Recent Developments on Drug Addiction, CRISPR and Herbal Treatment Options

Editor
Ali BİLGİLİ

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CHAPTER I

The Role of the Blood-Brain Barrier in Drug Addiction

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Özkan ŞİMŞEK[2]

Introduction

Drug addiction is on the rise worldwide. Today, there are many different substances that can be addictive, such as alcohol, nicotine, cocaine, cannabis, ecstasy, amphetamines, opioids and morphine derivatives (Gökler and Koçak, 2008).

Substances such as drugs and alcohol trigger the development of addiction in people by causing changes in specific brain regions.

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In particular, these substances affect the blood-brain barrier (Sajja et al., 2016).

The blood-brain barrier is a special structure that acts as a protective barrier for the central nervous system (CNS). It preserves the brain tissue from potentially harmful substances, toxins and pathogens. The blood-brain barrier is created by specialized cells and compounds on the vascular inner surface that surround the brain tissue. This barrier helps to maintain the delicate balance in the brain while allowing brain cells to maintain their normal function. (Gültürk et al., 2007). However, during the process of drug addiction, certain drugs have the ability to penetrate brain tissue by passing through the blood-brain barrier. These substances can affect nerve cells, disrupt nerve conduction and impair the function of neurons, leading to chemical imbalances in the brain. This leads to physiological and psychological health problems in people who consume substances (Sharma et al., 2009; Kousik et al., 2012; Pimentel et al., 2020).

The blood-brain barrier is one of the most important structures affected in drug addiction and is associated with individual dependence. The objective of this chapter is to explain the relationship between drug addiction and the blood-brain barrier in detail.

Structure of the Blood-Brain Barrier

The blood-brain barrier, a special structure, prevents infections and dangerous chemicals from entering the brain and guarantees that the brain receives enough nutrition. This structure consists of endothelial cells, basement membrane, pericytes and astrocytes, which are arranged in tight junctions (Tajes et al., 2014, Daneman and Prat, 2015).

Endothelial cells are squamous epithelial cells that make up the walls of blood vessels. The endothelial cells in the blood-brain barrier differ from those in other tissues in the connections they make with each other (Aird, 2007a; Aird, 2007b). Astrocytes are the type

of glial cells that surround neuronal structures and establish a cellular connection between the neuronal circuit and the blood vessels (Abbott et al., 2006). Pericytes are another barrier cell found on the surface of the microvascular endothelial wall, and are integrated into the vascular basement membrane. These cell structures serve the function of the blood-brain barrier through tight and adherens junctions (Breviario et al., 1995; Lampugnani et al., 1999; Altıntaş and Benbir, 2004).

Functions of the Blood-Brain Barrier

The primary role of the blood-brain barrier is to preserve the homeostasis of the brain. This barrier keeps the CNS from circulating peripheral toxins and immune factors (Abbott et al., 2010). It also contributes to homeostasis by regulating cerebral blood flow through the neurovascular junction by releasing local vasoregulators such as noradrenaline, nitric oxide and endothelin (Kousik et al., 2012).

The passage of most molecules is restricted by various factors such as lipid solubility, polarity or particle size. Because of this, they need transporters to get through the blood-brain barrier. Simple diffusion, facilitated diffusion, active transport, and vesicular transport are the several forms of transfer that take place in the blood-brain barrier (Gültürk et al., 2007). These transport systems move metabolic waste products from the brain into the blood and provide the brain with nutrients from the blood (Kousik et al., 2012).

Factors Affecting the Blood-Brain Barrier

We can divide the factors that influence the blood-brain barrier's permeability into two parts: physiological and pathological factors. The physiological factors that influence this permeability include the state of the substance in the blood, the surface area, the fat solubility of the substance, the presence of cellular transport systems, the continuity of the barrier, the concentration of the substance in the blood and the duration of its stay in the blood

(Yılmaz, 2006). In addition, factors such as aging, gut microbiota, environmental stress, metabolism, sleep and diet can also affect the blood-brain barrier's permeability and the interactions between the cellular components (Northrop and Yomomato, 2015; Altınsoy and Dikmen, 2023). Infections, oxidative stress, stimulant drugs, neurodegenerative diseases, diabetes and traumatic brain injuries are among the pathological factors that impair the blood-brain barrier (Kousik et al., 2012). Hypertension, radioactivity, edema, inflammation, ischemia and reoxygenation are among the pathological factors that increase the blood-brain barrier's permeability. These factors have the effect of altering the bioavailability of drugs (Germano and Tomasello, 2001).

Drug Addiction

In general terms, addiction is the irresistible desire for an object, person or being, even though it is known to be physically or psychologically harmful to the person (Koob and Le Moal, 2008). Substance addiction is defined as the improvement in tolerance to a substance as a result of the misuse of a substance and the gradual increase in its intake, whereby the person continues to consume this substance despite the fact that it causes many problems and various physiological effects occur in the person when the intake is reduced or stopped (Uzbay, 2009). Drugs, alcohol, nicotine, opioids and benzodiazepines are widely used and addictive substances.

Drugs can be addictive by affecting the chemical balance in the brain. Cocaine, ecstasy, methamphetamine (MET), lysergic acid diethylamide (LSD) and inhalants (such as aromatic hydrocarbons and butyl nitrite) are the most commonly consumed drugs in the world (Galloway, 1992; Seiden et al., 1993; Steele et al., 1994; Örken and Tavşanlı, 2014).

Alcohol is a widely used substance and a central nervous system depressant that can lead to addiction. Chronic alcohol consumption can trigger a condition known as alcohol dependence. Alcohol dependence is defined as the inability to control alcohol

consumption and the need for alcohol. Studies have shown that 85.6% of people aged 18 years and older have consumed alcohol at some point in their lives (Vore and Deak, 2021).

Nicotine, which is contained in cigarettes, cigars, pipes and other tobacco products, is an addictive stimulant. It contributes to the development of addiction by influencing the release of neurotransmitters in the brain. The degree of addiction depends on how long, how much, how heavily and what type of tobacco the person uses, how deeply they inhale, their health and genetic factors (Karacalar et al., 2014).

Opioids are drugs that are known for their strong pain-relieving effect. These include prescription opioids (oxycodone, hydrocodone, codeine) and the illegal drug heroin. Recently, the recreational use of opioids such as oxycodone and hydrocodone for pain relief has become more common than the intravenous use of heroin. Opioids reduce the sensation of pain by acting on the pain receptors in the brain while providing an increased sense of pleasure and relaxation. These effects indirectly promote the development of addiction (Snyder, 2004). It has also been shown that long-term abuse of benzodiazepines (such as diazepam, alprazolam, lorazepam), which are used to treat anxiety, insomnia and some psychiatric disorders, can lead to physical and psychological dependence (Lader, 2011).

Studies have shown that people primarily turn to these addictive substances when they are in a tense and stressful situation to relieve anxiety and stress, to feel happy and to relax. When the substance is suddenly discontinued in people who have become addicted, withdrawal symptoms such as severe pain, vomiting, diarrhea, sweating, insomnia and a runny nose have been observed in those affected. These symptoms can be mild or fatal depending on the type of substance used or the person (Karacalar et al., 2014; Uzbay, 2009).

The Role of the Blood-Brain Barrier in Drug Addiction

Studies have shown that addictive chemicals such as cocaine, methamphetamine, morphine, heroin, alcohol, and nicotine can lead to functional impairments by altering the blood-brain barrier's transport channels and endothelial cell connections. Furthermore, it has been observed that these compounds enhance the permeability of the blood-brain barrier, hence facilitating the entry of peripheral toxins into the brain. Pimentel et al. (2020) demonstrated that disruption of the blood-brain barrier triggers neuroinflammatory pathways by raising astroglial activation. This, in turn, increases the blood-brain barrier's permeability and the central nervous system's sensitivity to external molecules. This leads us to the conclusion that distinct processes underlie how addictive substances affect the blood-brain barrier.

Stimulant Drugs

It has been demonstrated that cocaine damages the basement membrane and neurovascular capillaries and increases the blood-brain barrier's permeability by 50% (Sharma et al., 2009). The loss or modification of tight junction protein complexes is one feature of cocaine-induced disruption of the blood-brain barrier. Clinical research indicates that cocaine addicts have a similar breach of the blood-brain barrier in the basal ganglia and enhanced HIV penetration into the central nervous system. The central nervous system dysfunction linked to cocaine usage is caused by the continual loss or conformational alterations of tight junction proteins and the reorganisation of basement membrane fibres, which leaves the brain vulnerable to toxins entering from the periphery. Blood-brain barrier dysfunction may be significantly impacted by cocaine-induced neuroinflammation, which is caused by an increase in inflammatory agents and endothelial adhesion molecules. Studies have indicated a rise in endothelial leukocyte adhesion molecule 1, intracellular adhesion molecule 1, vascular cell adhesion molecule 1, and platelet endothelial cell adhesion molecule 1 (Gan et al., 1999; Chang et al., 2000; Fiala et al., 2008).

Ecstasy, synthetic amphetamine derivative, interacts with serotonin and dopamine transporters similarly to other amphetamines, increasing monoamine release from nerve terminals (Sulzer et al., 2005). Ecstasy's neurotoxic effects may be exacerbated by disruption of the blood-brain barrier, according to recent studies. Rats given acute ecstasy treatment showed increased immunoglobulin G immunostaining and Evans-Navisi or trypan blue leakage in several cortical regions, including the striatum, cerebellum, and hippocampal regions. (Yamomoto and Bankson, 2005; Sharma and Ali, 2008). The blood-brain barrier was found to be disrupted as soon as acute ecstasy was administered and continued for up to 10 weeks following the injection. enhanced endogenous albumin parenchymal penetration and enhanced astrocyte and microglia activation were linked to the blood-brain barrier's increased permeability after ecstasy therapy. Additionally, it has been noted that ecstasy increases the expression of proinflammatory cytokines in brain tissue, such as IL-1β (Torres et al., 2011).

Again, studies have demonstrated that abusing ecstasy causes oxidative stress (Yamamoto and Bankson, 2005). According to Quinton and Yamamoto's (2006) research, ecstasy-induced increased release of DA and 5-HT resulted in the production of hazardous metabolites from ROS, DAQ, and 5-HT oxidation. Toxic free radical buildup makes brain tissue more vulnerable to ischemia injury and sets off a series of signals that result in malfunction of the blood-brain barrier, brain edoema, and neuroinflammation (Gu et al., 2012). Studies have shown that methamphetamine causes the blood-brain barrier's paracellular permeability to increase and the endothelial barrier's tightness to decrease. Methamphetamine disrupts brain homeostasis in this way by causing hyperthermia, which in turn damages the blood-brain barrier (Pimentel et al., 2020).

In a study, rats given a single high dosage of methamphetamine for 90 minutes showed increased blood-brain barrier permeability to endogenous IgG; however, this effect

subsided three days later. The hippocampal and amygdalae showed elevated immunoglobulin G levels, but only in mice who developed seizures and hyperthermia. Within 24 hours of methamphetamine exposure, lower dosages of methamphetamine also increased Evans blue permeability. These early elevations in permeability of the blood-brain barrier could be associated with heat (Northrop and Yamamato, 2015). Following methamphetamine consumption, transient alterations in the blood-brain barrier's structural makeup have also been noted. After a day, it was discovered that mice given a single, high dosage of methamphetamine decreased the expression of structural tight junction proteins in the blood-brain barrier, such as occludin, claudin-5, and zona occludens (Martins et al., 2011).

The impact of oxidative stress on the breakdown of the blood-brain barrier caused by methamphetamine was examined in a different investigation. Meth exposure reduced the expression of occludin, claudin-5, and ZO in cultured brain microvascular endothelial cells. It also reduced transendothelial electrical resistance, which was thought to be a sign of enhanced paracellular permeability (Northrop and Yamamato, 2015).

Morphine, one of the addictive drugs, causes neurotoxicity through proinflammatory cytokine activity, dysregulation of intracellular calcium release, and reactive oxygen species due to myosin light chain protein kinase activation. These changes impact the homeostasis and permeability of the blood-brain barrier (Kousik et al., 2012).

Heroin, which has a high lipophilicity, allows it to pass through the blood-brain barrier more quickly than morphine. Heroin consumption causes the blood-brain barrier to penetrate at a pace that is about 100 times faster (Boix et al., 2013).

Alcohol

Alcohol, which is more addictive than narcotic drugs, has been found to increase oxidative stress by phosphorylating myosin light chain and tight junction proteins. Leukocyte migration across the

blood-brain barrier is then enhanced and transendothelial electrical resistance is subsequently reduced as a result. It has been demonstrated to do this by causing neuroinflammation and blood-brain barrier disruption (Haorah et al., 2007; Muneer et al., 2012).

Wei et al. (2022) examined the effects of chronic alcohol use on the blood-brain barrier integrity by giving mice alcohol for 30 days. The study's findings revealed that the blood-brain barrier's integrity was compromised and that its permeability had considerably increased. Other studies have also found that long-term alcohol exposure leads to gaps between cells and damages some structural proteins on the brain's endothelial cell line (Carrino et al., 2021; Wei et al, 2022).

Nicotine

Studies on nicotine addiction have shown that nicotine consumed over a longer period of time leads to a disruption of the tight junction proteins and thus to an imbalance of ions. As a result, neuronal damage such as ischemic hypoxia and cerebral edema can be observed (Pimentel et al., 2020).

Nicotine is a soluble, tiny chemical that diffuses quickly through the blood-brain barrier and interacts directly with brain endothelial cells' nicotinic acetylcholine receptors. Studies conducted in vivo and in vitro have revealed a notable decrease of tight junction proteins, including occludin, ZO-1, claudin-3, and JAMs. Nicotine affects the blood-brain barrier's tight junctions as well as the transport and receptor systems necessary for the barrier's regular operation (Pimentel et al., 2020). Additionally, nicotine can set off inflammatory signals that have a direct impact on the blood-brain barrier's integrity. Research indicates that nicotine can facilitate leukocyte migration across the blood-brain barrier and boost the expression of inflammatory cytokines and endothelial adhesion molecules, especially during neuroinflammation (Bradford et al., 2011).

Conclusion

Addictive drugs that alter the blood-brain barrier's transport channels and endothelial cell connections, such as cocaine, ecstasy, methamphetamine, heroin, alcohol, and nicotine, can lead to functional problems. These drugs have various methods of rupturing the blood-brain barrier, and they also lead to major health issues aside from addiction. Because of this disruption, addictive chemicals cross the blood-brain barrier, affect the brain's reward centres, and cause an increase in dopamine release, which makes the user feel good. Therefore, increased permeability of the blood-brain barrier can facilitate the development of addiction and support the maintenance of addiction. In addition, some drugs can impair the blood-brain barrier, leading to increased oxidative stress and inflammation in the brain. This can damage brain tissue and lead to the development of neurological diseases.

In conclusion, the blood-brain barrier is of great importance in supporting the addiction cycle in substance users and causing serious health problems in addicted individuals. Therefore, a better understanding of the mechanisms of the blood-brain barrier in relation to drug addiction will make an important contribution to the treatment and prevention of addiction. Again, uncovering these mechanisms through new research is important to prevent the problems caused by addiction and to develop new treatment strategies for existing patients.

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CHAPTER II

Treatment of Crıspr ın Therapy

Okan AYKAÇ
Ceylan ÖZSOY

Introductıon

Clustered Regularly Interspaced Palindromic Repeats (CRISPR) is a gene-editing technology that corrects errors in the genome and turns genes on and off in organisms more cheaply and faster than other biotechnological methods. In particular, the CRISPR phase Cas9 variant is used more actively and obviously compared to other variants.

It has been previously mentioned that it is used to repair damaged DNA and ameliorate genetic disorders in mice. It has been reported that human embryos will be modified in the same way. It is informed that it has begun to be used effectively in the treatment of infectious diseases for example HIV, gene therapy, and in the treatment of other diseases [1].

This technology will also lead to more different gene editing technologies to be created in the future. New technology treatment methods such as CRISPR play a very important role in today's world. Rapidly-development has been achieved with the studies carried out in recent years. Various models of genome editing have been established. It is promising for the future in insidiousness diseases such as cancer, and as it has been developed, insidiousness pathogenesis has been overcome. However, the incomplete resolution of CRISPR limits its use and requires further research. Over time, it is thought that these problems will be overcome as the safe and responsible use of CRISPR increases. For these reasons, I have concluded that CRISPR gene technology is worthy of enlightenment and that I should use it in my work. The reason for the purpose and mission of this study has been stated in this way.

Crispr

Clustered regularly spaced palindromic repeats (CRISPR) Cas9 is a gene proofreading technology that causes great complexity in biomedical research. It makes it possible to correct errors in the genome and to turn genes on and off in cells and organisms inexpensively, quickly, and easily. It has a range of laboratory applications, including rapid generation of cell and animal models, useful genomic scans, and live imaging of the cellular genome [2].

It has previously been shown that it can be used to repair damaged DNA in mice and cure them of genetic disorders [3].

It has been informed that human embryos can also change in the same way. Other potential applications include treating infectious diseases such as HIV, gene therapy, and engineering autologous patient material to treat cancer and other diseases [4].

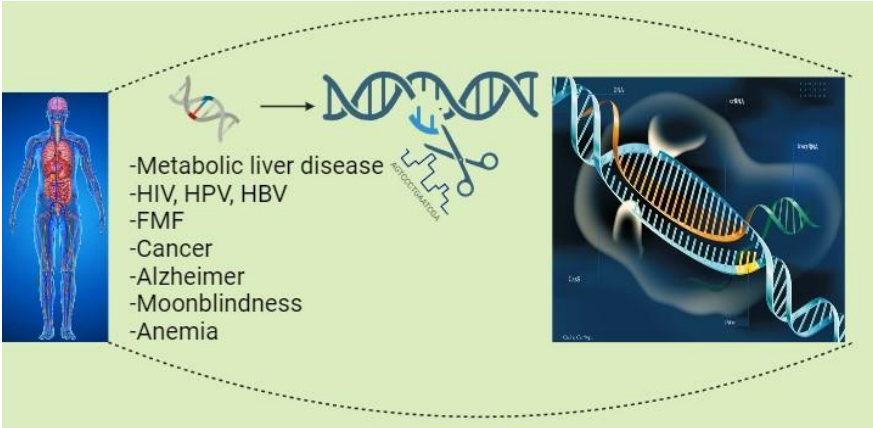

Figure 1. *CRISPR in therapy*

Bıologıcal Structure Of The System

Bıologıcal Structure

Many archaea and bacteria have evolved complex RNA-led adaptive defense systems encoded by CRISPR loci and associated CRISPR-related genes, providing acquired immunity anti bacteriophage infection and plasmid transfer [5].

During the immunization time interval as a result of exposure to invasive genetic elements from plasmids or phages, short fragments of foreign DNA are linked as new spacers to the CRISPR repeat-spacer sequence on the host chromosome, resulting in a genetic record of the infection and the host's future potential for the same invader. allows it to block the attack [6].

Subsequent transcription of the CRISPR sequence and enzymatic processing of precursor-CRISPR transcripts produce short, enhanced crRNAs via endonuclease cleavage. At the 5' end, crRNA contains short segments, a short segment of RNA complementary to a sequence of a foreign genetic unit, and at the 3' end is a portion of the CRISPR repeat sequence. Hybridizing between the crRNA short fragment and a complementary foreign

target fragment triggers sequence despecification of the offending DNA or RNA by Cas nucleases at the time of a second infection [7].

A pioneering feature of CRISPR-Cas systems is the assembly of engineered crRNAs with Cas proteins into crRNA effector compounds, which are used to interrogate DNA targets and eliminate overlapping sequences in foreign nucleic acids [8].

The short conserved motif sequence (2-5 bp), known as PAM, located next to the crRNA targeting sequence in the attacker DNA, has an crucial role in target DNA selection and degradation in many RISPR-Cas systems. [9].

Enzyme

Streptococcus pyogenes Cas9 (SpyCas9) is a multifunctional, multidomain, and large endonuclease. It cleaves dsDNA 3 base pairs above PAM via two kinds of nuclease sites (a RuvC-like nuclease domain that cuts the DNA bond that is complementary to the RNA sequence.). Added to its important role in the CRISPR effect, Cas9 also plays a different role in crRNA development and short fragment acquisition [10].

STRUCTURE OF THE ACTIVATOR COMPLEX

sgRNA BINDING AND AFTER

The principles of Cas9-sgRNA binding and guide RNA editing before target recognition are most eloquently explained by the crystal structure attached to the sgRNA. Comparison between apo-Cas9 and the structure with sgRNA shows that guide RNA binding forces Cas9 to a dramatic structural rearrangement from an inactive structure to an adequate structure such as DNA recognition, as suggested by less-resolution electron microscopy studies. The most important structural difference occurs in the REC lobe, specifically Hel-III, and moves about 65 Å by sgRNA splicing. However, Cas9 shows much smaller structural changes when binding to the target DNA and PAM sequence, indicating that most of the extensive

rearrangements occur before incorporation into the target DNA. This indicates that the guide RNA loading system is a serious regulator of Cas9 enzyme function, indicating that guide RNA loading is the central regulator of Cas9 enzyme function [11].

Target Search And Recognition

Relevant structures of Cas9-sgRNA bound to complementary single-stranded target DNA and partially without the DNA segment containing double-stranded PAM, obtained in recent years, and composite EM and crystal structure figures of Cas9-sgRNA bound to perfectly matching target dsDNA, Extensive structural information has been obtained regarding how Cas9-sgRNA recognizes substrate DNA. Related to these, Cas9-sgRNA has a mechanism to interact with the target ssDNA strand and was first learned from the ssDNA-bound structure [12].

In this structure, the target DNA strand mongrelizes with all 20 bases complementary gap sequences in the sgRNA via 20 Watson-Crick base pairs to form an RNA-DNA heteroduplex with an aberrant conformation, usually in the A form. The RNA-DNA hybrid is located in the central domain between the REC and NUC lobes and is recognized by Cas9 in a sequence-independent manner, indicating that Cas9 recognizes the distinct geometry of a guide-target heteroduplex from its nucleobases [13].

The molecular mechanism of PAM recognition is explained by the structure attached to the PAM duplex. In this construct, a Cas9 kinase (H840A) complexes with 83 nt sgRNA and a partially double-stranded target DNA, mimicking a partially truncated element in which it harbors the canonical 5'-TGG-3' PAM sequence on the non-target strand. It has a partially truncated off-target DNA strand and a strong target DNA strand. The PAM duplex is located in a positively charged space between the REC and NUC lobes, and the PAM-containing off-target strand is substantially present in the CTD. The first base in the PAM sequence is designated N and remains in base pairing with its conjugate but does not interact with

Cas9. Conserved PAM GG di-nucleotides are screened directly in the major groove via base-specific hydrogen bond interactions with two arginine residues (R1333 and R1335) present in a β-hairpin in the CTD. Because this direct base scan is formed via water-assisted hydrogen bond interactions in the major groove of DNA, it provides greater sequence specificity and discrimination than that observed with minor groove DNA recognition [14].

Both structures (PAM duplex-bound and dsDNA-bound) show that specific PAM-Cas9 interactions induce differences in local structure that repel the adjacent DNA helix strand and simplify subsequent Watson-Crick base pairing between the RNA guide and the target DNA region. In the PAM duplex-bound structure, it is stabilized by the phosphate lock loop in the CTD region of the linking phosphodiester group interacting with PAM, where the target DNA region makes a sharp turn just above the PAM [15].

This type of kink-turn configuration is necessary for the target site DNA to switch from pairing with the non-target site to pairing with the guide RNA. Overlapping of the sgRNA-bound and DNA-bound constructs shows that the phosphate lock loop exhibits multiple conformations and moves outward when bound to PAM [16].

Cryo-EM studies in which the Cas9-sgRNA complex binds with a 40 bp double-stranded DNA subunit have shown that Cas9 keeps both ends of the unfolded dsDNA in a longer helix [17].

In this true R-loop structure, the PAM-proximal end of the double helix is held in the same manner as the PAM-duplex and dsDNA bound crystal structures within the PAM interacting CTD region, while the PAM-distal end of the DNA target double helix is held between the Hel-III and RuvC nuclease sites. Cas9 performs a spherical twist of 180° to about 150° in the bound DNA segment, significantly bending the DNA helix and thereby deflecting the double helix. Although most non-target helices are unresolvable in this EM structure, the intensity indicates that the PAM-distal non-target helix follows a downward ditch trail behind the RuvC nuclease

domain, being attracted by the positive electrostatic region. DNA bending induced by Cas9 is reminiscent of DNA distortion induced by RNA polymerase at the time of transcription, possibly facilitating helix dissociation and inhibiting re-hybridization (R-loop collapse) [18].

Dna Targeting And Cutting

Currently, based on structural and mechanistic studies, we can construct a detailed model of Cas9 binding to guide RNA and activation by target DNA recognition. According to this model, guide RNA binding causes an efficient conformational rearrangement for Cas9 and converts the Cas9 enzyme from a passive state to a DNA-recognizing conformation. The RNA seed sequence is pre-ordered in an A-form conformation for target binding and strand invasion, and PAM recognition sites are pre-positioned for PAM interrogation [19].

Initial binding of Cas9 to PAM sequences allows the enzyme to rapidly scan potential target sequences. Once Cas9 obtains a potential target with the appropriate PAM, it initiates the uncoiling process and continues sampling the remaining target sequence. The phosphate lock cycle stabilizes the unfolded target DNA strand such that the first base of the target DNA sequence is oriented and oriented upstream for base pairing with the guide RNA. Cas9 interacts with oriented bases on the non-target helix to simplify uncoiling [20].

Guide-target base pairing and congruent conformational changes of Cas9 simplify guide RNA helix invasion beyond the seed domain. The non-seed region sequentially exits the restriction and base pairing moves towards the 5' end of the guide sequence. This progressive base pairing triggers conformational changes in Cas9 that indicate higher fitness and persists until it reaches an active state [21].

As a result, a complete coupling of the guide RNA and target DNA allows HNH to reach a stable and efficient conformation to cut

the target strand. This conformational change of HNH simultaneously causes a large conformational change in the loop linkers, which leads to the non-target helix to the RuvC catalytic center and enables concerted cutting. After cleavage, Cas9 remains tightly bound to the truncated target DNA until other cellular effects dislodge the enzyme for reuse [22].

Crispr And Drug Resistance Mechanism

The Role Of Virulence In The Crispr-Cas System

Many studies show that the CRISPR-Cas system has different functions besides defending bacteria against foreign invaders. This system is involved in the regulation of bacterial pathogenicity as well as directing endogenous transcription. For example, Francisella novicida, an agent that can cause disease in humans, replicates intracellularly by bypassing the host defense system. This bacterium has several mechanisms to destroy host macrophages and other defense cells. Upon ingestion of macrophages, F. novicida integrates into the phagosome, a domain with antimicrobial and defense recognition receptors [23].

Toll-like receptor 2 (TLR2) is one of the receptors that can detect bacterial lipoproteins (BLPs). Activation of TLR2 causes initiation of a pro-inflammatory response, attracting and activating defense cells. As a result, it aids clearance of the bacterial pathogen [24].

F. novicida uses Cas9, sacRNA, and tracrRNA as regulators to repress BLP expression. This pathogen can thus survive within the host by inhibiting the activation of TLR2. However, compared to Cas9, sacRNA and tracrRNA deletion mutants, F. novicida lacking these regulators has been reported to induce a severe inflammatory response. Furthermore, it was determined that these mutants were unable to productively infect mice, highlighting the importance of the CRISPR-Cas system as a virulence regulator in F. novicida [25].

The Role Of Crispr-Cas System In Antimicrobial Resistance

There are several studies related to the role of the CRISPR-Cas system in antimicrobial resistance. For example, this system supports the membrane integrity of *F. novicida* through the regulation of BLP. This results in the development of defenses against a variety of membrane stressors, including antibiotics [26].

Another study found a link between the competent systems (supporting gene acquisition) and the CRISPR system: competent strains of Aggregatibacter actinomycetemcomitans possessed CRISPR-Cas systems, while non-proficient bacterial strains lost their CRISPR defense system [27].

The present invention demonstrates that the competent system and exchange of CRISPRs promote genetic heterogeneity and the discovery of new bacterial species. Similarly, bacteria with a CRISPR system can acquire resistance and result in a higher-fit bacterial population than other species, as suggested by Levin et al. [28].

It is also important that the CRISPR system protects the guest genome against invaders to maintain genetic homeostasis. Foreign genetic materials such as plasmids and other conjugative elements may carry beneficial genes that may increase bacterial fitness in the environment, such as virulence and antibiotic resistance [29].

Most studies have found a non-positive association between the presence of plasmids and phages and the CRISPR-Cas system, as in Enterococcus, Campylobacter, and many clades A Streptococcus species. One study found that targeting of the plasmid by the CRISPR-Cas system caused an undesirable effect in terms of antibiotic resistance of *S. epidermidis* [30].

Effects Of Crispr In Clinical Treatments Pre-Clinical Studies

Cancer

Cancer is one of the most known deadly diseases, which can be demonstrated by the accumulation of epigenetic modifications in the genome. The disadvantage of conventional chemotherapy is the problem of non-specific targeting and defense against chemotherapeutic drugs. Therefore, it is necessary to uncover new molecular targets that can facilitate cancer treatment. It has been presented that mutated oncogenes and tumor suppressor genes in cancer cells may function in cancer treatment [31].

Smart therapeutic targets suggest that regulation of tumor suppressor genes can result in apoptosis in tumor cells, thereby inhibiting the proliferation of cancer cells, reducing cell motility, and inducing apoptosis. For example, Liu and colleagues observed that by editing tumor suppressor genes, namely hBax, E-cadherin, and p21, via CRISPR-Cas9, bladder cancer cells were effectively inhibited, cell motility decreased, and apoptosis was induced [32].

Mutation of epigenetic regulators has often been noted in myeloid malignancies. CRISPR-Cas9 technology has reported that in mouse xenografts, it corrected the additional sex comb-like 1 (ASXL1) gene and restored ASXL1 protein expression, significantly reducing the growth of leukemia cells. Moreover, the CRISPR-Cas9 system deleted the myeloid cell leukemia-1 (MCL-1) gene, a member of the emerging B cell lymphoma 2 (BCL2) gene class, in human Burkitt lymphoma (BL) cells to induce cell death. This suggests that the MCL-1 gene may be a new target for cancer therapy as it is involved in cell differentiation, proliferation, and tumorigenesis [33].

Cyclin-dependent kinases (CDKs) are important regulators of the cell cycle. Therefore, unregulated activation of CDKs may lead to tumorigenesis. CRISPR-Cas9 technology can silence the CDK11 gene for the treatment of osteosarcoma and silence the CDK7 gene

for the treatment of triple-negative breast cancer cells. This suggests that CDKs may be new targets for cancer therapy [34].

Immunological Disorders

The therapeutic role of CRISPR-Cas9 genome editing technology for immunological and allergic conditions has also been reported. It has been determined that the expression of cell surface glycoprotein MUC18 or CD146 is increased in alveolar macrophages in patients with chronic obstructive pulmonary disease (COPD) or asthma caused by viral or bacterial infection. Knockout of the MUC18 gene-induced pro-inflammatory chemokine interleukin-8 (IL-8) mimicking Toll-like receptor (TLR) agonists (i.e., TLR2, TLR3, and TLR4) in human primary nasal airway epithelial cells (AECs) stimulated by microbial infection. 8) decreased its level [35].

It has been observed that targeting receptor genes such as programmed cell death-1 (PD-1) can stimulate immune responses. PD-1 is a T cell surface protein associated with T cell activation. CRISPR-Cas9-mediated disruption of the PD-1 receptor gene in human primary T cells isolated from cancer patients resulted in enhanced interferon (IFN)-g production and increased cytotoxicity. This presents checkpoint inhibitors as new targets for cancer therapy [36].

X-linked hyper-immunoglobulin M (IgM) syndrome is an immunodeficiency disorder characterized by defects in CD40/CD40L signaling via non-regular class switching recombination and somatic hyper-mutation in B cells. It has been observed that CRISPR-Cas9 gene editing methods can correct mutations in the CD40 ligand. Cheong et al achieved IgH class switching by editing mouse and human Ig genes, which may support studies of B cell (normal and lymphoma) biology [37].

Cardiovascular Disorders

The proprotein convertase subtilisin/kexin type 9 (PCSK9) gene has a critical role in regulating cholesterol homeostasis. A gain-of-function mutation of the PCSK9 gene can result in hypercholesterolemia and associated atherosclerosis. Jiang et al have presented therapeutic targeting of the PCSK9 gene in mice via CRISPR-Cas9. Moreover, the CRISPR-Cas9 gene-editing tool has been reported to impair the low-density lipoprotein receptor (Ldlr) gene and express the PCSK9 gene in adult mice in atherosclerosis studies [38].

Tessadori and colleagues used a CRISPR-Cas9-based gene editing tool in a zebrafish model to correct human genetic cardiovascular disorders. These studies present the use of CRISPR-Cas9 as a potential gene editing tool against cardiovascular disorders, including conditions specifically associated with inherited lipid disorders [39].

Neurological Disorders

Huntington's disease (HD) is an autosomal inherited neurological disorder caused by the expansion of CAG repeats in exon 1 of the huntingtin (HTT) gene. The CRISPR-Cas9 nucleotide editing tool has been used effectively to selectively suppress the HTT gene in the mouse model. Other studies also support the use of the CRISPR-Cas9 gene editing system against HD conditions [40].

Alzheimer's disease (AD), being a neurodegenerative condition, causes progressive memory loss. Mutations in the presenilin 1 (PSEN1) and PSEN2 genes have been noted in familial Alzheimer's condition. PSEN1 is a protein that cleaves amyloid precursor protein (APP).

It is the catalytic subunit of the protease enzyme g-gamma octet and produces amyloid-b (Ab). A79V mutation in the PSEN1 gene decreases Ab40, increasing the Ab42/Ab40 ratio. Studies have noted the repair of A79V and L150P mutations in PSEN1 in the

induced pluripotent stem cell (iPSC) line derived from AD patients [41].

The CRISPR-Cas9 nucleotide assembly system was used to engineer the "T" point mutation with wild-type "C" nucleotide in the A79V-hiPSC line. Moreover, a mutation in the APP gene raises the b-secretase fraction of APP, resulting in abnormally increased levels of Ab in the brain. CRISPR-Cas9 has also been used to correct the APP allele, resulting in reduced Ab pathogenesis. CRISPR-Cas9-based gene editing against AD has been studied in detail by Rohn et al. In summary, CRISPR-Cas9 gene editing technology can be used as an effective strategy against genetically induced neurological disorders [42].

Metabolic Disorders

Metabolic liver disease (MLD) causes abnormal carbohydrate, protein, and fat metabolism due to a disorder of a carrier protein. In recent years, Villiger et al have corrected mutations in the phenylalanine hydroxylase (Pah)enu2 gene, an autosomal recessive liver disease called phenylketonuria (PKU) in mice, using CRISPR-Cas9 editing systems. In another study, computationally engineered hepatocyte-specific CRISPR-Cas9 was applied to target the mouse factor IX (F9) gene against an MLD-associated condition [43].

Yang et al corrected the X-linked deficiency in CRISPR-Cas9-mediated gene-editing-based OTC to correct urea cycle dysfunction in a baby mouse model [44].

Hereditary tyrosinemia (HT) is an autosomal recessive inherited disease associated with a deficiency of the enzyme fumarylacetoacetate hydrolase due to mutations in the Fah gene. HT type I (HTI) causes serious liver disorders such as cirrhosis, liver failure, and liver cancer due to toxin accumulation. A CRISPR technology-based therapeutic strategy for the correction of HT was one of the first known studies in mice demonstrating the delivery of CRISPR-Cas9 system components into adult mammalian organs [45].

In this study, rearrangement of the disease-causing mutation in the Fah gene was accomplished by hydrodynamic injection of Cas9 nuclease, sgRNA, and a donor oligonucleotide, making a significant advance in gene therapy. VanLith et al recorded hepatocyte-directed Fah gene repair in an HTI mouse model using CRISPR-Cas9 against metabolic liver diseases. CRISPR-Cas9-based correction of the Fah gene in the HTI mouse model provided weight stability in preventing liver cirrhosis in mice [46].

Hunter syndrome is a metabolic disorder that causes damage to the lungs, heart, and brain due to mutational dysfunction of an enzyme called iduronate-2-sulfatase (IDS). In the late 2010s, in vivo, genetic editing via ZFNs was registered for the treatment of Hunter syndrome [47].

This experiment is the first to display that in vivo gene-editing-mediated treatment of genetic diseases is possible. In the late 2010s, Sangamo Therapeutics (Richmond, CA, USA) reported ZFN-based in-body gene editing in people with Hunter syndrome. Despite achieving complex results, no adverse side effects were reported with this treatment. This initiative in the field of gene-editing-based therapy supports the possible use of CRISPR-Cas9 in the treatment of metabolic disorders. However, CRISPR-Cas9 technology against metabolic disorders requires efforts to enter clinical trials [48].

Hematological Disorders

Fanconi anemia is an ressesive-autosomal disorder caused by mutations in genes responsible for replication-dependent excision of interlane DNA cross-links. CRISPR-Cas9-mediated homology-directed recombination (HDR) in the FA gene highlights the application of genome editing to correct errors in the DNA repair pathway [49].

CRISPR-Cas9 correction of the disruptive mutation in the F (Fancf) gene and correction of the Fanconi anemia I (FANCI) gene have been reported in iPSCs derived from primary fibroblasts. Correction of FA mutations is associated with the therapeutic

approach of CRISPR-Cas9 against bone marrow failure. Richardson and colleagues discovered that single-strand template repair (SSTR) induced by human Cas9 requires the FA pathway for DNA interstrand cross-link repair in cells [50].

Sickle cell anemia (SC) is a hematological disease caused by the formation of abnormal hemoglobin S (HbS) protein because of a mutation in the b-globin gene. Studies show that CRISPR-Cas9 for SC anemia is a safe and promising gene therapy approach. CRISPR-Cas9-based correction of the Hbs gene in hematopoietic stem and progenitor cells (HSPCs) obtained from the blood of patients with SC disease confirmed the restoration of normal functional hemoglobin [51].

In an in vitro study, CRISPR-Cas9 components showed gene alteration in more than 18% of CD34+ cells. Similarly, CD34+ HSPCs obtained from the bone marrow of SC anemia patients have been noted to be corrected by CRISPR-Cas9 technology. Ye and colleagues generated a heritable fetal hemoglobin (HPFH) genotype in normal HSPCs using the CRISPR-Cas9 approach for safe autologous transplantation for patients with SC disease and β-thalassemia [52].

β-Thalassemia is a common genetic hematological disorder, caused by decreased synthesis of the β globin chains of the hemoglobin tetramer and lowers hemoglobin production. Correction of β-thalassemia cough mutation (IVSII-1G>A) in iPSCs using Cas9, treatment of the mutated gene with piggyBac transposon-modified donor vector method, provides increased specificity and accuracy. This could be an important treatment modality for stem cell-based gene therapy against monogenic diseases in future clinics [53].

Ophthalmic Disorders

Retinitis pigmentosa (RP), hereditary pigmented retinal dystrophy, can cause vision loss. There are some mutations associated with this disease, including mutations in the RP1,

rhodopsin (RHO), and RP GTPase regulator (RPGR) genes. The CRISPR-Cas9 gene editing technique can restore the Rho (S334) gene with guide RNA (gRNA)-Cas9 plasmid via subretinal injection, resulting in restoration of visual function by arresting retinal degeneration in mice [54].

Suzuki et al used CRISPR-Cas9 to treat visual status in mice using homology-independent targeted insertion (HITI), referred to as HITI. This demonstrated that in vivo gene editing can be performed using CRISPR-Cas9 technology [54].

Multiple mutations at different genetic loci have been recorded in the case of cataracts (clogged crystalline lens). Moreover, αA-crystallin gene mutations are associated with autosomal recessive cataracts [55].

To determine the role of the αA-crystalline gene in congenital cataracts, Yuan and colleagues used CRISPR-Cas9 to generate mutations in the αA-crystalline gene in an animal model. In these years, it was noted that missense mutation of GJA8 was also associated with congenital cataracts [56].

Yuan and colleagues created a GJA8 gene knockout rabbit model using the CRISPR-Cas9 system to study congenital cataracts in humans. Currently, the in vivo therapeutic cure of blindness via CRISPR-Cas9 is in a clinical trial [57], which will be discussed later in this review.

Viral Infection

HIV is a pathogen that targets the human immune system and causes acquired immunodeficiency syndrome (AIDS). Although AIDS is now available thanks to active antiretroviral therapy (HAART), a complete cure for AIDS is a major concern. Expression of the HIV-1 gene has been reported to be stimulated by long terminal repeats (LTRs), which are identical repeating sequences of DNA, and promote the insertion of retroviral DNA into the host

chromosome. LTR-driven viral transcription can be altered by genetic variation in the binding sites of LTRs [58]

It has been shown that CRISPR-Cas9 can mutate the LTRs in the DNA of the HIV-1 provirus and cause the lysis of the latent HIV-1 provirus. In negative feedback regulation of HIV-1, the Cas9 gene is placed under the control of a minimal HIV-1 promoter to express Cas9 in HIV-1 infectious cells. This can also minimize complications due to unusually high Cas9 expression in cells. It has been shown that CRISPR-Cas9-mediated gene editing can inhibit multiple steps of HIV-1 infection [59].

Hepatitis B Virus (HBV). HBV is a known infectious disease that causes chronic hepatitis, which is common worldwide. Covalently closed circular DNAs (cccDNAs) of HBV have been found in contaminated cells and cccDNA is predicted to be a potential therapeutic target for correction of HBV infection [60].

It has been discovered that the CRISPR-Cas9 nuclease disrupts chromosomally integrated HBV sequences by enabling the action of episomal cDNA in reporter cell lines. Dong et al. reported that CRISPR-Cas9 can reduce HBV cDNA by inhibiting viral replication [61].

Ramanan et al have also shown that CRISPR-Cas9 suppresses HBV by cutting viral DNA [62].

Wang et al used the RNA interference (RNAi) method using a sgRNA-microRNA (miRNA)-gRNA tool with Cas9 to inhibit HBV replication. Therefore, disruption of the HBV genome via CRISPR-Cas9 technology may be a promising approach as anti-HBV therapy [63].

Hepatitis -C-Virus. HCV is a single-stranded RNA (ssRNA) virus and is the result of hepatitis C, an inflammatory condition of the liver. The Cas9 endonuclease enzyme of the Gram-negative bacteria known as Francisella novicida, known as FnCas9, can target endogenous RNA. The CRISPR-FnCas9 system has been used to inhibit HCV in eukaryotic cells [64].

Human Papillomavirus (HPV). HPV is a double-stranded DNA (dsDNA) virus that infects mucous cells or skin. This pathogen, which causes a sexually transmitted disease, is responsible for 10-15% of cancer cases in women. RNA-guided endonuclease offers a therapeutic approach against HPV [65].

It has been reported that cervical cancer treatment can be applied by targeting HPVE6 through CRISPR-Cas9. CRISPR-Cas9 technology can also be targeted to conserved regions of HPV6/11 E7 genes, demonstrating the therapeutic potential of gene editing against genital warts [66].

EpsteinBarr Virus . EBV is a double-stranded DNA (dsDNA) virus that spreads through saliva and causes mononucleosis. CRISPR-Cas9 technology has been used for genome editing of EBV in human cells by altering the BART promoter gene, which encodes viral miRNAs [67].

Ma et al. emphasized that 87 lymphoblastoid cell lines (LCL) and 57 BL genes are important for survival and growth in LCL and BL cells. Ephrin receptor tyrosine kinase A2 (EphA2) facilitates the integration of EBV into human cells [68].

CRISPR-Cas9-mediated knockout experiments have shown that EphA2 has an extracellular domain that can bind with the EBV-glycoprotein gHgL for entry into the cell. Therefore, EphA2 may be a new potential target for therapeutic development [69].

Clinical Studies

China conducted the first ex vivo clinical trial using gene editing with CRISPR-Cas9 techniques in non-metastatic small-cell lung cancer patients [70].

They targeted the PD-1 gene on T cells in the patients' peripheral blood, edited it by electroporation of sgRNA and Cas9 plasmid, and reinjected it into the patients. In recent years, they noted that upregulated T cells were bring into being in the peripheral blood of all patients who received infusions. They determined that this

method was feasible and safe, but more advanced gene editing technologies were needed to improve treatment effectiveness [71].

In recent years, Stadtmauer and colleagues demonstrated the results of a phase 1 human CRISPR-Cas9 technology-based clinical trial in three patients with advanced refractory cancer. They removed the TRAC and TRBC genes, which encode the endogenous TCR chains, and the PDCD1 gene, which encodes the PD-1 regions, from the T lymphocytes taken from the patients, thereby increasing anti-tumor immunity. Moreover, they reported a transgene (NY-ESO-1) capable of recognizing tumors. It was noted that these T lymphocytes were well tolerated up to 9 months after reuptake by patients. Nevertheless, chromosomal translocation was identified but appeared to decrease over time [72].

Another clinical trial was conducted presenting CAR T cell therapy for emerging or refractory hematological malignancies due to CD19+ tumor cells. Integration of two different CARs (i.e., CD19 and CD20 or CD22) into the TRAC locus of T cells (capable of recognizing CD19+ cells) was performed. Moreover, the use of gene-disrupted allogeneic universal CD19 specific CAR T cells (UCART019), in which endogenous TCR and B2M genes are disrupted by electroporation of CRISPR RNA, is also in clinical trials in patients with relapsed or refractory CD19+ leukemia and lymphoma. This system can minimize immunogenicity by avoiding the possibility of vaccination versus host disease (GVHD). However, the results are not yet known. As a result, the FDA approved a clinical trial of CTX130, a CRISPR-Cas9-modified allogeneic T-cell line targeting CD70 against hematological malignancies and renal cell carcinoma [73].

In 2019, the treatment of SC disease and β-thalassemia was successfully recorded by Sangamo through disruption of the BCL11A gene via CRISPR-Cas9 into stem cells isolated from the peripheral blood of hemoglobinopathy patients [74].

BCL11A is a transcription regulator that suppresses the expression of the β-globin gene. In another semi-successful trial,

CCR5-deleted hematopoietic stem cells were transplanted into HIV-1 and acute lymphoblastic leukemia patients. Although the desired goal was not achieved, the important side

no effects were seen. The study presented the need to increase the efficiency of CCR5 degradation in lymphocytes [75].

In an in vivo clinical trial recorded in 2019, the CRISPR-Cas9 gene therapy-based drug AGN-151587 was administered by subretinal injection directly into the eye to cure a rare condition of blindness called Leber congenital amaurosis 10 (LCA10), which is caused by mutations in the CEP290 gene. This trial is the first approach in which CRISPR-Cas9 gene editing therapy is applied directly to the human body. According to recent enrollment, there are approximately 19 interventional clinical trials with CRISPR-Cas9-mediated gene editing technology [76].

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CHAPTER III

Physiological, Biochemical, Pharmacological Properties Of Melissa Officinalis

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Introductıon

In medical culture, there are many knowledge and practices about the importance of plants in treatment. Plants are one of the first sources used to relieve many medical conditions such as pain, pain, and oedema. Although the demand for natural products has decreased due to synthetic products with the industrial revolution and industrialization process, the tendency towards medicinal aromatic plants has increased again in recent years, and medicinal aromatic plants and spices have become a priority research area.

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Dozens of immunostimulant, anti-inflammatory, analgesic, hemostatic, cell membrane protective properties of plants are being intensively researched to develop more effective and safer treatment modalities (Aslan, 2016).

Medicine is the whole process of maintaining homeostatic balance and restoring it if it is disturbed (Aslan, 1999). Homeostasis is provided by buffer systems in the organism, antioxidant, anti-inflammatory, antihistamine, antibacterial, anticarcinogenic pathways (Dündar, 1999-1). One of the important pillars of homeostasis that has been recognized in recent years is oxidant-antioxidant balance. To protect the oxidant-antioxidant status, which is in equilibrium under normal physiological conditions, from the effects of internal and external stressors, endogenous antioxidants are present in the cells and exogenous antioxidants such as phytochemicals and vitamins are used (Dündar, 1999-2). Antioxidant plants and phytochemicals, the number of which is counted in tens of thousands, support homeostasis by protecting the organism against oxidative stress through many different pathways and contribute to the survival of cells in physiological conditions (Evcimen, 2015).

As a medicinal aromatic plant with widespread use in folkloric traditional medicine as well as in conventional medicine applications, the herb (Melissa officinalis) draws attention with its physiological, biochemical, botanical, and pharmacological properties. This chapter provides updated information on Melissa officinalis to researchers in related fields of academia and industry, and students studying medicine, veterinary medicine, pharmacy, etc. with reference to clinical data.

Melissa is Greek for "bee", probably due to the plant's ability to attract bees. A specific adjective for the species, "officinalis", means "used in medicine", indicating the historical medicinal use of this species. Melisa, which has a common name derived from the word "balsamon" meaning "balsam" or "oily, fragrant resin", is

mentioned in historical works with names such as "baume, balme, baulm" (Baytop, 1996).

The local names of plants such as yellow herb, snowdrop, nettle is usually made according to a known feature. Sometimes, they are named according to their physiological effects, such as snakeroot, woundwort, hemorrhoid. However, it is sometimes seen that different plants with the same physiological and symptomatic effects are named with the same names. In our country, at least 20 different plants that are thought to be effective in relieving hemorrhoid complaints are called "hemorrhoids". This situation shows that it would be more accurate and reliable to recognize medicinal aromatic plants with their Latin scientific names as well as their local names. Geographical conditions make our country a homeland for many medicinal aromatic plant species, and the plant diversity exceeding eleven thousand is an important indicator of this. Medicinal aromatic plants, of which dozens of species are subject to economy and trade today as medicine, food, nutritional support, and cosmetics, have an important area of use in sectors such as chemistry, cosmetics, medicine, spices, and food. Due to the therapeutic, protective, and vitalizing properties of bioactive chemical substances, humans and animals have tended to treat themselves with plants. Since the utilization of plants against diseases and disease agents has been a quest since the first human being, plants and plant extracts, extracts, glycosides, and essential oils have a very ancient use for healing purposes. The Roman naturalist Pliny wrote that sword grass planted close to beehives encouraged bees to return to the hive (Meyers, 2007). It is a perennial herbaceous plant with lemon odor, leaves are oval, flowers are white, corolla tube is curved, bearing four stamens, filaments are close to each other. The essential oil is oleum Melissa citronella. Although it is endemic to our country, it is a medicinal aromatic plant which is encountered relatively rarely but has started to be cultivated commercially. The homeland of the plant is Southern Europe. In the 7th century, it is thought to have been taken to Spain by the

Andalusians, cultivated in that region in the Middle Ages and used throughout Europe (Baytop, 1996).

Photography 1: Melisa officinalis (sword grass, balm, lemon balm)

Dioscorides, the author of De Materia Medica, pharmacologist and botanist, reports that the use of lemon balm has a history that goes beyond the ancient Greeks and Romans, that the first uses were in the form of a topical preparation applied for insect and bee stings, that it was used in the middle ages for purposes such as easy digestion, balanced psychology and facilitating sleep; Theophrastus, in his Historia Plantarum, records that the medicinal use of the plant dates back to 300 BC (Rees, 2001).

Believed to have a calming effect on the heart and body since ancient times, lemon balm is nowadays used in folk medicine as a

medicine that uses the plant itself (usually tea mixed with sedative herbs such as valerian), preparations in conventional medicine (for example, ointment made with its essence for topical application); It is also used in jams and jellies, fish and poultry feeds, as a flavoring in some drinks, in the production of perfume in cosmetics, in the production of lacquer in the furniture sector (Rees, A.M., 2001). Due to the widespread and active culture of lemon balm use, many authors, physicians, and herbalists have praised lemon balm, citing its calming, revitalizing, and other medicinal properties, for example Ibn Sina argued that lemon balm is successful in the treatment of depression and melancholy (Meyers, 2007).

Melissa Officinalis

It is an erect, branched, pubescent, herbaceous perennial, 28 to 95 cm or more tall. Leaves 18-95 X 12-75 mm, broadly ovate, rhomboidal, or elliptic, cuneate-cordate, acute, or obtuse, deeply crenate except at the base, covered with long or short fine hairs or semi-bare. Verticillastrums with 4-12 flowers. Bracteoles leaf-shaped, narrow, or broadly ovate, 3-10 x 1.2-7 mm. Calyx 6-10 mm, bearing short glandular hairs or long covering hairs, upper lip 2-3-toothed, the central tooth often short-pointed or absent, the teeth of the lower lip narrowly triangular lanceolate. Corolla 9-14 mm, whitish to light yellow, sometimes light lilac. The plant is flowering in June-September. It grows from sea level to 1800 m, in forest clearings, shrubs, maquis, stream banks, wastelands and roadsides. This family, which is represented by about 200 genera and 3200 species in global distribution, grows about 45 genera and 550 species in our country, many of which are used in medicine, pharmacy, cosmetics, and perfumery (Tanker, 2014).

Figure 1: Parts of Melissa officinalis L plant (www.plantpictures.de)

Melissa officinalis L. (Labiatae) is a herbaceous species of the Lamiaceae family originating from North Africa (Egypt, Tunisia, Morocco) and Southern Europe. For its specialized medicinal use, melissa is cultivated in countries such as France, Italy, Germany, Spain, Hungary, Hungary, USA (Toma, 2008); it is widely cultivated in West Asia, Serbia, and North Africa (Fahima, 2014).

Of the three subspecies of the genus lemon balm (ssp. officinalis, ssp. altissima, ssp. inodora), the lemon-scented ssp. officinalis, which has medicinal value, is naturally distributed in

Bursa, Bilecik, Bolu, İstanbul, Ankara, Ankara, Amasya, Samsun, Kütahya, Malatya, Erzincan, Tunceli and Muğla regions (Katar, 2008).

Place in Systematics, Family, Genus, and Species

The genus Melissa L. belongs to the subdivision Angiospermae, class Dicotyledonae, order Lamiales, family Labiatae (Lamiaceae) of the division Spermatophyta (Işık, 2010). Labiatae family plants are common in the Mediterranean basin. They are herbaceous plants or shrubs, have glandular hairs and contain essential oil. The characteristics of the family can be listed as follows:

1. Stem quadrangular, leaves simple, sometimes dissected, and decussate.

2. Flowers are vertisillastrum in each node.

3. Zygomorphic and bilabiate.

4. The essential oil is in the glandular hairs of the labiatae type, which are unicellular at the stem and eight-celled and scale-shaped at the head.

5. In hermaphrodite flowers calyx five-lobed persistent, sometimes bilabiate, corolla bilabiate, upper lip sometimes absent.

6. Stamens four, often dynamos, sometimes with two stamens.

7. Ovary with two carpels, four eyes and upper case, each eye with an ovule, stylus gynobasic.

8. Fruit a schizocarp consisting of four nuclei.

9. In some species the stamens are four, the filaments of equal length, some are dynamos; in some species there are two stamens.

Breed Characteristics

Plants of the genus Melissa are herbaceous perennials. Vertisillastrum are few or many-flowered. Calyx tubular

campanulate, bilabiate, upper lip flattened with three short teeth (in Turkish species the middle tooth is usually short pointed), lower lip with two teeth. Corolla tube curved to the center and bilabiate. Upper lip flat or cap-like, emarginate, lower lip three-lobed. Stamens are four and filaments are close to each other. Fruits are flat (Işık, 2010).

Species Characteristics

Latin name Melissa officinalis; Turkish name oğulotu, Melisa; English name; leman, balm;

parts used herba, folium. The subspecies of Melissa officinalis in Turkey differ from each other with some characteristics. Subspecies 1: Stem without long hairs; covered with short, fine glandular hairs, leaves cuneate at base, central tooth of upper lip of calyx broadly triangular (subspecies officinalis). Subspecies 2: Stems and leaves covered with dense, long, weak, or stiff-short hairs, with few glandular hairs, central tooth of the upper lip of the calyx very prominent and triangular (subspecies inodora). Subspecies 3: Stems and leaves covered with dense, long, weak, or stiff-short hairs, few glandular hairs, central tooth of the upper lip of the calyx short, pointed, or absent (subspecies altissima). Of these three subspecies, only the subspecies officinalis, which gives a lemon odor, is used for treatment, the other two subspecies are not used for medicinal purposes because they have an unpleasant odor (Işık, 2010).

Bioactive Subtances

Chemical substances such as β-pinene, Limonene, Linalol, Citronellal, Neral, Geraniol, Geranial, Geranyl acetate, β-caryophyllene, caryophyllene oxide are encountered in Melissa officinalis.

Table 1: Main phytochemical constituents of Melissa officinalis from different origins (Fahima, 2014).

Constituents	Various origins										
	This Work 2014	Serbia 2004	Slovak 1997	Cuba 1999	Egypt 1995	France 1998	Brazil 2005	Scotland 1995	Iran 2003	Turkey 2004	Greece 2005
β-pinene	-	-	-	-	-	-	-	-	-	-	18.2
Limonene	-	2.2	0.1	-	0.7	-	-	57.5	-	-	tr
Linalool	0.3	0.5	0.08	0.6	0.2	0.6	0.8	0.6	0.9	1.3	tr
Citronellal	6.3	13.7	11.3	0.2	13.3	39.5	-	24.9	12.9	2.9	-
Neral	30.2	16.4	22.2	29.9	19.7	20.4	39.3	4.3	24.5	5.8	-
Geraniol	0.6	3.4	-	-	4.2	0.2	-	5.7	0.7	0.4	-
Geranial	44.2	23.4	33.6	41.0	26.8	27.8	47.3	-	35.5	6.6	-
Geranyl acetate	-	0.8	5.9	4.4	1.8	0.6	1.5	-	7.1	-	-
β- Caryophyllene	1.3	4.6	4.2	-	4.9	2.4	0.9	-	4.9	14.2	15.3
Caryophyllene oxide	1.3	1.7	8.3	5.3	10.0	-	1.2	-	2.7	-	12.6

Six flavonoids were isolated from the leaves of Melissa officinalis L. Lamiaceae. Their structures were determined by spectral (UV, 1R, 1H NMR, 13C NMR and FAB MS) methods as luteolin, luteolin 7-O-beta-D-glucopyranoside, apigenin 7-O-beta-D-glucopyranoside, luteolin 7-O-beta-D-glucuronopyranoside, luteolin 3'-O-beta-D-glucuronopyranoside and luteolin 7-O-beta-D-glucopyranoside-3'-Obeta-D-glucuronopyranoside were identified. The last three glycosides (luteolin 7-O-beta-D-glucuronopyranoside, luteolin 3'-O-beta-D-glucuronopyranoside and luteolin 7-O-beta-D-glucopyranoside-3'-Obeta-D-glucuronopyranoside) were found for the first time in Melisa. Luteolin 7-O-β-D-glucopyranoside-3'-O-β-D-glucurono pyranoside is a new compound found in plants (Patora, 2002). Luteolin 3'-O-beta-D-glucronide, the major flavonoid of Melissa officinalis, was isolated from the leaves of the official subspecies of Melissa officinalis and characterized by spectroscopic analysis (Heitz, 2000).

Some of the activities of melissa are attributed to phenolic compounds occurring in melissa containing rosmarinic acid (Patora, 2002).

Active Substance Diagnostic Methods

Classical and high-tech methods frequently used in essential oil structural analysis include capillary GC and GC/MS methods (Venskutonis, 1995); HCT CID tandem MS methods (Toma, 2008). Enantioselective capillary GC is also described as an authentication study in case of adulteration (Holla, 1997). Enantioselective synthesis or asymmetric synthesis is a form of chemical synthesis. It is defined by the IUPAC (International Union of Pure and Applied Chemistry) system as a chemical reaction or reaction sequence in which one or more new purifying elements are formed in a substrate molecule, producing stereoisomeric (enantiomeric or diastereoisomeric) products in unequal amounts. Enantioselective synthesis, the synthesis of a compound by a method that favors the formation of a particular enantiomer or diastereomer, is a key process in modern chemistry.

It is particularly important for pharmaceuticals as different enantiomers or diastereomers of a molecule often have different biological activity. Methods such as EAE (Enzyme Assisted Extraction) and PLE (Pressurised Liquid Extraction) Liquid Tandem Mass Spectrometry (LC-MS/MS) (Miron, 2013), HPLC (Işık, 2010) are widely used in this field.

Volatile compounds

Carnat et al. conducted a study to compare the aromatic content of the infusion prepared from the dried leaves of Melissa officinalis with the content of the dried leaves before and after the preparation of the infusion. In this study, they showed that the dried leaves contained 0.32% essential oil. Citral (neral+ geranial) constituted 48%, citronellal 40% and β-caryophyllene 2% of the essential oil. In the infusion essential oil, which is rich in aldehydes

(90%), the amount of citral (74%) is high and citronellal is 16%. After infusion, the amount of citral (36%) decreased in the essential oil of the leaves, while citronellal was 43% (Carnat, 1998).

Phenolic Compounds

A study to compare the polyphenolic content of the infusion prepared from the dried leaves of Melissa officinalis with the content of the dried leaves before and after the preparation of the infusion. In this study, they showed that the dried leaves contained 11.8% polyphenolic compounds (11.3% hydroxycinnamic acid derivatives and 0.5% flavonoids). Among hydroxycinnamic acid derivatives, rozmarinic acid ranked first with 4.1% (Carnat, 1998).

Folin-Ciocalteu procedure was used for the determination of phenol content (Miron, 2013). The dried plants were distilled using a JEOL JMS DX-300 system with GC/MS investigation for oil extraction. All compounds were identified by comparing their mass spectra and retention indices with those available in the DENT database. Neral (29.9%) and geraniol (41%) were found to be the major chemical constituents in the oil of the dried plant Melisa officinalis subjected to GC/MS (Pino, 1999).

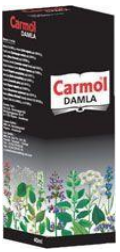
Biosynthesis

As a result of radioactive studies, caffeic acid and 3,4 dihydroxyphenylacetic acid side molecules were derived from phenylalanine and tyrosine, respectively. RA santase catalyses the phenylpropanoid pathway from 4-coumaryl-CoA by condensation of tyrosine-derived 4-hydroxyphenyllactic acid to 4-coumaryl-CoA and α-O-4-coumaryl-4'hydroxyphenyllactic acid (CHPL). RA is then formed by two successive hydroxylations of CHPL (Matsuno, 2002).

Use in Public Domain

The plant is an important medicinal aromatic plant with widespread use in folk medicine, pharmacy, perfumery, cosmetics,

and food industry. For these purposes, the essential oil, herb and leaves of the plant are mostly used (Katar, 2008); the tea form is preferred in folk medicine to benefit from its aromatic, antispasmodic and sedative effects and is widely used in headaches, insomnia, fevers, and colds (Jahanban, 2015). Melissa tea obtained from lemon balm leaves is used to treat migraine, insomnia and stomach disorders, to support the cardiovascular system, to improve memory; Melissa officinalis stands out in traditional medicine with its anxiolytic, hypnotic, sedative and spasmolytic aspects (Demirci, 2015); It draws attention as a protective and suppressive medicinal plant against headache, indigestion, intestinal pains, irritability (nervousness, anger), heart failure and depression (Fahima, 2014).

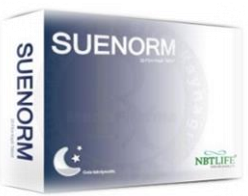


Carmol Drip

Nanna Herbal Syrup

Gasso Herbal Syrup

Vicol Herbal Syrup



Film Coated Tablet

Doppelherz VitaTonikum

Photography 2: Some products from Melissa Officinalis

Preliminary Clinical Trials on Melissa Officinalis

Anti-HIV activity: In a study evaluating the anti-HIV-1 activity of Labiatae aromatic plants in vitro, 45 extracts among 51 samples from 46 plant species showed significant inhibitory effect

against HIV-1-induced cytopathogenicity in MT-4 cells. Aqueous extracts of Melissa officinalis also showed these same effects (Yamasaki, 1998).

Antitumor effect: Glycosides and caffeic acid in isolated Melissa officinalis leaves directly inhibit the protein biosynthesis of elongation factor Eef-2. Therefore, this effect can be exploited by using the plant's protein biosynthesis inhibitors in specific antitumor preparations. This effect was studied in different human cancer cells and anticancer effects of Melissa officinalis extract were demonstrated (Galasinski, 1996). The results showed that one dose administration of the hydro-alcoholic extract of Melissa officinalis has a high potency in inhibiting the proliferation of different tumor cells; the optimal biological dose is more important than the maximum tolerance. Moreover, the anti-proliferation effect of Melissa officinalis seems to be tumor type specific; hormone-dependent cancers seem to be more sensitive to the antitumor effects of Melissa officinalis (Jahanban, 2015).

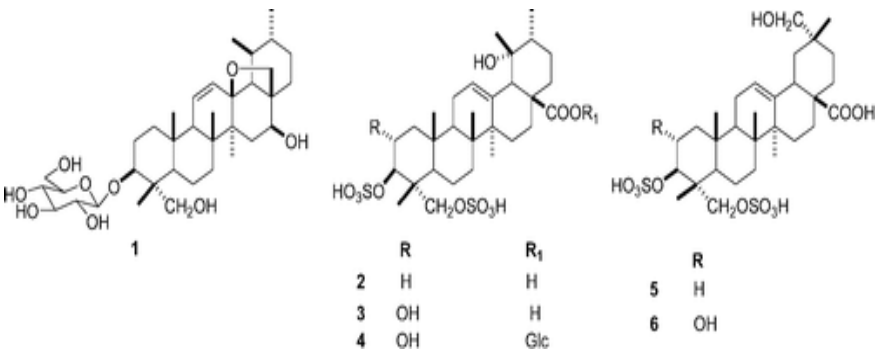


Figure 2: Antioxidant and antimicrobial melissa compounds (Mencherini, 2007)

Antiviral Activity Against Type-2 Herpes Simplex Virus: In studies to determine the effect of essential oil components of Melissa officinalis on the replication of type-2 Herpes Simplex virus in HEp-2 cells, different concentrations (25, 50, 100, 150 and 200 microg/mL) of essential oils were examined, and it was found that

M. officinalis essential oil was nontoxic for HEp-2 cells at concentrations up to 100 microg/mL, and slightly toxic above 100 microg/mL concentration. Antiviral activity at non-toxic concentrations was tested against HSV-2 and HSV-2 replication was inhibited. These findings suggest that Melissa officinalis extract contains bioactive substances with anti-HSV properties (Allahverdiyev, 2004).

Anti-kinetoplastid activity: Inhibition of parasites is clinically important. It has been reported that Melissa officinalis ethanol extract can be considered as a treatment option in these clinical conditions due to its anti-leishmania and anti-trypanosome properties (Cunha, 2016).

Anti-angiogenesis Activity: Melissa leaves extract, which exhibits anti-angiogenic activity, is thought to regulate the development of adipose tissue (Park, 2015).

Effect on Cardiovascular System: Maurice Messegue, one of the folk physicians, states that "the Arab physicians, who first understood the wonderful effects of the herb, claimed that the plant was heart-strengthening, heart-opening and suitable for strengthening all vital organs, relieving neurasthenia, internal distress, and nervous headaches". In a study on Melissa officinalis, the vasodilator effect of aqueous extract of Melissa officinalis on isolated rat aorta precontracted with phenylephrine and the possible mechanisms of this effect were investigated and it was thought that the plant may be useful for cardiovascular health (Işık, 2010). In a study conducted to elucidate the effects of Melissa officinalis aqueous extract on rat electrocardiogram (ECG), it was revealed that consumption of the plant caused significant changes in rat ECG (Joukar, 2015). In a study whose aim was to investigate the effect of melissa (Melissa officinalis L.) hydroalcoholic extract on CaCl2-induced arrhythmia in rats, heart rates, VPB, VT, VF frequency decreased significantly in the groups given Melissa officinalis extract compared to the control group. It has been reported that Melissa officinalis can be considered as an antiarrhythmic agent

because it reduces the incidence of VEA, VT and VF (Akhondal, 2015).

Antidepressant-like Effect: In a forced swimming experiment in rats, Melissa officinalis was reported to reduce the duration of immobility, and it was suggested that the water extract of Melissa officinalis showed a serotonergic antidepressant-like effect. These findings suggest that Melissa officinalis aqueous extract may suppress the development of depression and may support classical treatment with its serotonergic activity (Lin, 2015).

Memory Enhancing Effect: Melissa officinalis L. (Labiatae), which is frequently used in the treatment of neurological disorders in traditional medical practices, is known as a memory-enhancing plant. In a study investigating the role of the cholinergic system on the memory enhancement activity of Melissa officinalis extract, Melissa officinalis leaves were extracted with ethanol using maceration method, rats were given Melissa officinalis intraperitoneal injection at different doses (50-400 mg / kg) either alone or in combination with scopolamine (1 mg / kg) before a Morris water maze (MWM) training, then acetylcholinesterase enzyme (AChE) activity was measured. Administration of Melissa officinalis (200 mg/kg) significantly increased learning and memory in rats and the effect of the extract was not dose dependent. The findings suggest that Medulla officinalis may increase memory and the cholinergic property of the extract may contribute to memory improvement (Soodi, 2014).

Anti-inflammatory Effect: The application of gas chromatography and mass spectroscopy of essential oil obtained by hydro distillation to the leaves shows that Melissa officinalis L. essential oil has potential anti-inflammatory effect in the treatment of various diseases associated with inflammation and pain (Bounihi, 2013).

Antioxidant Activity: Melissa officinalis essential oil is thought to be a potential antioxidant with its free radical scavenging effect and properties that suppress lipid peroxidation and eliminate

its effects. In studies in this direction, the content profile of Melissa officinalis essential oil is determined by gas chromatography, mass spectrometry (GC-MS) and thin layer chromatography (TLC). The radical scavenging effect of the essential oil is shown by measuring the scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and OH radicals in the medium. The essential oil was reported to reduce OH radical generation (IC (50) = 1.74 microg/mL) and DPPH radical formation (IC (50) = 7.58 microg/mL). The most potent radical scavenging compounds were reported as monoterpenic aldehydes and ketones (neral/geranial, citronellal, isomentone and menthone), mono- and sesquiterpene hydrocarbons (E-caryophyllene). Strong inhibition of lipid peroxidation was observed in a dose-dependent manner, especially in the Fe (2+)/hydrogen peroxide induction system (94.59% for 2.13 microg/ml). The suppressive effect of the essential oil on lipid peroxidation is determined by its activity on Fe (2+)/ascorbate and Fe (2+)/hydrogen peroxide induction systems (Mimica-Dukic, 2004).

Melissa officinalis is a traditional herb widely used for its sedative, spasmolytic, antibacterial effects. The realization of the antioxidant properties of the plant is very recent. Melissa Officinalis essential oil has been shown to be antioxidant in several human cancer cell lines (A549, MCF-7, Caco-2, HL-60, K562) and mouse cell lines (B16F10). The antioxidant activity of the oil is explained by the reduction of 1,1- diphenyl-2-picryl-hydrazyl (DPPH). These results indicate that Melissa Officinalis can be used as an antioxidant and a potential antitumor (de Souza, 2004).

Antimicrobial activity: In a study investigating the antimicrobial activity of Melissa essential oil, a very strong effect was reported against 13 bacterial strains and 6 fungi; the most effective antibacterial activity was expressed in a multi resistant strain of Shigella sonei. Antifungal activity was demonstrated on Trichophyton species (Mimica, 2004).

Sedative effect: Many clinical studies related to sleep disorders report no statistically significant data (Cerny, 1999). In

support of these findings, double-blind placebo randomized cross-over controlled trials of lemon balm extract and double-blind placebo randomized cross-over controlled trials reported that there was no significant difference in effect between those using lemon balm extract and the placebo group (Schulz, 1998).

Effect on Herpes Labialis: The effect of lemon balm officinalis on patients suffering from herpes labialis has been studied, and it has been reported that the herb shortens the healing time; it can prevent typical herpes symptoms such as stabbing, burning, tingling, infection, and itching, prevent swelling, tension and erythema, and show a rapid effect. Lemon balm extract eliminated the resistance development of the herpes virus, and some indicators revealed that the intervals of herpes periods may be longer with lemon balm cream (Koytchev, 1999).

Effect on Premenstrual Syndrome: Premenstrual syndrome (PMS) is observed in women before menstruation with symptoms such as swelling in the breasts, abdominal gas, weakness, weight gain, lack of energy, headaches, depressive state, anxiety, and tension of varying severity and negatively affects the quality of daily life. Considering that the sedative effects of Melissa officinalis can be used to suppress and relieve PMS symptoms, the effect of Melissa officinalis capsules in this direction was investigated in a female high school student population; a statistically significant decrease in PMS symptoms was reported by repeated measurement tests. It was reported that Melissa officinalis may be effective in reducing PMS symptoms, but more studies are needed (Akbarzadeh, 2015).

Addiction and Withdrawal Syndrome Effect: Regarding the addiction and withdrawal caused by long-term use of Melissa officinalis, it has been reported that Melissa officinalis may cause addiction and withdrawal syndrome in patients in case of alternative use to drugs by many patients, so patients should be warned against this situation and their awareness should be increased (Demirci, 2015).

Effect in the treatment of severe dementia agitation: The results of some double-blind, placebo-controlled studies with Melissa are remarkable. Motor and psychological symptoms in dementia, especially in patients with cognitive impairments, are persistent, which is one of the main problems in managing the disease. Although studies indicate positive effects of aromatherapy using essential oils of plants, adequately powered placebo-controlled studies are not yet sufficient. In a placebo-controlled study with Melissa officinalis essential oil to determine the importance of aromatherapy for agitation in people with severe dementia, seventy-two people with clinically significant agitation in the context of severe dementia were subjected to aromatherapy with Melissa essential oil (N=36) and sunflower oil as placebo (N=36); Four-week periods of treatment were compared between the two groups on clinically significant changes in agitation (Cohen-Mansfield Agitation Inventory [CMAI]) and quality of life indices (percentage of time spent engaged in constructive activities, percentage of time spent in social recreation and constructive activities measured by Dementia Care Mapping). No significant side effects were observed and a 30% reduction in CMAI score was reported in 60% (21/35) of the active treatment group and 14% (5/36) of the placebo group. The quality of life of people receiving Melissa essential oil improved significantly; as a result, it was thought that aromatherapy with Melissa essential oil may be an effective and safe support for clinically significant agitation symptoms in patients with severe dementia (Ballard, 2002).

In conclusion, research on melissa officinalis, which is a very popular commercial medicinal aromatic plant in our country and in the world, continues for the bioactive active substances contained in the plant and their use for their symptom-relieving effects in various clinical situations. Although the plant is still widely used in traditional folk practices, some scientific studies suggest that the plant may have side effects and contraindications, so it should be used under physician control.

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