-modifying treatments (DMT) when administered in the right dosage [15].

There have been notable advancements in DMTs in recent years, nevertheless none of the current treatments effectively stop or eliminate symptoms associated with MS [16].

The most alarming adverse event is related to the application of natalizumab. This humanized monoclonal antibody targets VLA-4 integrin, preventing leukocyte migration across the blood-brain barrier. Regrettably, this can cause activation of the John Cunningham virus (JCV) which leads to progressive multifocal leukoencephalopathy (PML). This virus is frequent in society, possibly detected in about 50% of the population. The risk factors for developing progressive multifocal leukoencephalopathy (PML) are among others: over 2 years of treatment using natalizumab and prior chemotherapy or immunosuppressive therapy. These considerations can increase the risk of developing PML to 1 in 200 or even higher.

Life-threatening side effects of intravenous mitoxantrone administration include cardiovascular events and acute myelogenous leukemia. While treated with alemtuzumab it is possible to develop acute cytokine release syndrome manifested by fever, hypotension, diarrhoea, nausea, rash and headaches. Another noteworthy adverse effect in the case of utilized interferon beta 1b is a possibility that the immune system will start producing its own neutralizing antibodies to this drug. Other selected side effects of conventional treatment include liver damage, endocrinopathy, lymphopenia, adverse reactions in place of injection, hair loss, depression, flu-like signs, infections [4].

Presently, the available methods of symptomatic treatment include both pharmaceutical and non-pharmaceutical options. The primary strategy focuses on eliminating underlying causes, managing trigger factors, and using available treatments until relief is attained [4 17 18 19]. The specific symptoms of MS patients and their sample treatment are presented in the table below.

Symptoms of MS	**Sample treatment**
Fatigue	Amantadine
Cognitive impairment, mood changes	Antidepressants
The urinary track -urgency -nocturia	-Oxybutynin -Desmopressin
Sexual dysfunction	Sildenafil, alprostadil
Neuropathic pain	Gabapentin, pregabalin, amitriptyline
Muscle spasticity	Baclofen, tizanidine
Tremor, ataxia	Propranolol, levetiracetam
Constipation	Osmotic stimulants laxatives
Fecal incontinence	Loperamide

Figure 1 The specific symptoms of MS patients and their sample treatment [4].

Cannabinoids and the endocannabinoid system

Recently, the Cannabis plant has been causing numerous discussions in the medical world. It is known for its entertaining properties especially in varieties such as hemp, cannabis or marijuana which could appear in forms of cigarettes, hash pipes and sweets like brownies. Surprisingly, an increasing number of scientific studies are providing information about its potential usage as a treatment for various diseases.

This unique plant includes over 560 identified components, predominantly consisting of phytochemicals and terpenoids. The most noteworthy species of the cannabis plant, belonging to the *Cannabaceae* family are *Cannabis sativa, Cannabis indica,* and *Cannabis ruderalis* [20].

In *C. sativa* the major psychoactive component is called Δ-9-tetrahydrocannabinol (D9-THC) but it has a higher ratio of cannabidiol (CBD) to THC. Inversely occurs in *C.indica* [8 21 22 23]. CBD is the prominent non-psychoactive cannabinoid found in significant quantities in the leaves and flowers of the *Cannabis Sativa* plant [24]. It does not lead to psychosis, instead it affects pharmacologically on pain and spasticity [25]. Cannabidiol demonstrates promise in relieving symptoms associated with multiple sclerosis (MS) such as spasticity, inflammation, fatigue and depressive episodes, also helps patients increase their ability to move [26 27]. Further potential applications of CBD are currently being investigated in subsequent studies, focusing on its use as an agent for depression, psychosis, and anxiety. Encouraging are also first reports about CBD anticancer effect [28]. A crucial aspect of cannabinoid compounds is the entourage effect, where in the components in cannabis demonstrate greater efficacy when working together compared to when they are isolated [29]. This mainly concerns the interplay between terpenoids and phytocannabinoids present in the plant essence.

The endogenous cannabinoid system, comprising CB1 and CB2 receptors, is the mechanism through which cannabinoids exert their influence. It regulates biological processes, including memory, pain sensation, anxiety and food intake [30]. CB1 and CB2 are binds to adenylyl cyclase negatively and mitogen-activated protein kinase positively via the Gi/o protein [31].

Pathways of the endocannabinoid system are typically activated when endogenous cannabinoids (endocannabinoids) such as Anandamide (AEA) and 2-Arachidonoyl Glycerol (2-AG) bind to the CB1 and CB2 receptors [32]. CB1 and CB2 are located in the central and peripheral nervous systems and also occur in immune system [33]. CB1 receptors are discovered

largely in nerve endings in the central nervous system and certain peripheral tissues. They interact with a specific type of calcium and potassium channel. Due to their inhibition of ways that conduct pain in the brain and spinal cord, the CB1 receptors' primary function is to block neurotransmitter release. So, they play a significant role in mediating and relieving pain [8]. CB2 receptors are primarily found in immune cells, where they regulate cytokine production and the migration of immune cells [34]. CBD additionally affects the non-cannabinoids receptors GPCRs and ion channels**.** The modulation of these receptors determines pain management and reduces inflammation [8]. Moreover, cannabinoids have an affinity to opioid and serotonin receptors. Due to this, THC can be utilized to treat neuropathic and chronic pain, demonstrating anti-nausea and anti-inflammatory features. The combined effect of THC and CBD is beneficial because of their different modes of action [35]. They also can be a solution to persistent spasticity through CB1 receptors in the brain [36]. Enhanced secretion of endocannabinoids results in activation of CB1 receptors which, by limiting neurotransmitter release from presynaptic nerve terminals, decreases excessive neuronal excitability. Presumably this effect offers neuroprotection for the CNS [37].

The state of knowledge

According to some studies on experimental PC12 cells, scientists have found that activating CB2 receptors decreases the secretion of TNF-α and oxidative reactive oxygen species (ROS) [38]. Another group of researchers employed experimental autoimmune encephalomyelitis (EAE) evoked by myelin oligodendrocyte glycoprotein (MOG) in C57BL/6 mice, in the role of the model of multiple sclerosis. These studies have shown that CBD reduces the migration of T-cells and the activation of microglial cells, resulting in neuroprotection and immunomodulation of CNS in animal MS patterns and human cells tested in vitro. Studies in EAE have shown that CBD can effectively reduce the severity of symptoms, modulate immune responses, and protect against neuroinflammation and demyelination [24 28 39].

The review and meta-analysis performed by Whiting and partners highlighted that cannabinoids have a limited but potentially useful role in managing certain MS symptoms, particularly spasticity and pain. Regrettably, there is an increased risk of tolerable adverse effects, possibly leading to treatment discontinuation [40].

Lakhan et al chose to analyze 6 studies in terms of effectiveness in treating spasticity in MS patients using cannabis extract. Despite differences in used outcome measures, a consistent trend showing decreased spasticity in patients who received treatment [41].

A Phase III clinical trial called Multiple Sclerosis and Extract of Cannabis (MUSEC) was conducted to explore the effects of a standardized oral cannabis extract on providing symptomatic relief for persistent muscle stiffness and pain in adult patients with stable MS. The rate of relief from muscle stiffness was significantly higher in the Cannabis extract group compared to placebo at 12 weeks. Similar positive effects were noted for body pain, muscle spasms, and sleep quality [9].

The Cochrane review evaluates the effectiveness of cannabinoids, particularly focusing on spasticity and pain in people with multiple sclerosis (MS). Due to Cochrane studies, cannabinoids could be potentially beneficial in treatment of neuropathic pain. The evidence indicated that significantly more people treated with Nabiximols (one of the pharmaceutical agents contains cannabis) reported a reduction in spasticity compared to those receiving a placebo [42].

Furthermore, there is physiological evidence from at least one animal study supporting the anti-spastic properties of cannabinoids [43].

According to one academic work, cannabinoids showed a minor improvement in managing symptoms of bladder dysfunction [40]. Moderate-certainty evidence suggests that cannabinoids likely increase the number of patients reporting an improvement in their overall health status. At this point, treatment with cannabinoids likely results in a greater number of people experiencing noticeable improvement [42].

Retrospective study conducted by Michelle M. et al examined the effects of medical cannabis on symptoms in MS patients at a neurology outpatient center, analyzing data from 141 patients. After starting medical cannabis treatment, patients reported substantial improvement in MS symptoms, with 72% experiencing pain relief, 48% noting reduced spasticity and 40% seeing better sleep quality. Additionally, there was a notable decrease in the use of opioids following the initiation of using this medication. In case of adverse effects, most often reported was fatigue, in 11% of patients [44].

In studies utilizing an animal model of MS, the non-selective agonist for cannabinoid receptors (WIN55, 212-2) inhibits the entry of white blood cells into the central nervous system [45] and improves the course of the disease [46]. There is compelling evidence supporting the therapeutic potential of cannabinoids in animal models of MS.

The study by Chiurchiù et al demonstrated the beneficial effects of externally administered endocannabinoids, phytocannabinoids, and synthetic cannabinoids in alleviating motor symptoms and improving disease outcomes by reducing neuroinflammation [47].
Surprisingly, there's a suggestion that CBD exhibits an antidepressant-like effect in animal models, with its efficacy depending on the dosage [48].

Medications with cannabis extract

Products available on the market containing in their composition cannabis:

1. Nabiximols- generic name Sativex. It is an oromucosal spray with a 1:1 ratio of THC and CBD. Nabiximols contains THC, CBD, other cannabinoids and terpenoids dissolved in ethanol in the appropriate proportions. To avoid psychoactive effects that appeared while smoking cannabis, the preferred route of administration is via oromucosal spray [8]. The combined effect of THC and CBD is beneficial because of their different modes of action [35]. Applying nabiximols provides improvement in symptoms like spasticity, neuropathic pain, sleep and has minimal impact on HRQoL [8]. In the treatment of MS-related tremors and ataxia it does not fulfill its therapeutic role, just as in the case of disability and progression assessment [49] [50]. Sativex is authorized in Poland for alleviating symptoms in adult patients experiencing moderate to severe spasticity caused by MS [51].

2. Dronabinol is marketed under the same generic name and is accessible in 3 doses as an oral capsule. Dronabinol first appeared for chemotherapy-induced vomiting and nausea treatment. Moreover, was used in anorexia and weight loss in AIDS patients [8]. Dronabinol proves to be effective and safe in pain management in MS patients in long-term treatment [52]. It may also have a positive impact on difficulties in sleeping [8]. According to the CAMS trial, dronabinol does not rectify bladder symptoms in MS patients [53].

3. Nabilone primary was approved by FDA as an alternative for the treatment of chemotherapy-induced vomiting and nausea. Additionally, it was utilized as Parkinson's disease medication, neuropathic and chronic cancer pain. It is found to be effective in decreasing spasticity, pain, enhancing bladder functioning and quality of life in MS patients [8] [15]. In one research, authors mentioned ongoing study about evaluating the effectiveness of Nabilone in treating acute pain in people with inflammatory bowel disease [15].

Adverse effects and safety concerns

The most common adverse effects occurring in mild to moderate severity of using cannabis products are dizziness, fatigue, dry mouth, weariness and gastrointestinal disturbances [8]. No serious adverse events leading to discontinuation were reported, indicating a good tolerance profile. The occurrence of side effects is based on the dosage of cannabis required to effectively reduce one of the primary symptoms of MS- spasticity [41]. Research on medical marijuana indicates that THC can cause adverse effects at relatively low doses (15-30 mg). In contrast, CBD can be administered in much higher doses, up to several hundred milligrams, without the need to discontinue treatment due to side effects [40 54 55]. Surprisingly, it was concluded that the majority of reported adverse drug reactions were not associated with use of cannabis as medication [56].

Acute cannabis use can lead to negative effects such as hyperemesis syndrome, poor coordination and performance, anxiety, suicidal thoughts or tendencies, and psychotic symptoms. Long-term use may result in mood disturbances, worsening of psychotic disorders, cannabis dependency, withdrawal symptoms, cognitive impairments, and cardiovascular and respiratory issues [57]. The effects discussed above mainly relate to the recreational use of cannabis. Regarding the medical application of this substance, repeated studies have consistently demonstrated that high doses (up to 1,500 mg per day) and prolonged usage are well tolerated by humans [54]. Moreover, there's evidence suggesting that CBD could mitigate the adverse psychoactive effects of THC, while also augmenting its beneficial therapeutic effects [41 58].

In a recent epidemiological study conducted by Piper et al, it was revealed that among individuals who regularly used opioids, more than three-quarters (77%) reported a reduction in their opioid consumption since initiating cannabis use [59].

The primary concern revolves around the risk of addiction. Estimates suggest that approximately 9% of individuals who use cannabis may develop a dependency on the substance [60].

Like other medications used by patients, utilization of medical cannabis has a potential to develop interactions with other pharmaceuticals while simultaneous treatment. The in vitro metabolism of exogenous cannabinoids indicates involvement of hepatic cytochrome P450. Administering ketoconazole, which inhibits the CYP450 cytochrome, alongside cannabis extract, led to higher maximum serum concentrations THC and CBD. In contrast, when

rifampin, a strong inducer of this cytochrome, is administered concurrently, it results in lower levels of THC and CBD.

In clinical trials, dronabinol did not show clinically significant drug interactions. However, additive pharmacodynamic effects might occur when combined with other agents that have similar effects. Sedatives, alcohol, and antihistamines can enhance sedation, while tricyclic antidepressants and sympathomimetics can increase the risk of tachycardia [61] [62].

Nano-cannabinoids

Nanotechnology is a new way of using nanoparticles to administer substances easily to targeted areas despite their unfavorable pharmacokinetics. This technique permits accurate dosage control and specific delivery, improving treatment efficacy and minimizing toxicity. Nanomaterials in therapeutic systems are emerging as a valuable strategy for treating MS. They provide neuroprotection and improved efficacy by penetrating the blood-brain barrier (BBB) [63]. Cannabinoids are susceptible to auto-oxidation and degradation, affected by conditions like light and temperature. Nano-based technology is designed to enhance their stability, lower the dosage needed for treatment and pass the blood-brain barrier. This approach also reduce toxicity and side effects to normal neural tissues and cells [64] [65]. Although nanomedicine based on cannabinoids offers many benefits, it has not yet gained broad acceptance as a fully established treatment for multiple sclerosis patients [15].

Legal considerations

The WHO acknowledges the illegality status of cannabinoids (UN Convention of 1961). However, from a medical perspective, it believes that research in this field should be continued [66].

On November 1, 2017, Poland changed the legal status of herbal cannabis, allowing it to be used as a pharmaceutical raw material for making prescription drugs (the Act of Counteracting Drug Addiction). Medical doctors can now prescribe cannabis under the same regulations as other controlled substances [67]. There are no set limits on the amount of cannabis that can be possessed for medical use. Although, it should not exceed the quantity needed for 90 days of treatment, as determined by the THC dosage on the prescription, without any upper limit. This rule does not apply to other cannabinoids, such as CBD [68].

The Ministry of Health (MoH) did not approve reimbursement for any medications containing cannabinoids. Unfortunately, this means that the entire cost of treatment falls on the patient. Hordowicz M et al extensively discuss the legal status and principles of medical marijuana use in Poland. They also highlight the complexities of the regulations, the lack of official guidelines and the uncertainty among medical doctors regarding their knowledge of managing patients undergoing cannabis therapy. Additionally, they compare survey results from other European countries about this topic. The authors emphasize the need for further research and training for doctors, as nearly all survey participants expressed a desire to expand their knowledge on the subject and the need for clear governmental guidelines [69].

Cannabis treatment is registered in some states in the USA to control spasticity in MS, but also in Dravet's and Lennox-Gastaut's syndromes [70]. Epidiolex, a medication containing pure CBD, is utilized in epilepsy treatment [28]. It is worth noting that hemp-related laws are established by individual states in the USA, leading to differences in prohibitions, mandates, and allowable quantities. Federal law and DEA regulations have created challenges in obtaining cannabis for research purposes in the USA [71]. This becomes a significant obstacle considering the urgent need to conduct further research in the USA.

Szulc, in one of his creations evaluating the health effects of cannabis and the views of psychologists on its legalization, cited a study by Salomonsen-Sautel et al. This study revealed that 74% of teenagers in the United States have used medical cannabis prescribed to someone else [72]. This aspect certainly requires appropriate government regulations and careful consideration from the medical community to prevent overuse of medical products containing cannabis.

Conclusions

The medical use of cannabis is widely debated around the world, with both supporters and critics. It also divides the scientific community. Ongoing clinical trials investigating combined therapy involving THC and CBD are underway, given that it is a relatively new treatment approach [41]. While numerous studies show promising results regarding the effectiveness of cannabis-based treatments, an equal number of studies suggest the opposite. Each of these studies, however, has its own limitations [66]. Due to the uncertain clinical benefits and the variability in studies and results, it is wise not to make definitive conclusions about the effectiveness of cannabinoids in treating MS symptoms. Considering their safety, the limited

evidence of other effective treatments and the growing availability in certain areas, it might be tempting to consider including cannabinoids as part of the treatment options for MS [40].
The molecular impact of cannabinoids on MS, along with their roles in neuroprotection and immunomodulation, indicates that cannabinoids go beyond merely treating symptoms. Despite these promising reports, cannabidiol influence on the immune system of MS patients needs to be more investigated. To fully understand the risks of chronic medicinal cannabis use, long-term studies are needed to evaluate the potential adverse effects over extended period.

According to the detailed Cochrane analysis, there is a probable increase in neurological and psychiatric disorders associated with cannabinoid use, indicating these as potential risks of treatment. The evidence suggests that cannabinoids might not improve quality of life metrics significantly in MS patients- with the use of Health-Related Quality of Life (HRQoL) cannabinoids appear to have little to no effect on overall HRQoL. Nevertheless, it highlights the need for further high-quality, long-term studies to better understand the therapeutic potential and safety profile of these compounds in managing MS symptoms [42]. The study concluded that while there is limited efficacy of cannabinoids for treating spasticity, pain, and bladder dysfunction in MS, these drugs are relatively safe in terms of serious adverse events.

The results support the cautious use of cannabinoids in MS treatment, suggesting that while there are potential benefits, the overall impact on symptom relief may be limited, and the risk of adverse effects, particularly minor ones that affect patient tolerance, is considerable [40].
Lakhan and others show potential utility of cannabis extracts in managing spasticity and related symptoms in MS [41]. However, more high-quality studies need to be performed to establish clearer guidelines.

The aim of this review was to assess the effectiveness of cannabis extracts in treating MS, with the goal of enhancing knowledge about the potential efficacy, safety, and limitations of this medication.

Abbreviations:

MS multiple sclerosis

CNS Central nervous system

HLA-DR Human Leukocyte Antigen – DR isotype

MHC major histocompatibility complex

NICE The National Institute for Health and Excellence

HRQoL Health-related Quality of life

THC Δ9-tetrahydrocannabinol

DMT disease-modifying treatments

VLA-4 very late antigen-4

JCV John Cunningham virus

PML progressive multifocal leukoencephalopathy

CBD cannabidiol

C. Sativa Cannabis sativa

C.indica Cannabis indica

CB1,2 Cannabinoid receptor 1,2

GPCR G protein-coupled receptors

AEA Anandamide

2-AG 2-Arachidonoyl Glycerol

EAE experimental autoimmune encephalomyelitis

TNF-α tumor necrosis factor alpha

AIDS acquired immunodeficiency syndrome
FDA Food and Drug Administration
CYP450 cytochrome P450
UN United Nations
MoH Ministry of Health
USA United States of America
BBB Blood-Brain Barier

Disclosures

Author's contribution

Conceptualization: Aleksandra Rykucka, Natalia Zozula

Methodology: Marcin Wąs

Software: Justyna Kiełbasa

Check: Zuzanna Tomczewska, Agata Kowalczyk, Aleksandra Latała

Formal analysis: Iga Ślesicka

Investigation: Katarzyna Bil

Resources: Magda Przestrzelska

Data curation: Marcin Wąs

Writing-rough preparation: Aleksandra Rykucka, Magda Przestrzelska

Writing-review and editing: Zuzanna Tomczewska, Agata Kowalczyk

Visualization: Aleksandra Latała, Justyna Kiełbasa

Supervision: Iga Ślesicka, Katarzyna Bil

Project administration: Aleksandra Rykucka, Natalia Zozula

All authors have read and agreed with the published version of the manuscript.

Conflict of interest: The authors declare no conflict of interest.

Funding: This review has not received any external funding.

Statement of institutional review committee: not applicable

Informed consent: not applicable

Data availability: not applicable

Acknowledgments: not applicable

References

1. Browne P, Chandraratna D, Angood C, et al. Atlas of Multiple Sclerosis 2013: A growing global problem with widespread inequity. *Neurology*. (2014) 83:1022-4;https://doi.org/10.1212/WNL.0000000000000768.

2. Noseworthy J. Progress in determining the causes and treatment of multiple sclerosis. *Nature 399 A40-A47*. 1999;https://doi.org/10.1038/399a040.

3. Coles A, Alastair D, Compston S, et al. Alemtuzumab vs. interferon beta-1a in early multiple sclerosis. *N Engl J Med*. Oct 2008;https://doi.org/10.1056/NEJMoa0802670:1786-801;359(17).

4. Doshi A, Chataway J. Multiple sclerosis, a treatable disease. *Clin Med (Lond)*. 2016 Dec; 16(Suppl 6): s53–s59;https://doi.org/10.7861/clinmedicine.16-6-s53.

5. Lassmann H, Brück W, Lucchinetti C. The immunopathology of multiple sclerosis: an overview. *Brain Pathology*. 2007;17(2):210-218;https://doi.org/10.1111/j.1750-3639.2007.00064.x.

6. Belbasis L, Bellou V, Evangelou E, Ioannidis JPA, Tzoulaki I. Environmental risk factors and multiple sclerosis: an umbrella review of systematic reviews and meta-analyses". *Lancet. Neurology.* 2015 Mar;14(3):263-73;https://doi.org/10.1016/S1474-4422(14)70267-4.

7. Isobe N, Madireddy L, Khankhanian P, et al. An ImmunoChip study of multiple sclerosis risk in African Americans. *Brain.* 2015 Jun; 138(6): 1518–1530.;https://doi.org/10.1093/brain/awv078.

8. Haddad F, Dokmak G, Karaman R. The Efficacy of Cannabis on Multiple Sclerosis-Related Symptoms. *Life (Basel).* 2022 May 5;12(5):682.;https://doi.org/10.3390/life12050682.

9. Zajicek J, Hobart J, Slade A, Barnes D, Mattison P. Multiple Sclerosis and Extract of Cannabis: results of the MUSEC trial. *J Neurol Neurosurg Psychiatry.* 2012;83:1125-1132;https://doi.org/10.1136/jnnp-2012-302468.

10. Lublin F. New multiple sclerosis phenotypic classification. *Eur Neurol.* 2014:72 Suppl 1:1-5.;https://doi.org/10.1159/000367614.

11. Kurtzke J. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology.* 1983;33(11):1444-1452;https://doi.org/10.1212/wnl.33.11.1444.

12. Walczak A, Arkuszewski M, Adamczyk-Sowa M. Rozszerzona Skala Niepełnosprawności (EDSS, Expanded Disability Status Scale) — według J. Kurtzkego. *Polski Przegląd Neurologiczny*. 2017;13;1(https://journals.viamedica.pl/polski_przeglad_neurologiczny/article/view/53968):32-35.

13. Perry M, Swain S, Kemmis-Betty S, Cooper P. Multiple sclerosis: summary of NICE guidance. *BMJ*. 2014; 349 :g5701;https://doi.org/10.1136/bmj.g5701.

14. Hauser S, Cree BA. Treatment of Multiple Sclerosis: A Review. *The American Journal of Medicine*. July 2020DOI:https://doi.org/10.1016/j.amjmed.2020.05.049;https://doi.org/10.1016/j.amjmed.2020.05.049.

15. Nouh R, Kamal A, Oyewole O, et al. Unveiling the Potential of Cannabinoids in Multiple Sclerosis and the Dawn of Nano-Cannabinoid Medicine. *Pharmaceutics.* 2024; 16(2):241.;https://doi.org/10.3390/pharmaceutics16020241.

16. Ziemssen T, Derfuss T, de Stefano N, et al. Optimizing treatment success in multiple sclerosis. *J Neurol*. (2016) 263:1053–65;https://doi.org/10.1007/s00415-015-7986-y.

17. Zajicek J, Fox P, Sanders H, et al. Cannabinoids for treatment of spasticity and other symptoms related to multiple sclerosis (CAMS study): multicentre randomised placebo-controlled trial. *The Lancet*. November 2003;https://doi.org/10.1016/S0140-6736(03)14738-1.

18. Rice G, Incorvaia B, Munari L, et al. Interferon in relapsingremitting multiple sclerosis. *Cochrane Database Syst. Rev.* 2001;https://doi.org/10.1002/14651858.CD002002.

19. Schwab N, Schneider-Hohendorf T, Wiendl H. Therapeutic uses of anti-α4-integrin (anti-VLA-4) antibodies in multiple sclerosis. *International Immunology*. January 2015;https://doi.org/10.1093/intimm/dxu096:47-53;27;1.

20. Dąbrowski G, Skrajda M. Cannabinoids from Cannabis sp.: mechanism of their activity and potential health benefits in human body. *Journal of Education, Health and Sport*. August 2017;7(8):936-945;https://doi.org/10.5281/zenodo.995625.

21. Breijyeh Z, Jubeh B, Bufo S, Karaman R, Scrano L. Cannabis: A Toxin-Producing Plant with Potential Therapeutic Uses. *Toxins*. 2021, 13, 117;https://doi.org/10.3390/toxins13020117.

22. Bonini S, Premoli M, Tambaro S, et al. Cannabis sativa: A comprehensive ethnopharmacological review of a medicinal plant with a long history. *J. Ethnopharmacol.* 2018, 227, 300–315.;https://doi.org/10.1016/j.jep.2018.09.004.

23. Hillig K, Mahlberg P. A chemotaxonomic analysis of cannabinoid variation in Cannabis (Cannabaceae). *Am J Bot.* 2004 Jun;91(6):966-75;https://doi.org/10.3732/ajb.91.6.966.

24. Furgiuele A, Cosentino M, Ferrari M, al e. Immunomodulatory Potential of Cannabidiol in Multiple Sclerosis: a Systematic Review. *J Neuroimmune Pharmacol*. (2021) 16, 251–269;https://doi.org/10.1007/s11481-021-09982-7.

25. VanDolah H, Bauer B, Mauck K. Clinicians' Guide to Cannabidiol and Hemp Oils. *Mayo Clin Proc.* 2019;94(9):1840-1851.;https://doi.org/10.1016/j.mayocp.2019.01.003.

26. Sosnoff J, Rudroff T. Cannabidiol to Improve Mobility in People with Multiple Sclerosis. *Front Neurol.* 2018; 9: 183.;https://doi.org/10.3389/fneur.2018.00183.

27. Zwibel H. Contribution of impaired mobility and general symptoms to the burden of multiple sclerosis. *Adv Ther.* 2009;26(12):1043-1057;https://doi.org/doi:10.1007/s12325-009-0082-x.

28. Maayah Z, Takahara S, Ferdaoussi M, Dyck J. The anti-inflammatory and analgesic effects of formulated full-spectrum cannabis extract in the treatment of neuropathic pain associated with multiple sclerosis. *Inflamm Res.* 2020 Jun;69(6):549-558;https://doi.org/10.1007/s00011-020-01341-1.

29. Russo E. Taming THC: potential cannabis synergy and phytocannabinoid–terpenoid entourage effects. *Br J Pharmacol*. (2011) 163:1344–64.;https://doi.org/10.1111/j.1476-5381.2011.01238.x.

30. Puighermanal E, Busquets-Garcia A, Maldonado R, Ozaita A. Cellular and intracellular mechanisms involved in the cognitive impairment of cannabinoids. *Philos Trans R Soc Lond B Biol Sci.* 2012 Dec 5; 367(1607): 3254–3263;https://doi.org/10.1098/rstb.2011.0384.

31. Pertwee R. Pharmacology of cannabinoid CB1 and CB2 receptors. *Pharmacology & Therapeutics*. 1997;https://doi.org/10.1016/S0163-7258(97)82001-3:129-180;74.

32. Meyer H, Lee F, Gee D. The Role of the Endocannabinoid System and Genetic Variation in Adolescent Brain Development. *Neuropsychopharmacology.* 2018 Jan;43(1):21-33;https://doi.org/10.1038/npp.2017.143.

33. Ingram G, Pearson RO. Cannabis and multiple sclerosis. *Pract Neurol.* 2019 Aug;19(4):310-315.;https://doi.org/10.1136/practneurol-2018-002137.

34. Gong J, Onaivi E, Ishiguro H, et al. Cannabinoid CB2 receptors: Immunohistochemical localization in rat brain. *Brain Research*. 2006;https://doi.org/10.1016/j.brainres.2005.11.035:10-23;1071.

35. Überall M. A Review of Scientific Evidence for THC:CBD Oromucosal Spray (Nabiximols) in the Management of Chronic Pain. *J Pain Res.* 2020 Feb 14;13:399-410.;https://doi.org/10.2147/JPR.S240011.

36. Fischedick J. Identification of terpenoid chemotypes among high (−)-trans-Δ9-tetrahydrocannabinol-producing Cannabis sativa L. cultivars. *Cannabis and Cannabinoid Research*. 2017;https://doi.org/10.1089/can.2016.0040:34-47;2.

37. Wegener N, Koch M. Neurobiology and systems physiology of the cannabinoid system. *Pharmacopsychiatry*. 2009;42 Suppl 1:S79-S86.;https://doi.org/10.1055/s-0029-1216346.

38. Iuvone T, Esposito G, Esposito R, Santamaria R, Di Rosa M, Izzo A. Neuroprotective effect of cannabidiol, a non-psychoactive component from Cannabis sativa, on β-amyloid-induced toxicity in PC12 cells. *Journal of Neurochemistry.* 2004 89: 134-141.;https://doi.org/10.1111/j.1471-4159.2003.02327.x.

39. Kozela E, Lev N, Kaushansky N, et al. Cannabidiol inhibits pathogenic T cells, decreases spinal microglial activation and ameliorates multiple sclerosis-like disease in C57BL/6

mice. *British Journal of Pharmacology*. 2011 1507-1519;https://doi.org/10.1111/j.1476-5381.2011.01379.x.

40. Whiting P, Wolff R, Deshpande S, et al. Cannabinoids for Medical Use: A Systematic Review and Meta-analysis. *JAMA.* 2015;313(24):2456–2473;https://doi.org/doi:10.1001/jama.2015.6358.

41. Lakhan S, Rowland M. Whole plant cannabis extracts in the treatment of spasticity in multiple sclerosis: a systematic review. *BMC Neurol*. 9, 59 (2009);https://doi.org/10.1111/j.1476-5381.2011.01379.x.

42. Filippini G, Minozzi S, Borrelli F, Cinquini M, Dwan K. Cannabis and cannabinoids for symptomatic treatment for people with multiple sclerosis. *Cochrane Database Syst Rev.* 2022 May 5;5(5):CD013444;https://doi.org/10.1002/14651858.CD013444.pub2.

43. Baker D, Pryce G, Croxford J, et al. Endocannabinoids control spasticity in a multiple sclerosis model. *FASEB J.* 2001, 15(2): 300-302.;https://doi:10.1096/fj.00-0399fje.

44. Rainka M, Aladeen T, Mattle A, et al. Multiple Sclerosis and Use of Medical Cannabis: A Retrospective Review of a Neurology Outpatient Population. *International Journal of MS Care*. 2023; 25 (3): 111–117;https://doi.org/10.7224/1537-2073.2022-006.

45. Arévalo-Martín A, Vela JM, Molina-Holgado E, Borrell J, Guaza C. Therapeutic action of cannabinoids in a murine model of multiple sclerosis. *J. Neurosci.* 2003; 23, 2511–2516;https://doi.org/10.1523/JNEUROSCI.23-07-02511.2003.

46. Croxford JL, Miller SD. Immunoregulation of a viral model of multiple sclerosis using the synthetic cannabinoid R+WIN55,212. *J. Clin. Invest.* 2003; 111, 1231–1240.;https://doi.org/10.1172/jci200317652.

47. Chiurchiù V, Van Der Stelt M, Centonze D, Maccarrone M. The endocannabinoid system and its therapeutic exploitation in multiple sclerosis: clues for other neuroinflammatory diseases. *Prog. Neurobiol.* 2018; 160, 82–100.;https://doi: 10.1016/j.pneurobio.2017.10.007.

48. El-Alfy A, Ivey K, Robinson K, et al. Antidepressant-like effect Δ9-tetrahydrocannabinol and other cannabinoids isolated from Cannabis sativa L. *Pharmacol Biochem Behav*. 2010; 95:434–42;https://doi.org/10.1016/j.pbb.2010.03.004.

49. Koppel B, Brust J, Fife T, et al. Systematic review: Efficacy and safety of medical marijuana in selected neurologic disorders: Report of the Guideline Development Subcommittee of the American Academy of Neurology. *Neurology*. 2014, 82, 1556–1563;https://doi.org/10.1212/WNL.0000000000000363.

50. Ball S, Vickery J, Hobart J, et al. The CUPID trial: A randomised double-blind placebo-controlled parallel-group multi-centre trial of cannabinoids to slow progression in multiple sclerosis. *Health Technol. Assess.* 2015, 19, 1–187;https://doi.org/10.3310/hta19120.

51. *Sativex SmPC 2012.* https://rejestrymedyczne.ezdrowie.gov.pl/api/rpl/medicinal-products/29034/characteristic. Accessed 24 May 2024.

52. Schimrigk S, Marziniak M, Neubauer C, Kugler E, Werner G, Abramov-Sommariva D. Dronabinol is a safe long-term treatment option for neuropathic pain patients. *Eur. Neurol.* 2017, 78, 320–329.;https://doi.org/10.1159/000481089.

53. Zajicek J, Fox P, Sanders H, et al. Cannabinoids for treatment of spasticity and other symptoms related to multiple sclerosis (CAMS study): Multicentre randomised placebo-controlled trial. *Lancet*. 2003, 362, 1517–1526.;https://doi.org/10.1016/S0140-6736(03)14738-1.

54. Bergamaschi M, Queiroz R, Zuardi A, Crippa J. Safety and side effects of cannabidiol, a Cannabis sativa constituent. *Curr Drug Saf.* 2011;6(4):237-249.;https://doi:10.2174/157488611798280924.

55. Malan TJ, Ibrahim M, Lai J, Vanderah T, Makriyannis A, Porreca F. CB2 cannabinoid receptor agonists: pain relief without psychoactive effects?. *Curr Opin Pharmacol.* 2003;3(1):62-67.;https://doi.org/10.1016/s1471-4892(02)00004-8.

56. Wade D, Makela P, House H, Bateman C, Robson P. Long-term use of a cannabis-based medicine in the treatment of spasticity and other symptoms in multiple sclerosis. *Mult Scler.* 2006;12(5):639-645.;https://doi.org/10.1177/1352458505070618.

57. Laurent K, Roux P, Rolland B, et al. Acute and long-term effects of cannabis use: a review. *Curr Pharm Des.* 2014;20(25):4112-4118.;https://doi.org/10.2174/13816128113199990620.

58. Niesink R, van Laar M. Does Cannabidiol Protect Against Adverse Psychological Effects of THC?. *Front Psychiatry.* 2013;4:130.;https://doi.org/10.3389/fpsyt.2013.00130.

59. Piper B, DeKeuster R, Beals M, et al. Substitution of medical cannabis for pharmaceutical agents for pain, anxiety, and sleep. *Journal of Psychopharmacology.* 2017;31(5):569-575.;https://doi.org/10.1177/0269881117699616.

60. Lopez-Quintero C, Pérez de los Cobos J, Hasin D, et al. Probability and predictors of transition from first use to dependence on nicotine, alcohol, cannabis, and cocaine: results of the National Epidemiologic Survey on Alcohol and Related Conditions (NESARC). *Drug Alcohol Depend.* 2011;115(1-2):120-130.;https://doi.org/10.1016/j.drugalcdep.2010.11.004.

61. Stout SM CN. Exogenous cannabinoids as substrates, inhibitors, and inducers of human drug metabolizing enzymes: a systematic review.. *Drug Metab Rev.*. 2014;46(1):86-95.(doi:10.3109/03602532.2013.849268).

62. Sativex oral mucosal spray. electronic Medicines Compendium (eMC). May 2015.. Available at: www.medicines.org.uk/emc/ medicine/23262. Accessed May 22, 2024.

63. Zhang Q, Dai X, Zhang H, Zeng Y, Luo K, Li W. Recent advances in development of nanomedicines for multiple sclerosis diagnosis. *Biomed Mater.* 2021 Feb 17;16(2):024101.;https://doi.org/10.1088/1748-605X/abddf4.

64. Onaivi E, Singh Chauhan B, Sharma V. Challenges of cannabinoid delivery: how can nanomedicine help?. *Nanomedicine (Lond).* 2020;15(21):2023-2028.;https://doi.org/10.2217/nnm-2020-0221.

65. Durán-Lobato M, Álvarez-Fuentes J, Fernández-Arévalo M, Martín-Banderas L. Receptor-targeted nanoparticles modulate cannabinoid anticancer activity through delayed cell internalization. *Sci Rep.* 2022 Jan 25;12(1):1297.;https://doi.org/10.1038/s41598-022-05301-z.

66. Pinkas J, Jabłoński P, Kidawa M, Wierzba W. Use of marijuana for medical purposes. *Ann Agric Environ Med.* 2016;23(3):525-528;https://doi.org/10.5604/12321966.1219200.

67. Internet-Based Law System Act of the 7th of July 2017 Amending the Act on Counteracting Drug Addiction and the Act on Reimbursement of Medicines, Foodstuffs for Special Nutritional Purposes and Medical Devices. https://isap.sejm.gov.pl/isap.nsf/DocDetails.xsp?id=wdu20170001458. Accessed 22 May 2024.

68. Internet-Based Law System: Announcement of the Minister of Health of the 20th of January 2021 on the Announcement of the Consolidated Text of the Regulation of the Minister of Health on Narcotic Drugs, Psychotropic Substances, Precursors Category 1 and Pr. https://isap.sejm.gov.pl/isap.nsf/download.xsp/WDU20210000166/O/D20210166.pdf. Accessed 22 May 2024.

69. Hordowicz M, Jarosz J, Czaplińska M, Leonhard A, Klimkiewicz A. Polish Physicians' Perspectives on Medical Cannabis Policy and Educational Needs: Results of An Online Survey. *J Clin Med.* 2021 Sep 30;10(19):4545.;https://doi.org/10.3390/jcm10194545.

70. Russo E. Cannabis Therapeutics and the Future of Neurology. *Front. Integr. Neurosci.* 2018; 12:51.;https://doi.org/10.3389/fnint.2018.00051.

71. Bridgeman M, Abazia D. Medicinal Cannabis: History, Pharmacology, And Implications for the Acute Care Setting. *P T.* 2017;42(3):180-188(PMCID: PMC5312634).

72. Szulc M. The health consequences of marijuana use in the light of research and the proposal standardize the position of psychologists to the problem of the legalization of cannabis, formulated on the basis of the Code of Ethics and Professional Psychologists. *Alcoholism and Drug Addiction*. 2013; 26(4): 381–401 (in Polish).

73. Maayah Z, Takahara S, Ferdaoussi M, Dyck J. The anti-inflammatory and analgesic effects of formulated full-spectrum cannabis extract in the treatment of neuropathic pain associated with multiple sclerosis. *Inflamm Res.* 2020 Jun;69(6):549-558.;https://doi.org/10.1007/s00011-020-01341-1.