Review https://doi.org/10.47748/tjvr.1570036

TJVR 2025; 9 (1): 75-88

Turkish Journal of Veterinary Research

https://dergipark.org.tr/tr/pub/tjvr

e-ISSN: 2602-3695

Integrative treatment method in Veterinary Medicine: Hirudotherapy

Fatma Çoruk [1] Hüseyin Ayhan [1,2]

[1] *Department of Traditional, Complementary and Integrative Medicine, Biotherapeutic Products Research and Development Program, Ankara Yıldırım Beyazıt University, Ankara, Turkey*

[2] *Department of Medical Laboratory Techniques Program, Health Services Vocational School, University of Ankara Yıldırım Beyazıt, Çubuk, Ankara, Türkiye.*

Correspondence: *Hüseyin Ayhan (hayhan@aybu.edu.tr)*
Received: 19.10.2024 Accepted: 22.02.2025

ABSTRACT

Traditional and complementary medicine methods have a long history in veterinary medicine. Medicinal leech therapy (hirudotherapy) is one of these methods. Medicinal leeches are sanguivorous invertebrates. While sucking blood to feed, they secrete saliva into the host's body. They contain over 100 bioactive molecules with analgesic, anti-inflammatory, anti-platelet, anticoagulant, and antimicrobial extracellular matrix degeneration effects. Consequently, the leeches treat the host by exhibiting a phlebotomic effect, a range of pharmacological effects due to bioactive molecules, and a reflex stimulation effect at the bite site. Recently, leeches have been used successfully in veterinary medicine to treat many diseases of animals, especially dogs, cats, and horses. The most common indications for the use of leeches are venous congestion, acute and chronic arthritis, laminitis in horses, diseases associated with inflammation of tendons, ligaments and fascia, vertebral diseases, and treatment of scars. Despite its frequent application in different countries, there needs to be more scientific literature on the veterinary use of leeches. This article aims to encourage further scientific investigation to expand the veterinary use of leech therapy, which is a cost-effective and relatively safe alternative to other methods, and to elucidate the potential effects of leech secretions.

Keywords: *Veterinary leech therapy, Hirudotherapy, Medicinal leech, Leech saliva*

INTRODUCTION

The domestication of animals has enabled humans to use them as a sustainable source of food and livelihood and companions in various forms of transportation, freight transport, everyday life, and warfare. This situation has resulted in prioritizing animal health on a par with humans (Aslan, 2023). The fact that over 60% of infectious diseases are zoonotic and that epidemics can rapidly globalise and become pandemics due to the rapidity of contemporary communication is further evidence that veterinary health is of great importance (Maden, 2020).

Both medicine and veterinary medicine aim to achieve and maintain equilibrium within the body. The role of a physician is to maintain homeostasis, to rebalance it if it is disturbed, and to ensure the maintenance of mental and cognitive well-being. The practices carried out for the prevention of physical diseases, diagnosis, treatment and cure of these diseases have collectively developed a body of knowledge that forms the basis of today's veterinary and medical science (Aslan, 2023). Veterinary medicine has been supported by traditional medical practices for thousands of years, with preparations, drugs, and applications have been made against hundreds of medical conditions. These include parasite control; vertebrae, neck, and head diseases; joint, foot, and bone diseases; lameness; eye diseases; mouth, jaw, and facial muscle disorders; skin and subcutaneous connective tissue disorders; diseases of the chest and abdomen; dental diseases, stomach, and intestinal diseases (Brennan, 2001; Sinmez, 2011). One such traditional method is leech treatment, also known as hirudotherapy (Ben-Yakir, 2008a).

Hirudotherapy involves the use of blood-sucking leeches. The use of medicinal leeches has a vast historical background, extending from Ancient Egypt to the Ottoman Empire and Europe. The earliest written records date back to 200-130 BCE in *Alexipharmaca*, a medical poem by Nicander of Colophon, who lived in what is now Menderes, Izmir. During the Roman period, Galen extensively employed leech therapy for bloodletting (Wells et al., 1993; Whitaker et al., 2004). In Islamic medicine, Ibn Sina (Avicenna) in *Al-Qanun fi't-Tibb* described leeches as an effective treatment for deep vascular diseases and skin disorders, emphasizing their ability to draw blood from vessels that could not be accessed by cupping therapy. Similarly, Ottoman physicians, including Sabuncuoğlu Şerefeddin and Sanizade Mehmet Ataullah Efendi, provided detailed records on leech applications for a wide range of conditions, from fevers in children to post-surgical wound healing (Uzel, 1992; Kahya, 2009; Acıduman, 2009). In Europe, hirudotherapy was extensively practiced during the Napoleonic era, particularly in France and England, where it was used for brain, kidney, and liver diseases, ophthalmic disorders, rheumatism, epilepsy, and inflammatory conditions (Rolleston, 1959; Adams, 1988). However, with the rise of modern medicine in the late 19th century, leech therapy declined in favor of synthetic pharmaceuticals and surgical advancements (Whitaker et al., 2004). Historically, medicinal leeches have also been used in veterinary medicine for the treatment of various conditions, including acute equine laminitis, canine aural hematoma, laryngitis, and prolapsed uterus in cattle (Ben-Yakir, 2008a).

Hirudotherapy encompasses a range of biological properties inherent in medicinal leeches. Until the end of the nineteenth century, the only known property was that leeches feed on the blood of their hosts (phlebotomic property). However, with the discovery of anticoagulant substances in the secretion extract by Dr. John Haycraft in 1883 and subsequent studies, it became evident that leeches release a range of bioactive substances, particularly anticoagulants, anti-inflammatory agents, and anesthetics, into the host's body while sucking blood. The main aim of therapy is now to use the potential benefits of leech saliva secreted during leech feeding (Weinfeld et al., 2000; Alaama et al., 2024).

In veterinary medicine, medicinal leeches are applied to obstructed or inflamed areas of the animal body, particularly in cases where conventional treatment methods are ineffective or have insufficient efficacy (Buote, 2014; Sobzcak and Kantyka, 2014). In recent times, leeches have been used with considerable success in veterinary medicine for the treatment of a wide range of diseases affecting animals, particularly dogs, cats, and horses. The popularity of its use in microsurgery and reconstructive surgery in human medicine has led to its adoption in veterinary medicine for reconstructive surgery on swollen faces, limbs, and digits following successful arterial revascularization but limited venous repair (Ben-Yakir, 2008a; Fernee, 2011). The efficacy of leeches in the healing of inflammatory conditions, peripheral venous and arterial diseases, non-granulating wounds or ulcers in aged and immunocompromised animals, tendon injuries and muscle fatigue, and many other diseases has been demonstrated (Ben-Yakir, 2008a; Sobzcak and Kantyka, 2014; Kermanian et al., 2022).

Currently, leech therapy, which has become a prominent practice in both human and animal medicine and a valuable raw material in the cosmetic and pharmaceutical industries, is endorsed by international organizations and demonstrates a growing prestige within mainstream health services (Rados, 2004; Anonym, 2023). However, the scientific literature on the veterinary use of leeches remains limited, and standardized treatment protocols have yet to be established.

This review systematically examines the literature on veterinary hirudotherapy using databases such as PubMed, Scopus, Web of Science, and Google Scholar. Search terms included 'hirudotherapy', 'veterinary hirudotherapy', 'medicinal leeches in veterinary medicine', 'leech therapy in animals', and 'hirudotherapy and veterinary application'. Inclusion criteria were peer-reviewed studies published between 2000-2024 focusing on the veterinary use of leech therapy. Studies not related to veterinary applications or lacking sufficient data were excluded.

The objective of this review is threefold: firstly, to contribute to the existing literature on the veterinary use of leeches by providing a comprehensive analysis of their therapeutic applications; secondly, to increase awareness of this treatment method among veterinary professionals and researchers; and thirdly, to highlight potential research gaps and future directions for studies in this field. By critically evaluating the available scientific data, this review aims to establish a foundation for further clinical research and the integration of hirudotherapy into veterinary practice.

1. Medicinal Leeches

In 1758, Linnaeus classified medicinal leeches in his monograph *Systema Naturae*, assigning the name *Hirudo medicinalis* to emphasize their therapeutic applications. Today, the classification of *H. medicinalis* and other medicinal leech species places them within Kingdom Animalia, Phylum Annelida, Class Clitellata, Order Arhynchobdella, Family Hirudinidae, and Genus Hirudo (Sawyer, 1986; Gileva and Mumcuoğlu, 2013). The subclass *Hirudinea* comprises over 800 species, though only about 15 are used in medical treatments (Kvist et al., 2013; Ünal et al., 2023). While *Hirudo medicinalis* is the most widely recognized species, several other members of the *Hirudo* genus are also used for medicinal purposes, including *Hirudo verbana*, *Hirudo orientalis*, *Hirudo troctina*, *Hirudo nipponia*, and the newly identified *Hirudo sulukii*, endemic to Turkey. Additionally, medicinal leeches outside the *Hirudo* genus, such as *Hirudinaria manillensis* and *Macrobdella decora*, are also utilized in therapeutic applications (Sağlam et al., 2016; Trontelj and Utevsky, 2005; Utevsky et al., 2010).

- *Hirudo medicinalis*: Found across Central and Western Europe, including the United Kingdom, Lithuania, and Ukraine (Elliott and Kutschera, 2011).
- *Hirudo verbana*: Distributed in the Balkans, Eastern Mediterranean, Moldova, Ukraine, Russia, Turkey, and Armenia (Elliott and Kutschera, 2011).
- *Hirudo orientalis*: Native to Transcaucasia, Iran, and Central Asia (Trontelj and Utevsky, 2005; Utevsky et al., 2010).
- *Hirudo troctina*: Found in North Africa and parts of Spain (Hechtel and Sawyer, 2002).
- *Hirudo nipponia*: Found in Asia (Whitman, 1886)
- *Hirudo sulukii*: Recently discovered in southeastern Turkey, specifically in Kara Lake (Adıyaman), Sülüklü Lake (Gaziantep), and Segirkan Wetland (Batman) (Sağlam et al., 2016).

Furthermore, within the Turkish context, both state institutions and private farms are engaged in the production of medicinal leeches. The purpose of this production is to satisfy both domestic and foreign demand.

Medicinal leeches feature a cylindrical, dorsoventrally flattened body comprising 33-34 segments and are hermaphroditic. They feed by ingesting the blood of mammals or, on occasion, that of frogs, tadpoles, and small fish (Blair, 1927; Sawyer, 1986). These animals have photoreceptors, comprising five pairs of eyes, as well as sensilla that function as chemoreceptors (for chemicals in the air and water) and sensilla that function as mechanoreceptors (for vibrations or sounds). These allow them to locate potential prey and hosts (Nesemann and Neubert, 1999; Phillips et al., 2020). The body segments are differentiated into anterior suckers at the beginning and posterior suckers at the end. The posterior sucker is usually larger than the anterior sucker and only helps with surface attachment and movement (Mann, 1962; Lukin,1976). The anterior sucker, consisting of a buccal cavity and jaws, is used for attachment and feeding. The leech has three rigid jaws arranged in a tri-radiated configuration. About 100 pyramidal, sharp teeth in each jaw are used to cut the skin, leaving a characteristic Y-shaped bite. On the side of each tooth, close to the piercing tip, is an opening through which saliva is injected into the skin (Orevi et al., 2000; Ayhan et al., 2021). The row of teeth in each jaw works like a saw to cut open the host's body surface. After piercing the skin and injecting its saliva, the leech begins to feed on the blood flowing through the destroyed vessels and lymph from the interstitial space, which is then sucked into the crop by the pumping action of the pharyngeal muscles while gradually releasing the salivary chemicals responsible for the therapeutic benefits of hirudotherapy following the bite (Hildebrandt and Lemke, 2011). Adult leeches typically feed for approximately 30 to 40 minutes, ingesting approximately 10 to 15 milliliters of blood (Sawyer, 1986). After feeding, the blood is stored in the crop, which contains ten pairs of lateral pockets, providing additional storage space (Wenning et al., 1980). The blood is combined with anticoagulation secretions during ingestion. The blood in the digestive tract is preserved by endoenzymes, and blood cells are concentrated by excreting plasma from the ingested blood. After feeding, leeches have been observed to survive for approximately a year without sucking blood (Wenning and Cahill, 1989; Hildebrandt and Lemke, 2011).

Furthermore, the digestive process is facilitated by endosymbiotic bacterias, such as *Aeromonas veronii* and *Aeromonas hydrophila*, which are present within the leech's digestive tract (Graf, 1999; Özbey et al., 2020). In medicinal leeches, the body surface is adapted in such a way as to facilitate gas exchange. The leeches respire by taking in oxygen from the water during locomotion, and they also respire by attaching the posterior sucker to something and undulating their body (Mann, 1962; Kaestner, 1967). The nervous system of medicinal leeches comprises a pair of cerebroid ganglia, 21 body ganglia, and seven caudal ganglia. These ganglia contain thousands of microglial cells (Le Marrec-Croq et al., 2013). The body of leeches typically possess both male and female reproductive systems, with the former consisting of tube-like male reproductive organs (including the duct for sperm transport) and the female reproductive organs located caudally. Following mating, full embryonic development occurs within a cocoon. In the natural environment, eggs in cocoons are laid in moist soil on the banks of inhabited ponds. After hatching from the cocoon, juvenile leeches enter the aquatic environment, where they grow by feeding on the blood of aquatic animals (Kutschera and Elliott, 2014).

2. Mechanism of Action

The therapeutic effects of medicinal leeches on their hosts while feeding can be broadly categorised as follows: a bloodletting effect during active blood absorption and passive wound oozing; the injection of biologically active substances into the host with saliva; and neuro-reflex (cuto-visceral) effects (Isakhanjan, 2003). To date, a substantial body of scientific literature has been produced on the secretions and mechanisms of action of leeches. In particular, more than 100 specific bioactive molecules with different molecular masses and their mechanisms of action have been identified in their secretions (Baskova et al., 2004). The mechanisms of action have been categorised to facilitate comprehension; however, these mechanisms are closely interrelated and should be considered as a whole. Following a leech bite, the leech must create a suction pathway by degrading the extracellular matrix. It must then inhibit platelet function and anticoagulant action to prevent adhesion, aggregation, and coagulation. The leech must also increase blood flow, protect itself by showing antimicrobial activity, and avoid detection in the host's body by exhibiting analgesic and anti-inflammatory action. Furthermore, the bioactive molecules present in leech saliva have been demonstrated to exhibit anti-cancer properties (Sig et al., 2017; Shakouri and Wollina, 2021).

2.1 Extracellular matrix degradation, increasing blood flow, and antimicrobial effect

The enzymes collagenase and hyaluronidase are produced by leeches immediately following a bite, facilitating the absorption and diffusion of their bioactive compounds (Sig et al., 2017). Hyaluronidase reduces the viscosity of tissues and renders them more permeable to injected fluids, thereby increasing the rate of fluid absorption. This effect allows the resorption of excess fluids and extravasated blood in the tissues, thereby increasing the efficacy of local anesthetics. Hyaluronidase thus increases the diffusion of all salivary secretions (Hovingh and Linker, 1999). Furthermore, it has antibiotic properties (Hallaji et al., 2019). Hyaluronidase can be used to treat various complications associated with hyaluronic acid. Collagenase has been demonstrated to diminish scar tissue density and inhibit fibroblast formation in keloids and hypertrophic scars (Rigbi et al., 1996).

Leech saliva contains the vasodilator acetylcholine and histamine-like molecules, as well as a carboxypeptidase A inhibitor that increases blood flow and reduces local edema. Furthermore, acetylcholine induces relaxation of the endothelial muscles. These molecules facilitate the continuity of microcirculation by transporting oxygenated blood to areas of compromised circulation, thereby restoring normal circulation (Segal, 2005; Herlin et al., 2016).

Destabilase has been observed to possess β-glycosidase activity, which enables it to break down β1-4 bonds in the bacterial cell walls' peptidoglycan layer directly (Zavalova et al., 2003). Lysozyme, frequently present in lacrimal fluid and both human and animal saliva, has exhibited the same action as described above (Franken, 1989). In addition to the enzymatic action of glycosidases, non-enzymatic components also contribute to the antimicrobial activity (Zavalova et al., 2006). The bacteriostatic action of the substance is effective against several bacterial species, including *Escherichia coli, Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Indergand and Graf, 2000). Leech secretions include the powerful antibiotic chloromycetin, yet there is a paucity of knowledge regarding this compound (Wilmer et al., 2013).

2.2 Analgesic and anti-inflammatory effects

The kallikrein system plays a significant role in the inflammatory response and is associated with the coagulation cascade. Bradykinin, a pro-inflammatory protein, kallikreins, and kininogen (a precursor to kinin) are components of the complex system known as the kallikrein-kinin system, which is produced in different organs. Bradykinin functions by increasing vascular permeability, which induces vasodilatation and intensifies pain (Sharma and Al-Sherif, 2011; Kashuba et al., 2013). Antistasin and hirustasin found in leech saliva act as potent factor Xa inhibitors and have an inhibitory effect on the kinin-kallikrein system (Nutt et al., 1991). Factor Xa is a prothrombin activator and plays a critical role in the common pathway of the coagulation cascade (Schenone et al., 2004). Some studies have shown that some kininases and antistasins may inhibit the kinin kallikrein mechanism, an important nociceptive pathway (Nutt et al., 1991). In addition, it has been observed in animal experiments that antistasin prevents vascular graft thrombosis, accelerates reperfusion, reduces restenosis after balloon angioplasty of atherosclerotic femoral artery, and affects mitosis of aortic smooth muscle cells in cell culture as a result of selective factor Xa inhibition (Zaidi et al., 2011).

Mast cell tryptases are serine proteases found in cell granules, and their release causes inflammatory reactions in animals. Strong correlations exist between these effects and leukocyte activation, chemotaxis, the kinin-kallikrein system, vasoactive activities, and the ensuing pain-producing interactions. Allergy and inflammatory conditions like arthritis, asthma, and anaphylaxis can be caused by chemicals that are secreted by mast cells (Vitte, 2015; Caughey, 2016). Three isoforms of leech-derived tryptase inhibitor (LDTI), known as a, b, and c, function by preventing mast cell proteolytic enzymes from acting. In addition to specifically inhibiting mast cell tryptase, the serine protease inhibitor LDTI also inhibits trypsin and chymotrypsin (Campos et al., 2004).

Eglins (elastase-cathepsin G leech inhibitors) are small proteins found in leeches that exhibit potent inhibitory activity against chymotrypsin and subtilisin-like serine proteinases acting on non-cationic substrates. A single leech contains approximately 20 μg of eglin. Eglin C has been identified as a potential therapeutic agent for treating inflammatory diseases. Its efficacy has been demonstrated in the management of shock and emphysema in experimental models (Seemuller et al., 1986; Zaidi et al., 2011). Eglins also improve diabetic neuropathy due to their anti-inflammatory effect on the nerve and improve microangiopathy by increasing blood circulation through the effect of many other bioactive substances in saliva, which are the main mechanisms of the positive impact on diabetic wounds (Dwivedi, 2012).

Additionally, the saliva of leeches contains bdellins. Bdellins are a class of substances that act as inhibitors of trypsin, plasmin, and sperm acrosine. They have anti-inflammatory effects. Furthermore, they act as proteinase inhibitors. In particular, bdellin A can be utilised as a plasmin inhibitor to regulate bleeding (Fink et al., 1986; Seemuller et al., 1986). These substances, which are secreted by leeches, are efficacious in the treatment of inflammation-induced diseases, anaphylaxis, allergy, and pain management in animals.

2.3 Anticoagulation effects

To maintain blood flow into their host's body while extracting blood, leeches inject anticoagulant chemicals. Leech secretions contain bioactive compounds that act at distinct sites in the coagulation cascade, a series of reactions that ultimately lead to the formation of a blood clot. Factor Xa plays a role in breaking the chain reaction, destabilase has a fibrinolytic effect, and hirudin primarily functions as a thrombin inhibitor (Sig et al., 2017).

Hirudin irreversibly binds to thrombin, thereby inhibiting blood coagulation by blocking all biological functions caused by thrombin (Markwardt, 1955; Bichler and Fritz, 1991). The administration of hirudin or its derivatives may be indicated for the prophylaxis and treatment of postoperative venous thrombosis, particularly in the context of cardiac surgery; the augmentation of fibrinolytic therapy and/or angioplasty to prevent re-occlusion; and flap surgery. Hirudin and its analogs may be utilized in the treatment of vascular diseases, including strokes and myocardial infarction (Zaidi et al., 2011; Alaama et al., 2024). Factor Xa inhibitor is an anticoagulant, and it also plays a critical role in the treatment of osteoarthritis and rheumatoid arthritis (Das et al., 2014).

The monomerising activity of destabilase has the potential to degrade stable fibrin by isopeptidolising α and γ fibrin chains linked by ε (γ

Glu) Lys isopeptide bonds (Baskova et al., 2001). It can be concluded that the anticoagulant effect of bioactive substances is of great importance in the therapeutic management of diseases associated with anticoagulant deficiency in humans and animals.

2.4 Antiplatetelet effect

Normally, in animals and humans, when the wall of a blood vessel is damaged, it causes the spread and release of collagen particles targeting the von Willebrand factor (vWF). After the collagen particles are released, a plug is formed in the vessel following a series of events, and the bleeding is stopped. This reaction also initiates another chain in which substances such as thromboxane A2, platelet activation, and the coagulation cascade are released (Kumar et al., 2015). In leech secretions, various molecules (saratin, calin, and apyrase) react against different parts of this chain (Das, 2014). Saratin affects only the first stage of platelet adhesion and inhibits the collagen-vWF reaction. Some animal studies have shown promising results with recombinant saratin molecule as a potential local therapeutic agent for antithrombotic treatments and atherosclerosis (Gronwald et al., 2008).

Calin exhibits rapid action (within 10 minutes) on collagen, effectively suppressing platelet adhesion to collagen-coated microcarrier particles and collagen-induced platelet aggregation (Munro et al., 1991). In addition to preventing direct platelet-collagen contact, Calin also obstructs the binding of von Willebrand factor to collagen. This is believed to be one of the initial stages of thrombus formation in injured endothelial areas (Harsfalvi et al., 1995). A disruption to this mechanism may have antithrombotic effects. Research conducted on hamsters has demonstrated that calin selectively and in a dose-dependent manner prevents platelet aggregation brought on by collagen (Deckmyn et al., 1995). The chemicals released by leeches have the potential to suppress platelet aggregation by blocking the platelet receptor vWf and degrading collagen particles. This approach may prove effective in the management of micro thrombus formation following surgical procedures in animals.

A summary of the bioactive molecules mentioned above can be found in Table 1.

Table 1. Potential bioactive substances in leech secretions.

Mode of action	Bioactive Substance	Function
Analgesic and anti-inflammatory effect	Bdellins	Used as a plasmin inhibitor to control bleeding
	Hirustasin/ Antistasin	Prevents vascular graft thrombosis, accelerates reperfusion
	LDTI	Inhibits mast cells that occur in conditions such as arthritis and asthma
	Eglins	Improves diabetic neuropathy and promotes healing of diabetic wounds
	Carboxypeptidase A inhibitor	Inhibits carboxypeptidase A, accelerates blood flow
Anticoagulant effect	Hirudin	Hirudin inhibits thrombin. It is a powerful anticoagulant alternative to heparin
	Destabilase	Dissolves stabilised fibrin
	Factor Xa inhibitor	Inhibits Factor Xa
Extracellular matrix degradation	Hyaluronidase	Inhibits hyaluronic acid. Makes vessels more permeable to fluids
	Collagenase	Dissolves collagen particles, accelerates wound healing
Increasing blood flow	Histamine-like molecules	Increase blood flow
	Acetylcholine	Helps relaxation of endothelial muscles.
Anti-platelet function	Calin	Inhibits collagen-induced platelet aggregation
	Saratin	Potential agent for atherosclerosis
Antimicrobial effect	Destabilase	Destroys the peptidoglycan layer in the bacterial cell wall.

3. The application of Veterinary Hirudotherapy

3.1. Procedure

It is recommended that leeches for therapeutic use are obtained from hygienic leech farms that are certified to cultivate leeches for medicinal purposes. This is more effective than acquiring them from natural habitats as it protects against various pathogens, including viruses, bacteria, fungi, and parasites. Leeches taken from farms should be kept in groups of no more than 10-15 in glass jars of approximately 3 liters, covered with a layer of dense linen fabric, and held in place by an elastic band or secure plastic lid with small perforations to facilitate ventilation (Figure 1). The water in the container should be free of chlorine. The water in the container should be changed once or twice a week, and the container should be thoroughly cleaned once a month without using chemicals. The leech container should be kept in a dark and cool place, away from strong odors and vibrations. These conditions have been shown to support the survival of leeches for up to one and a half years (Gileva and Mumcuoğlu, 2013).

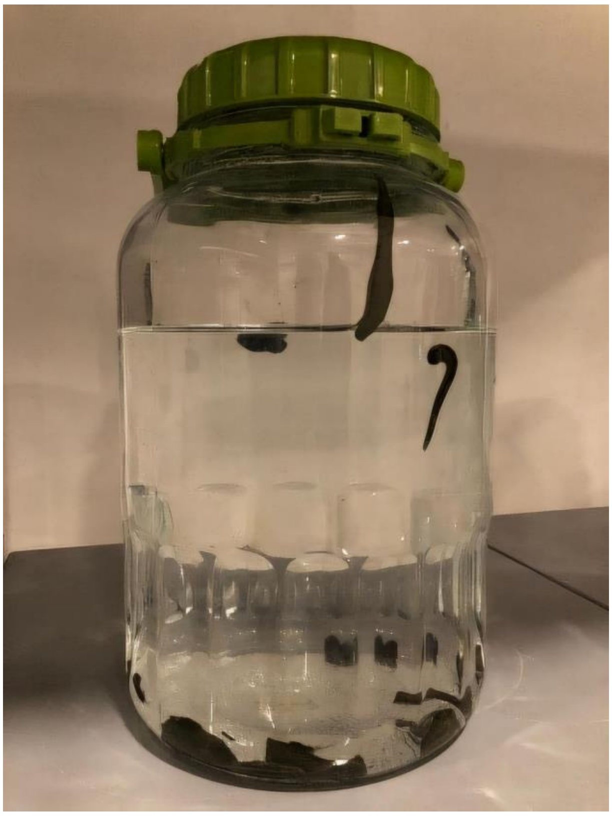

Figure 1. Glass container suitable for storage of leeches.

The number of leeches to be used in a therapeutic application is contingent upon the size and disease state of the animal. It is recommended that one leech be used for animals weighing less than 10 kg, 1- 2 leeches for animals weighing 10-15 kg, 3-4 leeches for animals weighing 30-40 kg, and 5-15 leeches for a horse (Sobczak and Kantyka, 2014). Before the application of leeches, it is imperative that the area be cleansed with sterile water, as the utilization of disinfectant or antiseptic solutions within the application area will impede the leech's ability to attach. In order to facilitate the attachment of the leech, it may be necessary to shave the treatment area, depending on the area to be applied. However, shaving is not a prerequisite for applications in auricular hematomas (Anonym, 2024a). Leeches are typically willing to feed due to their starvation before use. However, if the leech is reluctant to feed, pricking the skin with a small needle to induce bleeding facilitates its attachment, or applying a sugar and water solution can facilitate the attachment of the leech. Once the leech has attached itself, it is likely to remain in place until it has ingested sufficient blood, at which point it will fall off of its own accord. Nevertheless, monitoring the process to prevent the leech from relocating to an unintended area is essential. The feeding period for leeches in dogs and cats is typically 30 to 60 minutes, while in horses, it is 40 to 120 minutes. During this period, the leech expands to approximately five times its original size (Sobczak and Kantyka, 2014). It is of the utmost importance to allow the leech to feed until it detaches naturally; the leech should never be forcibly removed during this process. The application of force may result in the leech vomiting its stomach contents into the wound, which could potentially lead to infection. The optimal method for leech removal is to shake the leech's entire body several times gently or to gently loosen the posterior sucker with a fingernail, allowing the leech to descend due to its body weight (Michalsen et al., 2007). Following the application of the leech, it is important to maintain the bleeding of the tissue at the bite sites. The wound should be kept open in a clean environment.

Following application, leeches should not be reused on another patient. To mitigate the potential for complications, it is advised that physicians applying leeches disinfect them prior to use in accordance with established protocols, apply the leech on one occasion only, and then dispose of it. The most humane method of leech disposal is to initially place the leech in an 8% alcohol solution and then apply a stronger 70% solution (Ben-Yakir, 2008a).

3.2 Indications

Hirudotherapy is used in traditional and complementary medicine to treat a wide range of diseases and support existing therapies. The anticoagulant, vasodilator, thrombolytic, analgesic and anti-inflammatory properties of leeches can be used to treat various conditions (Ayhan and Mollahaliloğlu, 2018). Using leeches in veterinary medicine has the same indications as in human medicine. The most common applications of hirudotherapy in animals include flap surgery, orthopedic trauma, limb amputation, post-castration, venous congestion, presence of blood clots, poorly healing wounds, and inflammatory diseases (Sobczak and Kantyka, 2014; Abdisa, 2018; Kermanian et al., 2022).

The diseases in which the use of hirudotherapy is indicated in various animal species are as follows.

3.2.1. Cats

The therapeutic efficacy of medicinal leeches as an adjunct treatment for polycythemia vera has been demonstrated in veterinary medicine. In a cat diagnosed with polycythemia vera, leeches were applied as an alternative to more invasive procedures, such as phlebotomy, which was initially not feasible. Hirudotherapy aids circulation by reducing blood viscosity, thereby improving hemodynamics. However, the optimal dosage and duration of application vary depending on the patient and disease severity. Studies indicate that hirudotherapy contributes to the stabilization of blood cell levels and may play a supportive role in long-term disease management (Net et al., 2001).

Conventionally, polycythemia vera is treated with therapeutic phlebotomy and chemotherapeutic agents, such as hydroxyurea. While phlebotomy is effective in acutely reducing hematocrit levels, it necessitates frequent and repetitive procedures, which may be challenging in some clinical scenarios. Additionally, chemotherapeutic agents pose risks of immunosuppression and myelosuppression, requiring careful patient monitoring (Plumb, 2008). In contrast, hirudotherapy presents a minimally invasive alternative with fewer systemic side effects, making it a potentially beneficial complementary treatment in polycythemia vera management. However, further research is warranted to establish standardized protocols and confirm its long-term efficacy.

In another case, a cat was presented with acute hind limb paralysis and pain in these limbs. Furthermore, no femoral pulses could be discerned in either limb. The distal limbs were observed to be cold and bluish, and no bleeding was observed from the cut nail beds. Two medicinal leeches were applied to both sides of the inner thigh, close to the abdomen. Twenty-four hours after the leeches were applied, the cat exhibited a return of femoral pulses to normalcy, a reduction in pain, and the ability to walk without assistance. It was documented that other similar cases were presented to the clinic within two years, and each was successfully treated with medical leeches. (Ben-Yakir, 2008b).

Following a constrictive injury from a bandage, a cat with considerable swelling in its paw and impaired limb use was treated with leech therapy for four days, during which time a notable reduction in swelling was observed. The cat was able to use its limbs, and the constrictive wound healed further without any complications. In this case, it was reported that the cat's severe venous congestion had been resolved and that there was no further skin loss due to leech therapy (Figure 2) (Buote, 2014).

Figure 2. Leech treatment of cat paw with venous congestion (Buote, 2014).

Additionally, medicinal leeches are used in the treatment of conditions such as inflammation and diseases of the peripheral veins and arteries, including feline aortic thromboembolism. They are also used in FIV-positive cats with salivary gland diseases, including severe stomatitis, gingivitis, sialadenitis, and sialoadenosis, acute and chronic inflammatory responses of the oral mucosa and periodontal lesions. Furthermore, they are applied in the management of postoperative wound complications, discopathies, eczema, abscesses, strained ligaments, dysplasia of the knee, knee inflammation, neuritis, and mastitis (Sobczak and Kantyka, 2014; Anonym, 2024b).

3.2.2. Dogs

An aged dog presenting with fractures of all four metatarsal bones in its hindlimb underwent surgical intervention to stabilize the affected bones via internal fixation. Following the surgical procedure, leeches were applied to various regions of the dog's foot. As a consequence of the therapeutic intervention, the foot exhibited a notable degree of recovery (Fernee, 2011).

A 10-year-old spayed female Mastiff dog with an upper respiratory tract obstruction was intubated and received a propofol and fentanyl infusion, as well as a series of antibiotics, during the initial 18 hours of treatment. Subsequently, a total of 11 leeches were applied to the sublingual and cervical regions of the dog, resulting in a reduction in sublingual swelling observed 6-8 hours after the treatment. The patient was extubated at the 44th hour and subsequently discharged from the hospital (Trenholme et al.,2021).

A study conducted by Canpolat and Sağlam (2004) explored the use of medicinal leeches as a non-invasive alternative for treating aural hematomas. In their experiment, ear hematomas were induced in five adult dogs by traumatizing the arteries and veins of the outer ear surface. On the following day, three medicinal leeches were applied to each hematoma without anesthesia. The leeches were allowed to feed until they were fully engorged and detached naturally, with the procedure repeated daily for three days. The study reported that all cases exhibited complete resorption of the hematoma without any complications, demonstrating the potential of hirudotherapy as an effective alternative to conventional methods (Canpolat & Sağlam, 2004).

Aural hematomas are commonly managed through medical or surgical interventions, depending on their size and severity. Smaller hematomas may be treated conservatively with corticosteroids and drainage, whereas larger hematomas typically require surgical intervention as they are less responsive to therapeutic management. Surgical treatment involves waiting for the hematoma to coagulate over 8–10 days before surgical evacuation, but this approach carries risks such as fibrosis, chronic inflammation, and auricular deformation (Dubielzig et al., 1984; Cowley, 1976).

Compared to surgical intervention, hirudotherapy offers a less invasive and low-risk treatment, reducing the likelihood of fibrosis and structural deformities associated with surgical procedures. Additionally, the anticoagulant and anti-inflammatory properties of leech saliva aid in blood resorption and tissue healing, further supporting its use as a viable alternative or adjunct therapy in aural hematoma management. However, more clinical studies are needed to establish standardized treatment protocols and validate its long-term efficacy.

Furthermore, the treatment is indicated mainly in instances where the vessels are particularly thin, as well as in cases of mastitis and gastritis, the reduction of scrotal edema in adult dogs following a castration procedure, postoperative wounds, spinal osteoarthritis, discopathies, and cauda equina syndrome, hip and elbow dysplasia, neuritis, ear eczema, poor wound healing, postoperative scars, tendinitis, tenosynovitis (Sobczak and Kantyka, 2014; Ben-Yakir, 2008a).

3.2.3. Horses

Laminitis is a painful and debilitating inflammatory condition affecting the laminae of the horse's hoof, often leading to severe lameness and structural hoof damage. Conventional treatment typically includes NSAIDs (phenylbutazone), cryotherapy, and corrective farriery (Glöckner, 2002). While these methods aim to control pain and inflammation, many cases become chronic, and NSAID toxicity presents a concern (Rasch, 2010). Surgical interventions, such as deep digital flexor tenotomy, are reserved for refractory cases but have variable success rates (Obel, 1948).

An alternative approach using medicinal leeches has been investigated for its anticoagulant, anti-inflammatory, and vasodilatory properties. A study conducted in Germany between May 2008 and August 2009 examined the effects of hirudotherapy in 57 laminitic horses, with a total of 112 leech applications (Rasch, 2010). Results indicated that 84% of the horses exhibited clinical improvement, with some cases showing symptom relief within 48 hours. Unlike NSAIDs, which can contribute to renal and gastrointestinal complications, leech therapy resulted in only mild to moderate itching at the application site in 6 cases out of 112 (Rasch, 2010).

Compared to conventional therapy, hirudotherapy offers a less invasive, systemic toxicity-free alternative. By improving local circulation and reducing venous congestion in the hoof, medicinal leeches facilitate the resolution of inflammation without the adverse effects associated with NSAIDs. However, further research is required to determine long-term efficacy, develop standardized application protocols, and establish its role as a routine adjunctive therapy in laminitis management.

The team led by Dr Sagiv Ben-Yakir used leech therapy on four horses with acute laminitis. Four leeches were implanted into each affected leg. Following a 12-hour course of treatment, the horses were reported to have returned to their normal behavioral patterns (Ben-Yakir, 2006).

A haematoma was observed on the penis of a stallion whose penis had been kicked during mating. As the initial treatment was unsuccessful and exhibited delayed efficacy, a second course of treatment was initiated, comprising two applications of leeches with a five-day interval between each application. Following the administration of anti-inflammatory and antibiotic agents, the horse made a full recovery within 45 days. The stallion's erectile function was restored, and the stallion was able to resume reproductive activities (Nowicki et al., 2021)

In an interview with a leech practitioner, it was reported that the application of leeches was observed to be 80-90% effective in the treatment of mud fever in horses (Figure 3) (Bergsma, 2023).

Figure 3. Leech application in horse (Bergsma, 2023)

In addition to the previously mentioned conditions, leeches are also used in the treatment of ophthalmia, lymphangitis, congestion, tendinitis, tenosynovitis, ataxias, myositis, and spinal osteoarthritis in horses, as well as arthritis in the shoulder joint (Sobczak and Kantyka, 2014; Anonym, 2024b).

3.2.4. Ruminants

In a study examining the effects of hirudotherapy on hemostasis-plasma link in cows with clinical mastitis, two leeches were attached to the tissue of the diseased quarter of the udder for a period of three days, with each application occurring at 24-hour intervals for 40-50 minutes per therapy session. The results indicated that leech therapy had a corrective effect on the plasma-coagulation coupling of hemostasis (Soboleva, 2018).

The use of medicinal leeches for the treatment of mastitis, uterine prolapse, and inflamed vulva in other ruminants has also been reported (Ben-Yakir, 2008a; Haq et al., 2021).

3.3. Contraindications and complications

The following conditions are contraindications to the use of leeches in medical therapy: coagulopathy or animal on anticoagulant therapy, anemia and/or low blood pressure, hemophilia, the last stages of malignant tumour, pregnancy in the animal, lactation, bleeding in estrus time, immunosuppressive diseases and malignant skin abnormalities, fungal diseases or in animals with known allergic reactions to the active components of leech saliva (hirudin, hyluronidase, destabilase) (Sobczak and Kantyka, 2014).

It is important to note that there is a risk of transmission of *Aeromonas spp*. bacteria when leeches suck blood. These bacteria are carried in the leech's body and may cause infection if transmitted to a host. The presence of *Aeromonas spp*. and other gram-negative bacteria within the leech body can result in various bacterial infections, including intestinal disorders, pneumonia, septicemia, necrosis, and flap failure (Abdualkader et al., 2013). Another potential complication is the reuse of the leech, which carries the risk of contamination with various blood-borne diseases. Additionally, allergic reactions, unstoppable bleeding, and anaphylaxis may occur following leech application (Sobczak and Kantyka, 2014; Abdisa, 2018).

Some authors have proposed that prophylactic parenteral antibiotics may be administered to the animal prior to leech application or that leeches may be immersed in 0.02% chlorhexidine hydrochloride for 10 to 15 seconds before application to the treatment area to reduce the risk of infection (Haycox et al., 1995).

DISCUSSION and CONCLUSION

The bioactive compounds found in medicinal leeches have been utilized for centuries in the treatment of various diseases. Modern scientific research has confirmed the anti-inflammatory, anticoagulant, and circulatory-regulating effects of key bioactive molecules such as hirudin, eglins, bdellins, and destabilase (Sig et al., 2017; Shakouri and Wollina, 2021).

Laboratory studies on leech secretions have provided promising evidence regarding the therapeutic potential of hirudotherapy. However, the pharmacokinetic and pharmacodynamic properties of these bioactive compounds remain incompletely elucidated (Bayıroğlu, 2023; Erol, 2023).

Due to its minimally invasive nature, cost-effectiveness, and biological compatibility, hirudotherapy has been suggested as a potential alternative to conventional treatments, particularly in the management of joint diseases, vascular obstructions, and postoperative recovery (Abdualkader et al., 2013). Recent studies have highlighted its critical role in reducing venous congestion, accelerating soft tissue healing, and preventing postoperative complications following microsurgical procedures (Gileva and Mumcuoğlu, 2013).

In veterinary medicine, hirudotherapy presents a promising complementary treatment option. Nevertheless, the absence of standardized treatment protocols, interspecies variability in biological responses, and concerns regarding sterilization procedures pose significant limitations to its widespread clinical application. Therefore, further scientific investigations are warranted to establish the safety and efficacy of hirudotherapy as a viable therapeutic approach in veterinary practice. However, future research should focus on evaluating the long-term outcomes and safety profiles of leech therapy in veterinary patients.

In conclusion, hirudotherapy holds significant potential as a complementary and alternative treatment in veterinary medicine. However, further empirical evidence is required to substantiate its clinical efficacy and safety, and the development of standardized treatment protocols remains a priority. Consequently, future studies should prioritize controlled clinical trials and veterinary-specific applications to establish a robust scientific foundation for the widespread implementation of hirudotherapy.

REFERENCES

Abdisa T. Therapeutic importance of leech and impact of leech in domestic animals. MOJ Drug Des Devel Ther. 2018; 2(6):235-242.

Abdualkader AM, Ghawi AM, Alaama M, *et al.* Leech therapeutic applications. Indian J Pharm Sci. 2013; 75:127-137.

Acıduman A. Sanizade Mehmet Ataullah Efendi ve Mi'yaru'l Etibba adlı eserinde çocuk hastalıkları. Cocuk Sagligi ve Hastalik. Derg. 2009;52(1):42-52.

Adams SL. The medicinal leech: A page from Annelids of internal medicine. Ann Int Med. 1988;109(5):399-405.

Alaama M, Kucuk O, Bilir B, *et al.* Development of Leech extract as a therapeutic agent: A chronological review. Pharmacol Res-Mod Chin Med. 2024; 10:100355.

Anonym (2024a) What can leeches be used for? Biopharm Leeches. Available at: https://www.biopharm-leeches.com/what-can-leeches-be-used-for.html Accessed Sept 28, 2024.

Anonym (2024b). Ricarimpex Sangsues Médicinales. Available at: https://sangsue-medicinale.com/applications-medicales/applications-en-medecine-veterinaire/. Accessed Sept 9, 2024.

Anonym. Hiruline corporation. Available at: https://hiruline.com/en/international_community/. Accessed Oct 9, 2024.

Aslan R. Holistic applications in veterinary medicine. In: Çoğun HY ed. New Approaches in Veterinary Physiology. Turkiye: BIDGE Publications; 2023. pp.37-94.

Ayhan H, Mollahaliloğlu S. Tıbbi sülük tedavisi: Hirudoterapi. Ankara Med J. 2018; 18(1):141-148.

Ayhan H, Özyurt Koçakoğlu N, Candan S. Functional morphology of the suckers and teeth of the medicinal leech Hirudo verbana Carena, 1820 (Annelida; Clitellata; Hirudinida): A scanning electron microscope study. Microsc Res Tech. 2021; 84(12):2930-2935.

Baskova I, Zavalova LL, Basanova AV, Sass AV. Separation of monomerizing and lysozyme activities of destabilase from medicinal leech salivary gland secretion. Biochem (Mosc). 2001; 66(12):1368-1373.

Baskova IP, Zavalova LL, Basanova AV, Moshkovskii SA, Zgoda VG. Protein profiling of the medicinal leech salivary gland secretion by proteomic analytical methods. Biochem (Mosc) 2004; 69:770 - 775.

Bayıroğlu ME. Investigation of the efficacy of leech therapy (hirudotherapy) in the acute muscle strain model on rats [Master Thesis]. Ankara: Ankara Yıldırım Beyazıt Unìversìty; 2023.

Ben-Yakir S. Feline thromboembolism (Saddle Thrombus) treated by medical leeches. In: Small Animal and Exotics. Proceedings of the North American Veterinary Conference, Orlando, Florida, USA. 2008b; 22:8.

Ben-Yakir S. Hemoacupuncture, or the therapy that sticks. In: Small Animal and Exotics. Proceedings of the North American Veterinary Conference, Orlando, Florida, USA, 2008a; 22:13-15.

Ben-Yakir S. Veterinary hirudotherapy (VHT) or therapy that sucks. Ganzheitliche Tiermedizin 2006; 20:95-100.

Bergsma M (2023). Little organic pharmacies: Leeches for horses. Available at: https://horsegrooms.com/little-organic-pharmacies-leeches-for-horses/. Accessed Sept 24, 2024.

Bichler J, Fritz H. Hirudin, a new therapeutic tool? Ann Hematol 1991; 63:67-76.

Blair WN. Notes on Hirudo medicinalis, the Medicinal Leech, as a British Species. In Proceedings of the Zoological Society of London. Oxford, UK: Blackwell Publishing Ltd; 1927. pp.999-1002.

Brennan ML. Complete holistic care and healing for horses. The owner's veterinary guide to alternative methods and remedies. Buckingham: Kenilworth Press; 2001.

Buote NJ. The use of medical leeches for venous congestion. Vet Comp Orthop Traumatol. 2014; 27(03):173-178.

Campos IT, Silva MM, Azzolini SS, Souza AF, Sampaio CA, Fritz H. Evaluation of phage display system and leech-derived tryptase inhibitor as a tool for understanding the serine proteinase specificities. Arch Biochem Biophys. 2004; 425:87-94.

Canpolat İ, Sağlam N. Treatment of aural hematomas in dogs with the medicinal leech, hirudo medicinalis. Fırat Üniversitesi Doğu Araştırmaları Dergisi. 2004; 2(2):67-69.

Caughey GH. Mast cell proteases as pharmacological targets. Eur J Pharmacol 2016; 778:44-55.

Cowley F. Treatment of hematoma of the canine ear. Vet Med Small Anim Clin. 1976; 71:283.

Das BK. An overview on hirudotherapy/leech therapy. Ind Res J Pharm Sci. 2014; 1:33-45.

Deckmyn H, Stassen JM, Vreys I, *et al*. Calin from Hirudo medicinalis, an inhibitor of platelet adhesion to collagen, prevents platelet-rich thrombosis in hamsters. Blood 1995; 85:712-719.

Dubielzig RR, Wilson JW, Seireg AA. Pathogenesis of canine aural hematomas. J Am Vet Med Assoc. 1984;185(8):873.

Dwivedi AP. Case study of leech application in diabetic foot ulcer. Int J Res Ayurveda Pharm. 2012; 3(5):748-751.

Elliott JM, Kutschera U. Medicinal Leeches: Historical Use, Ecology, Genetics, and Conservation. Freshw Rev. 2011; 4(1):21-41.

Erol ME. Investigation of the effects of medicinal leech and medicinal leech saliva extract in rats with flap model [Master Thesis]. Ankara: Gazi University; 2023.

Fernee R. (2011). Leeches and their effective use in veterinary medicine. Available at: https://www.vettimes.co.uk/article/leeches-and-their-effective-use-in-veterinary-medicine/ Accessed Sept 29, 2024.

Fink E, Rehm H, Gippner C, *et al*. The primary structure of bdellin B-3 from the leech Hirudo medicinalis. Bdellin B-3 is a compact proteinase inhibitor of a "non-classical" Kazal type. It is present in the leech in a high molecular mass form. Biol Chem Hoppe Seyler. 1986; 367:1235-1242.

Franken C, Meijer CJ, Dijkman JH. Tissue distribution of antileukoprotease and lysozyme in humans. J Histochem Cytochem 1989; 37:493-498.

Gileva OS, Mumcuoglu KY, Hirudotherapy. Biotherapy-history, Principles and Practice: A Practical Guide to the Diagnosis and Treatment of Disease Using Living Organisms. Dordrecht: Springer Netherlands; 2013. pp.31-76.

Glöckner S. Eine retrospektive Studie über die Hufrehe bei Pferden: dargestellt an den Patienten der Klinik für Pferde, Allgemeine Chirurgie und Radiologie der Freien Universität Berlin aus den Jahren 1976–1995 [Doctoral thesis]. Berlin, Germany: Freie Universität Berlin; 2002.

Graf J. Symbiosis of Aeromonas veronii biovar sobria and Hirudo medicinalis, the medicinal leech: a novel model for digestive tract associations. Infect Immun. 1999; 67:1-7.

Gronwald W, Bomke J, Maurer T, *et al*. Structure of the leech protein saratin and characterization of its binding to collagen. J Mol Biol. 2008; 381:913-927**.**

Hallaji M, Parhamfar M, Raoufi E, Abtahi H. Cloning and high-level expression of the enzymatic region of phytase in E. coli. Int J Pept Res Ther 2019; 25(4):1431-1439.

Haq AU, Malik HU, Randhawa SS, Sudhan NA. Hirudotherapy in veterinary practice: A modern twist to ancient science. The Science World a Monthly e Magazine. 2021; 1(4):32-38.

Harsfalvi J, Stassen JM, Hoylaerts MF, *et al*. Calin from Hirudo medicinalis, an inhibitor of von Willebrand factor binding to collagen under static and flow conditions. Blood. 1995; 85:705-711.

Haycox CL, Odland PB, Coltrera MD, Raugi GJ. Indications and complications of medicinal leech therapy. J Am Acad Dermatol. 1995; 33:1053-1055.

Hechtel FOP, Sawyer RT. Toward a taxonomic revision of the medicinal leech *Hirudo medicinalis* Linnaeus, 1758 (Hirudinea: Hirudinidae): re-description of *Hirudo troctina* Johnson, 1816 from North Africa. J Nat Hist. 2002; 36(11):1269-1289.

Herlin C, Bertheuil N, Bekara F, Boissiere F, Sinna R, Chaput B. Leech therapy in flap salvage: systematic review and practical recommendations. Ann Chir Plast Esthet. 2016; 62:1-13.

Hildebrandt JP, Lemke S. Small bite, large impact-saliva and salivary molecules in the medicinal leech, Hirudo medicinalis. Naturwissenschaften. 2011; 98(12):995-1008.

Hovingh P, Linker A. Hyaluronidase activity in leeches (Hirudinea). Comp Biochem Physiol B Biochem Mol Biol. 1999; 124:319-326.

Ibn-i Sina. El-Kanun Fi't-Tıbb, Vol. 1, Translated by Esin Kahya. Atatürk Language and Culture Center, Ankara, 2009: 413-4.

Indergand S, Graf J. Ingested blood contributes to the specificity of the symbiosis of Aeromonas veronii biovar sobria and Hirudo medicinalis, the medicinal leech. Appl Environ Microbiol. 2000; 66:4735-4741.

Isakhanjan GS. On the reflex mechanism of action of hirudotherapy. In: Baskova IP ed. Treatment with Leeches and Preparations from Them. Luberzi: Girudo-Med; 2003. pp.22-23.

Kaestner A. Invertebrate zoology. A Division of John Wiley and Sons, New York, London, Sydney: Interscience Publishers; 1967. pp.597.

Kashuba E, Bailey J, Allsup D, Cawkwell L. The kinin-kallikrein system: physiological roles, pathophysiology and its relationship to cancer biomarkers. Biomarkers. 2013; 18(4):279-296.

Kermanian CS, Buote NJ, Bergman PJ. Medicinal leech therapy in veterinary medicine: A retrospective study. J Am Anim Hosp Assoc. 2022; 58(6):303-308.

Kumar V, Abbas AK, Aster JC. Robbins and Cotran pathologic basis of disease. 9th ed. Philadelphia: Elsevier Saunders; 2015.

Kutschera U, Elliott J. The European medicinal leech Hirudo medicinalis L.: Morphology and occurrence of an endangered species. Zoosyst Evol. 2014; 90(2):271-280.

Kvist S, Min, GS, Siddall, ME. Diversity and selective pressures of anticoagulants in three medicinal leeches (Hirudinida: Hirudinidae, Macrobdellidae). Ecology and Evolution. 2013; 3(4):918-933.

Le Marrec-Croq F, Drago F, Vizioli J, Sautière PE, Lefebvre C. The leech nervous system: A valuable model to study the microglia involvement in regenerative processes. J Immunol Res. 2013; 2013(1):274019.

Lukin EI. Leeches. Fauna USSR. Moscow: Academy of Science of the USSR; 1976.

Maden, M. COVID-19 Pandemisi: Sağlık iletişimi, tek sağlık ve biyogüvenlik. Turkiye Klinikleri J Vet Sci. 2020;11(2):81-91.

Mann KH. Leeches (Hirudinea). Their Structure, Physiology, Ecology and Embryology. Oxford, London, New York, Paris: Pergamon Press; 1962. pp.201.

Markwardt F. Untersuchunjen uber hirudin. Naturwissenschaften 1955; 42:537-538.

Michalsen A, Roth M, Dobos G, Aurich M. Medicinal leech therapy. Stattgurt, Germany: Thieme Medical Publishers; 2007. pp.58-65.

Munro R, Jones CP, Sawyer RT. Calin – a platelet adhesion inhibitor from the saliva of medicinal leech. Blood Coagul Fibrinolysis. 1991; 2:179-184.

Nesemann H, Neubert E. Annelida: Clitellata: Branchiobdellida, Acanthobdellea, Hirudinea. Heidelberg, Berlin: Spektrum Akademischer Verlag; 1999. pp.178.

Net CS, Arnoldq P, Glaus TM. Leeching as initial treatment in a cat with polycythaemia vera. J Small Anim Pract. 2001; 42(11):554-556.

Nowicki A, Jaworska J, Baranski W. Leech therapy in the treatment of a penile haematoma in a stallion. Vet Med-Czech. 2021; 66(6):266-271.

Nutt EM, Jain D, Lenny AB, Schaffer L, Siegl PK, Dunwiddie CT. Purification and characterization of recombinant antistasin: a leech-derived inhibitor of coagulation factor Xa. Arch Biochem Biophys 1991; 285:37-44.

Obel N. Studies on the Histopathology of Acute Laminitis [Doctoral Thesis]. Upsala: Swedish University of Agricultural Sciences; 1948.

Orevi M, Eldor A, Giguzin Y, Rigbi M. Jaw anatomy of the blood-sucking leeches, Hirudinea Limnatis nilotica and Hirudo medicinalis, and its relationship to their feeding habits. J Zool Lond. 2000; 250:121-127.

Özbey G, Sağlam N, Sproston E, Persad A, Otlu B. Identification of bacteria and select viruses harbored by medicinal leeches (Hirudo verbana). Int J Mol Clin Microbiol. 2020; 10(2):1339-1345.

Phillips AJ, Govedich, FR, Moser WE. Leeches in the extreme: Morphological, physiological, and behavioral adaptations to inhospitable habitats. Int J Parasitol Parasites Wildl. 2020; 12:318-325.

Plumb DC. Plumb's Veterinary Drug Handbook. 6th ed. 7 Publishing; 2008. pp.345-368.

Rados C. Beyond bloodletting: FDA gives leeches a medical makeover. FDA Consum. 2004; 38(5):9.

Rasch, K. Blutegeltherapie bei Hufrehe der Pferde. Zeitschrift für Ganzheitliche Tiermedizin. 2010; 24(01):24-29.

Rigbi M, Orevi M, Eldor A. Platelet aggregation and coagulation inhibitors in leech saliva and their roles in leech therapy. Semin Thromb Hemost. 1996; 22:273-278.

Rolleston JD. Francois-Joseph-Victor Broussais 1772 to 1832: his life and doctrines. Proc R Soc Med. 1959; 22: 405.

Sabuncuoğlu S. Cerrâhiyyetü'l-Hâniyye. Translated by I. Uzel. Atatürk Kültür, Dil ve Tarih Yüksek Kurumu, Türk Tarih Kurumu Yayınları; Ankara, 1992:91-104.

Sağlam N, Saunders R, Lang SA, Shain DH. A new species of *Hirudo* (Annelida: Hirudinidae): Historical biogeography of Eurasian medicinal leeches. BMC Zool. 2016; 1(1):1-13.

Sawyer RT. Leech biology and behavior. Oxford Clarendon Press; 1986.

Schenone M, Furie BC, Furie B. The blood coagulation cascade. Curr Opin Hematol 2004; 11:272-277.

Seemuller U, Dodt J, Fink E, Fritz H. Proteinase inhibitors of the leech Hirudo medicinalis (hirudins, bdellins, eglins). In: Barettand AJ, Salvesen G, eds. Proteinase Inhibitors. New York, NY: Elsevier Science Ltd.; 1986. pp.337-359.

Segal SS. Regulation of blood flow in the microcirculation. Microcirculation 2005; 12:33-45.

Shakouri A, Wollina U. Time to change theory; medical leech from a molecular medicine perspective leech salivary proteins playing a potential role in medicine. Adv Pharm Bull. 2021; 11(2):261-266.

Sharma J, Al-Sherif G. The Kinin System: Present and future pharmacological targets. Am J Biomed Sci. 2011; 3(2):156-159.

Sig AK, Guney M, Guclu AU, Ozmen E. Medicinal leech therapy-an overall perspective. Integr Med Res. 2017; 6(4):337-343.

Sinmez ÇÇ. Research on livestock raising and folkloric veterinary medicine in bozlak culture [Doctoral Thesis]. Konya: Selcuk Universïty; 2011.

Sobczak N, Kantyka M. Hirudotherapy in veterinary medicine. Ann Parasitol. 2014;60(2):89-92.

Soboleva EN. Effects of hirudotherapy on parameters of plasma Hhemostasis in cows with clinical Mastitis. Russ Agric Sci. 2018; 44(5):474-476.

Trenholme HN, Masseau I, Reinero CR. Hirudotherapy (medicinal leeches) for treatment of upper airway obstruction in a dog. J Vet Emerg Crit Care. 2021; 31(5):661-667.

Trontelj P, Utevsky SY. Celebrity with a neglected taxonomy: Molecular systematics of the medicinal leech (genus *Hirudo*). Mol Phylogenet Evol. 2005; 34(3):616-624.

Ünal K, Erol ME, Ayhan H. Literature review on the effectiveness of medicinal leech therapy in the wound healing. Ank Med J. 2023; 23(1):151-164.

Utevsky S, Zagmajster M, Atemasov A, *et al.* Distribution and status of medicinal leeches (genus *Hirudo*). J Nat Hist. 2010; 44(17-18):1085-1098.

Vitte J. Human mast cell tryptase in biology and medicine. Mol Immunol. 2015; 63:18-24.

Weinfeld AB, Yuksel E, Boutros S, Gura DH, Akyurek M, Friedman JD. Clinical and scientific considerations in leech therapy for the management of acute venous congestion: An updated review. Ann Plast Surg. 2000; 45(2):207-212.

Wells MD, Ralp T, Manktelow RT, Boyd JE. Bowen V. The medical leech: an old treatment revisited, Microsurg. 1993; 14: 183–6.

Wenning A, Cahill MA. Structural and ultrastructural features of the inlet and outlet regions of the urinary bladders of the leech, Hirudo medicinalis L. J Morphol. 1989; 201:285-291.

Wenning A, Zerbst-Boroffka I, Bazin B. Water and salt excretion in the leech. J Comp Physiol. 1980; 139:97-102.

Whitaker IS, Izadi D, Oliver DW, Monteath G, Butler PE. *Hirudo medicinalis* and the plastic surgeon. Br J Plast Surg. 2004; 57(4):348-353.

Whitman CO. The Leeches of Japan. Q. J. Microsc. Sci. 1886; 26(103):317– 416.

Wilmer A, Slater K, Yip J, Carr N, Grant J. The role of leech water sampling in choice of prophylactic antibiotics in medical leech therapy. Microsurg. 2013; 33(4):301-304.

Zaidi SM, Jameel SS, Zaman F, Jilani S, Sultana A, Khan SA. A systematic overview of the medicinal importance of sanguivorous leeches. Altern Med Rev. 2011; 16(1):59-65.

Zavalova LL, Artamonova II, Berezhnoy SN, *et al*. Multiple forms of medicinal leech destabilase-lysozyme. Biochem Biophys Res Commun. 2003; 306:318-323.

Zavalova LL, Yudina TG, Artamonova II, Baskova IP. Antibacterial non-glycosidase activity of invertebrate destabilase-lysozyme and of its helical amphipathic peptides. Chemotherapy. 2006; 52:158-160.

ACKNOWLEDGMENTS

Author contributions: HA and FÇ reviewed the literature about the topic, and both are participated in drafting and revising the manuscript. FÇ: Fatma Çoruk, HA: Hüseyin Ayhan.

Financial Disclosure: The authors declared that this study has received no financial support.

Conflict of Interests: The authors declared that there is no conflict of interests.

Additional information: All authors have read and agreed to the published version of the manuscript Correspondence and requests for materials should be addressed to HA.

Reprints and permissions information is available at https://dergipark.org.tr/tr/pub/tjvr/policy

Publisher's note Dergipark remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.