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WHAT IS NEED TO BE KNOWN ABOUT MEDICINAL LEECHES AND HIRUDOTHERAPY? : A COMPREHENSIVE REVIEW

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ABSTRACT

Since ancient times humans have used a variety of plants and animals to both prevent and treat ailments. Hirudotherapy is a traditional treatment modality using medicinal leeches. In both traditional and modern medicine, medicinal leeches have been important in the treatment of eczema, chronic ulcers, psoriasis, osteoarthritis, thrombosis, and hypertension. Hirudotherapy is a trustworthy, easy-to-use, helpful, and affordable treatment option for draining blood from deeper tissues in localized venous congestion related with surgical replantations and flap reconstructions. Hirudotherapy has also been commonly used to treat soft tissue oedema and haematomas in trauma situations. Throughout the feeding process, leeches secrete biologically and pharmacologically active components into the wound. This article aims to present a broad assessment of the potential use of hirudotherapy, indications, contraindications, and therapy-related complications. Providing detailed evidence-based information about the usage areas of hirudotherapy will help spread healthy medicinal leech practices and prevent possible complications, thus infectious diseases and public health problems.

Key Words; Medicinal leech, hirudotherapy, indication, contraindication, complication.

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Introduction

Humans have used plants and animals to prevent and treat diseases since prehistoric times (1, 2). Medicinal leeches have played an essential role in traditional and modern medicine (3, 4). The oldest legibly documented record of leeches used for therapeutic purpose was revealed in a painting of an Egyptian Tomb around 1500 BC. The odyssey of the hirudotherapy reached its peak in 17th and 18th centuries AD in Europe, while in the Arab era leeches were used only for bloodletting. (5).

Leeches are used not only in medicine but also in the production of drugs and cosmetics, as well as in veterinary medicine (3, 6, 7, 8). Revealing the biodiversity of their habitat by analysing the blood they sucked and, providing the opportunity to determine the trophic levels of the wetlands they inhabit thanks to their partial indicator properties are some of the not very well-known functions of medicinal leeches in addition to the health field (9, 10).

Today, more than 800 leech species have been described. However, very few of them function as "medicinal leeches" (11). Leech species that are frequently used in medicine and subject to international trade are in the genus *Hirudo* (12, 13). A total of seven species (*Hirudo medicinalis*, *Hirudo verbana*, *Hirudo orientalis*, *Hirudo troctina*, *Hirudo nipponia*, *Hirudo sulukii* and *Hirudo tianjinensis*) have been described in the genus *Hirudo*. Although there is a

suggestion that the leeches in the genus *Hirudo* in Asian geography should be classified under the genus *Hirudinaria*, according to the scientific reports and papers, leeches in the genus *Hirudo* are distributed in Europe, Asia, and North Africa (14-17). Leeches belonging to the genus *Macrobdella* are found in North America), *Richardsonianus* in Australia, *Whitmania* and *Hirudinaria* in Asia (4, 18, 19). Medicinal leeches live in freshwater wetlands, i.e., shallow water rich in aquatic plants (20, 21). They feed by sucking the blood of mammals, fish, amphibians, and birds (10, 22, 23).

Two medicinal leech species (*H. verbana* and *H. sulukii*) are prevalent in Turkish wetlands (16) (Figure 1). While *H. verbana* has a wide distribution in the Mediterranean and steppe zones from Western Europe to Türkiye and Uzbekistan (24), *H. sulukii* lives in the Southeastern Anatolia region of Türkiye (16, 25).

Although leeches have been used in traditional medicine since ancient times, scientific proof of the effectiveness of the active ingredients they secrete has made medicinal leeches one of the complementary treatment modalities of modern medicine (26).



Figure 1. Dorsal view of the medicinal leeches, *Hirudo sulukii* (above) and *H. verbana* (bottom), distributed in wetlands of Türkiye (Scale: 10 mm).

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The approval of the US Food and Drug Administration for the use of leeches in plastic and reconstructive surgery in 2004, and the approval of leech therapy in the treatment of certain diseases with the Regulation on Traditional and Complementary Medicine Practices (abbr: GETAT) by the Ministry of Health of Türkiye in 2014, set an actual threshold in modern medicine in terms of leeches (27-29). Authorized institutions that use leech therapy is responsible for the increase demand for leeches and this to over-collection, illegal utilization, and bio-smuggling attempts, which pose severe threats to the sustainability of the leech populations (10, 12, 21). In addition to the increasing demand for leeches, degradation of natural habitats and reduction of pastoral farming, an essential source of food for leeches, have led to a significant decline in leech populations (30-32). This situation has caused the medicinal leeches to become endangered and paved the way for them to be protected by international conventions, such as IUCN, CITES, Berne convention, and EU Habitat Directive (24, 33, 34). The international trade of medicinal leeches is conducted under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (12, 33). Medicinal leeches, the subject of the international leech trade, are mainly collected from Türkiye and to a lesser extent from the Krasnodar Territory in Russia (24). Although Türkiye is not yet in a leading position regarding the production of leeches under aquacultural conditions, it is the leading country in the international trade of leeches collected from the field? (24, 35). The leech export quota of Türkiye, which was 10,000 kg/year in 1996, has however gradually decreased due to the diminishing of medicinal leech populations in nature and was approximately 1,500 kg in 2022 (13, 36).

The increasing demand for leeches cannot be met adequately by either aquaculture facilities or nature. Therefore, leech prices are increasing unpredictably. The leech price has risen from 0.75 Turkish Liras/each (~USD 0.40) (year: 2013) to 25 Turkish Liras/each (~USD 1.30) (year: 2022) with the effect of the regulation of GETAT in Türkiye (13).

Due to the risk of pathogen transmission by leeches collected from nature, the law of GETAT approves using leeches only from leech farms that produce

sterile leeches (36). There are 11 licensed medicinal leech farm having a total of 7432 kg project capacity in Türkiye (37). Physicians are obliged to know the sterility status of leeches. The risk of pathogen transmission in leeches collected from nature, decreasing leech populations, and increasing leech prices have made medicinal leech breeding important (38, 39).

however, the fact that the breeding protocols are not well-known makes leech breeding unprofitable and cause the leech demand to be insufficiently met (29, 40). This again increases the possibility that the leeches used in hirudotherapy are those collected from nature. The present paper aims to summarize the morphology, biology, epidemiology of leeches, the active ingredients included in their saliva, the indications, contraindications, and complications known in hirudotherapy.

Function Of Leeches

Medicinal leeches, with their three-sided jaws and between 60 to 100 sharp teeth in each jaw, bite the skin, inject their saliva during blood feeding. Adult leeches can ingest blood up to ten times of their weight in a single meal (41).

It is shown that each leech in vitro can prevent between 50 to 100 mL of human blood from coagulating via their saliva (42).

Treatment effects of medicinal leeches depend on the amount of active components such as hirudin, hyaluronidase, histamine-like vasodilators, collagenase, and analgesic compounds that allow the blood to flow during the feeding and the post-attachment period (Table 1.) (26, 41).

Today, hirudin is produced in vitro by using recombinant DNA technology and is sold in the market as Lepirudin and Desirudin (43).

Mechanisms of action	Active components
Analgesic and anti-inflammatory effect	Antistasin (44-46), hirustasin (44, 45), ghilantens (47, 48), eglin C (45), leech-derived tryptase inhibitor (LDTI) (49), complement C1 inhibitor (50), guamerin and piguamerin (44, 45), carboxypeptidase inhibitor (45), bdellins and bdellastasin (45, 47).
Degrading extracellular matrix	Hyaluronidase and collagenase (44, 45, 51).
Strengthening blood flow	Acetylcholine (44, 45), histamine-like molecules (44, 45, 52).
Inhibition of platelet function	Saratin (44, 45, 51, 53), calin (44, 45, 51, 54), apyrase (44, 45, 51), decorsin (44, 45, 51, 55, 56).
Anti-coagulant effect	Hirudin (45, 51, 52, 57), gelin (45, 51), factor Xa inhibitor (44, 45, 51), destabilase (45, 51, 58-60), new leech protein-1, whitide, and whitmanin (60).
Anti-microbial effect	Destabilase (45, 51, 58-19, 61), chloromycetyn (44, 45, 51), theromacin, theromyzin, and peptide B (62, 63).

Table 1. Possible bioactive enzymes and mechanisms of action of leech saliva

The Principles of Medicinal Leech Application

Before beginning the treatment; the patient needs to be informed about both benefits and the possible risks of the treatment. Patients, their parents, or legal guardian should sign a written informed consent. As they have the potential to decrease immune response, cause immune suppression, increase the risk of excess bleeding, cause coagulopathy or prolong the prothrombin time, patients should not be treated concomitantly with vitamin E, aspirin, nonsteroidal anti-inflammatory drugs, heparin, warfarin, as well as herbal supplements like Ginkgo biloba, ginger, garlic, dong quai, and ginseng (64).

In order to reduce the risk of vasoconstriction, caffeine usage should be avoided. The leech saliva has an antithrombotic activity, which is also known with the use of heparin, aspirin, and/or dextran. A physician's order regarding the number of leeches to be applied, application frequency and site is mandatory. It is advised to prescribe antibiotic prophylaxis if leech are applied close to open wounds. (64-66).

Depending on the ailment and its severity the number of leeches to be applied and application

frequency may differ. Even though it is recommended to use a higher number, the number of leeches usually used is between 1 and 10. Two or more treatments per day might be necessary. The number of daily sessions depend from the amount of bleeding at the application site of the previous sessions. In case that leeches are not fully fed or they detach right after attachment, it is recommended to apply a higher number of leeches several times per day. According to the Iowa Head and Neck Protocol, leeches are applied every 2 hours (67, 68).

It is necessary to clean the application site before the treatment using normal sterile saline and soft soap, while any residue ointments or gels on the skin should be removed before the application, as they can affect the attachment and blood-feeding of the leech. Hirudotherapy should be given in a warm room, as the leeches feed better when the skin temperatures are between 33 °C to 40°C.

Leeches normally begin feeding immediately, but if that does not happen spontaneously, in case of vascular insufficiency the skin could be punctured with a sterile needle as the snoozing blood can stimulate them to attach. Leeches should be

observed during the attachment period, and if their size does not increase and no gut peristalsis can be observed, new leeches should be applied or the application site should be changed. The use of active and large leeches might be advisable (27, 64, 69).

Hirudotherapy sessions might vary between 20 and 120 minutes. Each leech can ingest between 5 and 15 mL of blood per session. Bleeding may continue whoever for up to 24 hours after the removal of leeches (67, 70).

Continuous monitoring of blood pressure, heart and respiratory rate, by a professional healthcare provider is necessary during hirudotherapy. Every 4 hours, patients need to be reevaluated hematologically by performing complete blood cell count, serum chemistry studies, prothrombin time, and partial thromboplastin time. If necessary patients should receive blood transfusions, when the hemoglobin level drops below 8g/dL (27, 64, 69-71).

It is possible that leeches can migrate to lesions or other healthy tissues or fall into adjacent area both during and after the treatment, as they are keen on hiding after feeding. Therefore, observation is necessary for also that reason. The leeches usually get fed enough and detached spontaneously. When the leech needs to be removed before being done feeding, if the leech gets forced to detach, it's teeth may be remainder, which may cause an infection. In this case, manipulating the leech's head using a swab saturated with vinegar, normal saline or alcohol usually induce detachment but disproportionate usage of these liquids might also cause blood to flow back into the bite site and lead to an infection, such as submucosal abscesses, erysipelas, ecchymosis and scarring (64, 70, 72).

To prevent bleeding, the bite areas can be cleaned regularly using a swab saturated with isotonic or heparin solution after detaching the leeches. It is possible to be seen that fluids are leaking from the leeches, as they drain the excess water to condense the red blood cells. The leeches killed with 70% ethyl alcohol, after putting into a biological waste bag when they are finished feeding and being

detached naturally (27, 65, 73).

Leeches should not be used on another patient as they carry the risk of blood-borne infections, and also, they would not like to feed again for a few weeks. Infection is one of the main complications that is documented, due to hirudotherapy, with an incidence range from 2.4 to 20%. Hence, prescription of prophylactic antibiotics is suggested strongly, and appropriate antibiotic is suggested strongly, and appropriate antibiotic prophylaxis showed a major reduction in the risk of infection, duration of hospital stay, and potential loss of flap or wounded tissues in some studies (64, 69-71).

Usage of dual antibiotics during the treatment and the use of a single antibiotic during the two weeks of follow-up have also been suggested for prophylaxis (64, 74, 75).

Fluoroquinolones are usually used for prophylaxis, as *Aeromonas hydrophilia* is sensitive to them. Nevertheless, it has been reported that resistant bacteria strains can cause post-leech therapy complications (74-76).

Bacteria such as *Morganella morganii*, *Proteus vulgaris*, *Serratia marcescens*, *Aeromonas sobria*, and *Vibrio fluvialis* were detected on the surface and/or inside the leeches. In case fluoroquinolones do not prevent an infection, appropriate cultures should be collected and third-generation cephalosporins, tetracyclines, aminoglycosides, or trimethoprim should be used (64, 71, 76, 77).

Medicinal Uses of Leeches

Contraindications, indications, and therapy-related complications of hirudotherapy are shown in Table 2-4 (44, 51, 64, 70, 73, 78-84).

Contraindication	
Children	Hematological malignancies (e.g. leukaemia)
Pregnancy and lactation	Haemophilia
Unstable medical status	Anaemia
Sepsis	Hemorrhagic diathesis
Arterial insufficiency	History of allergy to leeches
Hypotension	Severe allergic diathesis
Hepatobiliary diseases	Disposition to keloid scar formation
Cachexia	HIV-infection
Patient refusal to undergo hirudotherapy (individual intolerance to leeches) and to accept blood transfusions if necessary	In those using anti-coagulants, immunosuppressants, and some vasoactive substitutes such as Ginkgo biloba

Table 2. Contraindications of hirudotherapy

Indication	Field of Application
Following reconstructive surgeries	To reduce venous congestion in flap tissues, free and pedicled flaps (6, 70, 79, 85-101).
Phlebitis and thrombosis	To draw blood from deeper layers in varicose veins and to dissolve thrombus in postphlebotic syndrome and ecchymosis (79, 99, 102, 103).
Peripheral arterial diseases	To restore blood flow in the affected areas (e.g. peripheral arterial occlusion) (80, 99, 104).
Post-operative blood clotting	To help re-attachment of severed fingers (amputated digits), toes, ears, nasal tips and penile replantation (45, 79, 86, 90, 105-115)
Coronary arterial thrombosis and ischemic heart disease	To reduce the blood viscosity (79, 85).
Wound healing	To suck the excessive blood, reduce tissue swelling, and contribute to healing by allowing oxygen-rich blood to reach the area until normal circulation can be restored (116, 117).
Swelling and haematoma	To reduce the swelling in excessive periorbital and lingual swelling, sublingual and massive lingual haematoma, macroglossia (85, 88, 118-129).
Analgesic and anti-inflammatory purposes	To relieve the pain and reduce inflammation in varicose veins, leg ulcers, phlebitis, thrombophlebitis, joint diseases like osteoarthritis and epicondylitis, vertebrogenic pain syndromes/lower back pain, lumbar pain in renal cell carcinoma and leiomyosarcoma (6, 78, 79, 103, 130-146).
Chronic skin diseases	Psoriasis, eczematous dermatitis, chronic ulcers, epidermoid cysts, cutaneous leishmaniasis. (79, 85, 147-149)
Respiratory diseases	To use the advantage of the mucolytic action of leech saliva for asthma, acute rhino pharyngitis, and spasmodic coryza (150).
Dentistry and gum diseases	To reduce bacterial growth and inflammation in abscess and also in root canal treatment (41, 85, 151, 152)
Compartment syndrome	To reduce pressure on the neurovascular structures (6, 127, 153).
Sialoadenitis	To reduce inflammation and swelling (6).
Cancer diseases	To provide anti-metastatic activity by <i>Haementaria officinalis</i> in lung cancer and anti-proliferative activity by <i>Hirudinaria manillensis</i> in vitro against small cell lung cancer and to reduce penoserotral oedema in prostate adenocarcinoma (80, 85, 154).
Other less commonly uses	Hypertension, migraines, complications of diabetes mellitus, proliferative vitreous retinopathy, acute and chronic otitis, tinnitus, tympanophonia, peripheral cochleavestibular disorders of vascular origin, nasal polip, spasmodic urethral stricture (85, 155-159).

Table 3. Indications of hirudotherapy

Complication	
Infection	Flap necrosis, extensive muscular necrosis, cellulitis, septic shock (160-166).
Bleeding	Periorbital ecchymosis and subconjunctival hemorrhage (167).
Allergy	Kounis syndrome (168).
Migration	Accidentally penetration in the eye (26, 78).
Other	Thrombotic microangiopathy and acute renal failure (169), lichen planus (170), cutaneous pseudolymphoma (171), atraumatic hemarthrosis (172).

Table 4. Therapy-related complications of hirudotherapy

Discussion

Hirudotherapy is a trustworthy, easy-to-use, helpful, and affordable treatment option for draining blood from deeper tissues in localized venous congestion related with surgical replantations and flap reconstructions. Hirudotherapy can be used in skin diseases such as eczema, chronic ulcers, and psoriasis, as an adjuvant treatment for atherosclerotic heart disorders, coronary thrombosis, hypertension, and varicose veins. In cases of trauma, hirudotherapy has also been used to treat hematomas and swollen soft tissues (41).

Purchasing leeches from reliable commercial sources can reduce the risk of infectious complications and prevent blood-borne infections such as hepatitis viruses (173).

Hirudotherapy has an indication for venous congestion but is contraindicated for arterial insufficiency. Therefore, it must be differentiated cautiously before starting the treatment (64, 108).

Although mechanisms of action and active components still await further exploration, their fields of use in particular medical conditions is clear. Indications and possible complications, also efficient antibiotic prophylaxis against *Aeromonas* and application frequency should be assessed carefully (174).

Conclusion

Hirudotherapy is a precious traditional technique with potent biochemical effects. It can be a crucial part of multidisciplinary approach for patients who do not respond to conventional treatment methods, although it is not a stand-alone treatment method.

Hirudotherapy is becoming more and more popular, and as a result, medicinal leech populations in nature are under tremendous collection pressure, possibly to the point of extinction.

Hirudotherapy practices should be legislated by health authorities.

Providing detailed evidence-based information about the usage areas of hirudotherapy will help spread healthy medicinal leech practices and prevent possible complications, thus infectious diseases and public health problems.

REFERENCES

- 1- Yuan, H., Ma, Q., Ye, L., & Piao, G. (2016). The traditional medicine and modern medicine from natural products. *Molecules*, 21(5), 559.
- 2- Miranda, J. J. M. (2021). Medicinal plants and their traditional uses in different locations. In *Phytomedicine* (pp. 207-223). Academic Press.
- 3- Whitaker, I. S., Rao, J., Izadi, D., & Butler, P. E. (2004). Historical Article: *Hirudo medicinalis*: ancient origins of, and trends in the use of medicinal leeches throughout history. *British Journal of Oral and Maxillofacial Surgery*, 42(2), 133-137.
- 4- Elliott, J. M., & Kutschera, U. (2011). Medicinal leeches: historical use, ecology, genetics and conservation. *Freshwater Reviews*, 4(1), 21-41.
- 5- Munshi, Y., Ara, I., Rafique, H., & Ahmad, Z. (2008). Leeching in the history--a review. *Pakistan journal of biological sciences : PJBBS*, 11(13), 1650-1653.
- 6- Buote, N. J. (2014). The use of medical leeches for venous congestion. *Veterinary and Comparative Orthopaedics and Traumatology*, 27(03), 173-178.
- 7- Sobczak, N., & Kantyka, M. (2014). Hirudotherapy in veterinary medicine. *Annals of Parasitology*, 60(2), 89-92.
- 8- Bergh, A., Lund, I., Boström, A., Hyytiäinen, H., Asplund, K. (2021). A Systematic Review of Complementary and Alternative Veterinary Medicine: "Miscellaneous Therapies". *Animals*, 11(12), 3356.
- 9- Kazancı, N., Ekingen, P., Dügel, M., & Türkmen, G. (2015). Hirudinea (Annelida) species and their ecological preferences in some running waters and lakes. *International Journal of Environmental Science and Technology*, 12(3), 1087-1096.
- 10- Williams, K. M., Barkdull, M., Fahmy, M., Hekkala, E., Siddall, M. E., & Kvist, S. (2020). Caught red handed: iDNA points to wild source for CITES-protected contraband leeches. *European Journal of Wildlife Research*, 66(5), 1-10.
- 11- Kvist, S., Min, G. S., & Siddall, M. E. (2013). Diversity and selective pressures of anticoagulants in three medicinal leeches (Hirudinida: Hirudinidae, Macrobdellidae). *Ecology and Evolution*, 3(4), 918-933.
- 12- Sağlam, N. (2011). Protection and sustainability, exportation of some species of medicinal leeches (*Hirudo medicinalis* L., 1758 and *Hirudo verbana* Carena, 1820). *Journal of FisheriesSciences.com*, 5(1), 1-15.
- 13- Ceylan, M., & Çetinkaya, O. (2017). Investigation on the collection and economy of medicinal leeches from wetlands around Lake Eğirdir, Turkey. *Türkiye Parazitoloji Dergisi*, 41(2), 96.
- 14- Utevsky, S. Y., & Trontelj, P. (2005). A new species of the medicinal leech (Oligochaeta, Hirudinida, *Hirudo*) from Transcaucasia and an identification key for the genus *Hirudo*. *Parasitology Research*, 98(1), 61-66.
- 15- Sket, B., & Trontelj, P. (2007). Global diversity of leeches (Hirudinea) in freshwater. *Hydrobiologia*, 595, 129-137
- 16- Sağlam, N., Saunders, R., Lang, S. A., & Shain, D. H. (2016). A new species of *Hirudo* (Annelida: Hirudinidae): historical biogeography of Eurasian medicinal leeches. *BMC Zoology*, 1(1), 1-12.
- 17- Wang, H., Meng, F. M., Jin, S. J., Gao, J. W., Tong, X. R., & Liu, Z. C. (2022). A new species of medicinal leech in the genus *Hirudo* Linnaeus, 1758 (Hirudiniformes, Hirudinidae) from Tianjin City, China. *ZooKeys*, (1095).
- 18- Phillips, A. J., & Siddall, M. E. (2009). Poly-paraphyly of Hirudinidae: many lineages of medicinal leeches. *BMC evolutionary biology*, 9(1), 1-11.
- 19- Zavalova L.L., Artamonova I.I., Berezhnoy S.N. Multiple forms of medicinal leech destabilase-lysozyme. *Biochem Biophys Res Commun*. 2003;306:318-323.
- 20- Sawyer, R. T. (1986). *Leech Biology and Behavior*, Vol: I, II, III. New York.
- 21- Ceylan, M., & Çetinkaya, O. (2021). Size and structure of the Mediterranean medicinal leech, *Hirudo verbana* populations inhabiting wetlands around Lake Eğirdir, Turkey. *Ege Journal of Fisheries and Aquatic Sciences*, 38(4), 437-447.
- 22- Neubert, E., & Neseemann, H., (1999). *Annelida, Clitellata: branchiobdellida, Acanthobdellea, Hirudinea*. Spektrum Akademischer Verlag, Berlin.
- 23- Ceylan, M., Çetinkaya, O., & Kvist, S. (2021a). Function of the waterfowl nests as reproduction and living areas for leeches (Annelida: Hirudinea). *Animal Reproduction Science*, 232, 106816.
- 24- Trontelj, P., & Utevsky, S. Y. (2012). Phylogeny and phylogeography of medicinal leeches (genus *Hirudo*): Fast dispersal and shallow genetic structure. *Molecular Phylogenetics and Evolution*, 63(2), 475-485.
- 25- Ceylan, M., Küçükkara, R., Erbatur, İ., Karataş, E., Tunç, M., & Sağlam, N. (2021b). Growth, survival and reproduction of the Turkish medicinal leech, *Hirudo sulukii*. *Invertebrate Reproduction & Development*, 65(1), 57-68.
- 26- Sig, A. K., Guney, M., Guclu, A. U., & Ozmen, E. (2017). Medicinal leech therapy—an overall perspective. *Integrative medicine research*, 6(4), 337-343.
- 27- Mumcuoglu, K. Y. (2014). Recommendations for the use of leeches in reconstructive plastic surgery. *Evidence-Based Complementary and Alternative Medicine*, 2014, 205929.

- 28- Ceylan, M., Çetinkaya, O., Küçükbara, R., & Akçimen, U. (2015). Reproduction efficiency of the medicinal leech *Hirudo verbana* Carena, 1820. Turkish Journal of Fisheries and Aquatic Sciences, 15(3), 411-418.
- 29- Karataş, E., Ceylan, M., & Dernekbaşı, S. (2022). Effects of mammalian blood with different glucose levels on reproduction, growth and survival of the southern medicinal leech, *Hirudo verbana* Carena, 1820. Animal Reproduction Science, 107030.
- 30- Utevsky, S., Zagmajster, M., Ateasov, A., Zinenko, O., Utevska, O., Utevsky, A., & Trontelj, P. (2010). Distribution and status of medicinal leeches (genus *Hirudo*) in the Western Palaearctic: anthropogenic, ecological, or historical effects?. Aquatic Conservation: Marine and Freshwater Ecosystems, 20(2), 198-210.
- 31- Sağlam, N. (2018). The effects of environmental factors on leeches. Advances in Agriculture and Environmental Science, 1(1), 1-3.
- 32- Arias, A., Surugiu, V., Carballeira, R., Popa, O.P., Popa, L.O., Utevsky, S. (2021). Unravelling the extent of diversity within the Iberian medicinal leeches (Hirudinea: *Hirudo*) using molecules and morphology. Biology, 10(4), 315.
- 33- CITES (2022a). Convention on international trade in endangered species of wild fauna and flora: Appendices I, II and III valid from 22 June 2022. Retrieved from <https://cites.org/eng/app/appendices.php> (Accessed on 25.07.2022).
- 34- IUCN (2022). IUCN Red List of Threatened Species. Retrieved from <https://www.iucnredlist.org/search?taxonomies=121982&searchType=species> (Accessed on 25.07.2022).
- 35- CITES (2022b). Convention on the International Trade in Endangered Species of Wild Flora and Fauna Trade Database. Retrieved from <https://trade.cites.org> (Accessed on 25.07.2022).
- 36- Anonymous (2021). Ministry of Agriculture and Forestry of Türkiye, General Directorate of Fisheries and Aquaculture, 2022 export quota announcement for Eel and Medicinal Leech. Retrieved from <https://www.tarimorman.gov.tr/BSGM/Duyuru/148/Yilan-Baligi-Ve-Tibbi-Suluk-Icin-2022-Yili-Ihracat-Kotalari-Belirlenmistir> (Accessed on 25.07.2022).
- 37- BSGM (2022). Aquaculture facilities in Türkiye. Retrieved from <https://www.tarimorman.gov.tr/BSGM/Belgeler/Icerikler/Su%20%C3%9Cr%C3%BCnleri%20Yeti%C5%9Ftiricileri%C4%9Fi/Su-Urunleri-Tesisleri-2019.pdf> (Accessed on 21.12.2022).
- 38- Malek, M., Jafarifar, F., Roohi Aminjan, A., Salehi, H., & Parsa, H. (2019). Culture of a new medicinal leech: growth, survival and reproduction of *Hirudo orientalis* Utevsky and Trontelj, 2005 under laboratory conditions. Journal of natural history, 53(11-12), 627-637.
- 39- Manav, M., Ceylan, M., & Büyükçapar, H. M. (2019). Investigation of reproductive efficiency, growth performance and survival of the southern medicinal leech, *Hirudo verbana* Carena, 1820 fed with mammalian and poultry blood. Animal reproduction science, 206, 27-37.
- 40- Ceylan, M. (2020). Effects of maternal age on reproductive performance of the southern medicinal leech, *Hirudo verbana* Carena, 1820. Animal reproduction science, 218, 106507.
- 41- Jha, K., Garg, A., Narang, R., & Das, S. (2015). Hirudotherapy in Medicine and Dentistry. *Journal of clinical and diagnostic research : JCDR*, 9(12), ZE05–ZE7.
- 42- HELDT T. J. (1961). Allergy to leeches. *Henry Ford Hospital medical bulletin*, 9, 498–519.
- 43- Massart, D., Sohawon, S., & Noordally, O. (2009). Les sangsues [Medicinal leeches]. *Revue medicale de Bruxelles*, 30(5), 533–536.
- 44- Das B.K. An overview on hirudotherapy/leech therapy. *Ind Res J Pharm Sci*. 2014;1:33–45.
- 45- Zaidi S.M., Jameel S.S., Zaman F., Jilani S., Sultana A., Khan S.A. A systematic overview of the medicinal importance of sanguivorous leeches. *Altern Med Rev*. 2011;16:59–65.
- 46- Nutt E.M., Jain D., Lenny A.B., Schaffer L., Siegl P.K., Dunwiddie C.T. Purification and characterization of recombinant antistasin: a leech-derived inhibitor of coagulation factor Xa. *Arch Biochem Biophys*. 1991;285:37–44.
- 47- Moser M., Auerswald E., Mentele R., Eckerskorn C., Fritz H., Fink E. Bdelastasin, a serine protease inhibitor of the antistasin family from the medical leech (*Hirudo medicinalis*) *Eur J Biochem*. 1998;253:212–220.
- 48- Blankenship D.T., Brankamp R.G., Manley G.D., Cardin A.D. Amino acid sequence of ghilanten: anticoagulant-antimetastatic principle of the South American leech, *Haementeria ghilianii*. *Biochem Biophys Res Commun*. 1990;166:1384–1389.
- 49- Campos I.T., Silva M.M., Azzolini S.S., Souza A.F., Sampaio C.A., Fritz H. Evaluation of phage display system and leech-derived trypsin inhibitor as a tool for understanding the serine proteinase specificities. *Arch Biochem Biophys*. 2004;425:87–94.
- 50- Baskova I.P., Zavalova L.L. Proteinase inhibitors from the medicinal leech *Hirudo medicinalis*. *Biochemistry (Mosc)* 2001;66:703–714.
- 51- Abdullah S., Dar L.M., Rashid A., Tewari A. Hirudotherapy/leech therapy: applications and indications in surgery. *Arch Clin Exp Surg*. 2012;1:172–180.
- 52- 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.
- 53- Gronwald W., Bomke J., Maurer T. Structure of the leech protein saratin and characterization of its binding to collagen. *J Mol Biol*. 2008;381:913–927

- 54- Depraetere H., Kerekes A., Deckmyn H. The collagen-binding leech products rLAPP and calin prevent both von Willebrand factor and $\alpha 2\beta 1$ (GPIIb/IIIa)-I-domain binding to collagen in a different manner. *Thromb Haemost.* 1999;82:1160–1163.
- 55- Krezel A.M., Wagner G., Seymour-Ulmer J., Lazarus R.A. Structure of the RGD protein decorsin: conserved motif and distinct function in leech proteins that affect blood clotting. *Science.* 1994;264:1944–1948.
- 56- Seymour J.L., Henzel W.J., Nevins B.E.T.A.L., Stults J.T., Lazarus R.A. A potent glycoprotein IIb-IIIa antagonist and platelet aggregation inhibitor from the leech *Macrobodella decora*. *J Biosoc Sci.* 1990;265:10143–10147.
- 57- Clarke C.E.W. Medical therapeutics derived from leeches (Phy. Annelida; Cl. Hirudinea) *MacEwan University Student eJournal.* 2016;3(1)
- 58- Baskova I.P., Zavalova L.L., Basanova A.V., Sass A.V. Separation of monomerizing and lysozyme activities of destabilase from medicinal leech salivary gland secretion. *Biochemistry (Mosc)* 2001;66:1368–1373.
- 59- Zavalova L.L., Yudina T.G., Artamonova I.I., Baskova I.P. Antibacterial non-glycosidase activity of invertebrate destabilase-lysozyme and of its helical amphipathic peptides. *Chemotherapy.* 2006;52:158–160.
- 60- Dong H., Ren J.X., Wang J.J. Chinese medicinal leech: ethnopharmacology, phytochemistry, and pharmacological activities. *J Evid Based Complementary Altern Med.* 2016;2016:7895935.
- 61- Zavalova LL, Baskova IP, Lukyanov SA, Sass AV, Snezhkov EV, Akopov SB, Artamonova II, Archipova VS, Nesmeyanov VA, Kozlov DG, Benevolensky SV, Kiseleva VI, Poverenny AM, Sverdlov ED. Destabilase from the medicinal leech is a representative of a novel family of lysozymes. *Biochim Biophys Acta.* 2000; 1478(1): 69–77.
- 62- Tasiemski A., Vandenbulcke F., Mitta G. Molecular characterization of two novel antibacterial peptides inducible upon bacterial challenge in an annelid, the leech *Theromyzon tessulatum*. *J Biol Chem.* 2004;279:30973–30982.
- 63- Tasiemski A. Antimicrobial peptides in annelids. *Invertebrate Surviv J.* 2008;5:75–82.
- 64- Yantis MA, O’Toole KN, Ring P. Leech therapy. *Am J Nursing* 2009; 109: 36-42.
- 65- K. Y. Mumcuoglu, C. Pidhorz, R. Cohen, A. Ofek, and H. A. Lipton, “The use of the medicinal leech, *Hirudo medicinalis*, in the reconstructive plastic surgery,” *The Internet Journal of Plastic Surgery*, vol. 4, no. 2, 2007
- 66- K. Y. Mumcuoglu, L. Huberman, R. Cohen et al., “Elimination of symbiotic *Aeromonas* spp. from the intestinal tract of the medicinal leech, *Hirudo medicinalis*, using ciprofloxacin feeding,” *Clinical Microbiology and Infection*, vol. 16, no. 6, pp. 563– 567, 2010.
- 67- D. B. Chepeha, B. Nussenbaum, C. R. Bradford, and T. N. Teknos, “Leech therapy for patients with surgically unsalvageable venous obstruction after revascularized free tissue transfer,” *Archives of Otolaryngology*, vol. 128, no. 8, pp. 960–965, 2002.
- 68- Leech Therapy - Anticoagulation Protocols, 2019, <https://medicine.uiowa.edu/iowaprotocols/leech-therapy-anticoagulation-protocols> (Accessed on 29.07.2022)
- 69- Green PA, Shafritz AB. Medicinal leech use in microsurgery. *J Hand Surg Am* 2010; 35: 1019-1021
- 70- Mommsen J, Rodriguez-Fernandez J, MateosMicas M, et al. Avulsion of the auricle in an anticoagulated patient: Is leeching contraindicated? A review and a case. *Craniofacial Trauma Reconstr* 2011; 4: 61-68
- 71- Whitaker IS, Oboumarzouk O, Rozen WM, et al. The efficacy of medicinal leeches in plastic and reconstructive surgery: a systematic review of 227 reported clinical cases. *Microsurgery* 2012; 32: 240-250. 8. Nguyen MQ, Crosby MA, Skoracki RJ, et al. Outcomes of flap salvage with medicinal leech therapy. *Microsurgery* 2012; 32: 351-357
- 72- Knobloch K, Gohritz A, Busch K, Spies M, Vogt PM. *Hirudo medicinalis*-Anwendungen in der plastischen und rekonstruktiven Mikrochirurgie--eine Literaturübersicht [*Hirudo medicinalis*-leech applications in plastic and reconstructive microsurgery--a literature review]. *Handchir Mikrochir Plast Chir.* 2007 Apr;39(2):103-7.
- 73- O. S. Gileva and K. Y. Mumcuoglu, “Hirudotherapy,” in *Biotherapy—History, Principles and Practice: A Practical Guide to the Diagnosis and Treatment of Disease Using Living Organisms*, M. Grassberger, R. A. Sherman, O. Gileva, C. M. H. Kim, and K. Y. Mumcuoglu, Eds., pp. 31–76, Springer, Heidelberg, Germany, 2013.
- 74- Giltner CL, Bobenchik AM, Uslan DZ, et al. Ciprofloxacin-resistant *Aeromonas hydrophilia cellulitis* following leech therapy. *J Clin Micro* 2013; 51: 1324-1326. 18.
- 75- Patel KM, Svetska M, Sinkin J, et al. Ciprofloxacin-resistant *Aeromonas hydrophilia* infection following leech therapy: A case report and review of the literature. *J Plast Reconstr Aesthet Surg* 2013; 66: e20–22.
- 76- Bibbo C, Fritsche T, Stemper M, et al. Flap infection associated with medicinal leeches in reconstructive surgery: two new drug-resistant organisms. *J Reconstr Microsurg* 2013; 29: 457-460.
- 77- Irish, J. C., Gullane, P. J., Mulholland, S., & Neligan, P. C. (2000). Medicinal leech in head and neck reconstruction. *The Journal of otolaryngology*, 29(5), 327–332.
- 78- Haycox CL, Odland PB, Coltrera MD, Raagi GJ. Indications and complications of medicinal leech therapy. *J Am Acad Dermatol.* 1995;33(6):1053–55.

- 79- Ahmed T, Anwar M. Clinical importance of leech therapy. *Indian Journal of Traditional knowledge*.2009;8(3):443-45.
- 80- Hildebrandt J.P., Lemke S. Small bite, large impact—saliva and salivary molecules in the medicinal leech, *Hirudo medicinalis*. *Naturwissenschaften*. 2011;98:995–1008.
- 81- Singh A.P. Medicinal leech therapy (hirudotherapy): a brief overview. *Complement Ther Clin Pract*. 2010;16:213–215.
- 82- Whitaker I.S., Cheung C.K., Chahal C.A.A., Karoo R.O.S., Gulati A., Foo I.T.H. By what mechanism do leeches help to salvage ischaemic tissues? A review. *Br J Oral Maxillofac Surg*. 2005;43:155–160.
- 83- Hosnuter M., Demircan N., Unalacak M., Kargi E., Aktunc E., Babuccu O. Modern tıbbin yeniden keşfettiği bir alternatif tedavi metodu: hirudoterapi. *Türk Aile Hek Derg*. 2003;7:177–179.
- 84- Porshinsky B.S., Saha S., Grossman M.D., Beery I.P.R., Stawicki S.P.A. Clinical uses of the medicinal leech: a practical review. *J Postgrad Med*. 2011;57:65–71.
- 85- Abdualkader AM, Ghawi AM, Alaama M, Awang M, Merzouk A. Leech therapeutic applications. *Indian J Pharm Sci*. 2013;75(2):127–37.
- 86- Kreamer BA, Korber KE, Aquino TI, Engleman A. Use of leeches in plastic and reconstructive surgery—a review. *J Reconst Microsurg*. 2000;45:207–12.
- 87- E. Vural and J. M. Key, “Complications, salvage, and enhancement of local flaps in facial reconstruction,” *Otolaryngologic Clinics of North America*, 2001;34(4):739–751.
- 88- Arami, A., Gurevitz, S., Palti, R., Menachem, S., Berelowitz, M., & Yaffe, B. (2018). The Use of Medicinal Leeches for the Treatment of Venous Congestion in Replanted or Revascularized Digits. *The Journal of hand surgery*, 43(10), 949.e1–949.e5.
- 89- Vasei N, Jahangiri K. Leech therapy of nipple-areolar complex (NAC) congestion in reduction mammoplasty: A case report. *Clin Case Rep*. 2021 Oct 28;9(10):e05013.
- 90- Lee, Z. H., Cohen, J. M., Daar, D., Anzai, L., Hacquebord, J., & Thanik, V. (2019). Quantifying outcomes for leech therapy in digit revascularization and replantation. *The Journal of hand surgery, European volume*, 44(4), 414–418.
- 91- Cornejo, A., Shammass, R. L., Poveromo, L. P., Lee, H. J., & Hollenbeck, S. T. (2017). Institutional Outcomes of Leech Therapy for Venous Congestion in 87 Patients. *Journal of reconstructive microsurgery*, 33(9), 612–618.
- 92- Hullett JS, Spinnato GG, Ziccardi V. Treatment of an ear laceration with adjunctive leech therapy: A case report. *J Oral Maxillofac Surg* 2007;65:2112-4.
- 93- Kind GM, Buncke GM, Placik OJ, Jansen DA, D'Amore T, Buncke HJ Jr. Total ear replantation. *Plast Reconstr Surg* 1997;99:1858-67.
- 94- Cho BH, Ahn HB. Microsurgical replantation of a partial ear, with leech therapy. *Ann Plast Surg* 1999;43:427-9.
- 95- Wagenheim, G. N., Au, J., & Gargollo, P. C. (2016). Medicinal Leech Therapy for Glans Penis Congestion After Primary Bladder Exstrophy-Epispadias Repair in an Infant: A Case Report. *Urology*, 87, 193–195.
- 96- Soucacos, P. N., Beris, A. E., Malizos, K. N., Xenakis, T. A., & Georgoulis, A. (1994). Successful treatment of venous congestion in free skin flaps using medical leeches. *Microsurgery*, 15(7), 496–501.
- 97- Frodel, J. L., Jr, Barth, P., & Wagner, J. (2004). Salvage of partial facial soft tissue avulsions with medicinal leeches. *Otolaryngology--head and neck surgery : official journal of American Academy of Otolaryngology-Head and Neck Surgery*, 131(6), 934–939.
- 98- Zamboni, W. A., Lozano, D. D., Vitkus, K., Roth, A. C., Stephenson, L. L., Kenneaster, D., Suchy, H., Russell, R. C., Corcoran, J., & Yen, L. (1999). Single-vessel arteriovenous revascularization of the amputated ear. *Journal of reconstructive microsurgery*, 15(1), 9–13.
- 99- Bicher J, Fritz H. Hirudin, a new therapeutic tool? *Ann Hematol*. 1991; 63(2): 67–76.
- 100- Eldor A, Orevi M, Rigbi M. The role of the leech in medical therapeutics. *Blood Rev*. 1996; 10(4): 201–209.
- 101- O'Toole, G., Bhatti, K., & Masood, S. (2008). Replantation of an avulsed ear, using a single arterial anastomosis. *Journal of plastic, reconstructive & aesthetic surgery : JPRAS*, 61(3), 326–329.
- 102- Bapat RD, Acharya BS, Jukevar S, Dahanukar SA. Leech therapy for complicated varicose veins. *Indian J Med Res*. 1998;107:281–4.
- 103- Zarnigar AA. Clinical efficacy of leech therapy in varicose ulcer—a case series. *Unani Res*. 2011;1:31–8.
- 104- Afify, O., Alkhoury, S., & Lauder, N. (2021). Improving Symptoms of Peripheral Artery Disease With Hirudotherapy. *Cureus*, 13(7), e16270.
- 105- Weinfeld AB, Yuksel E, Boutros S, Gura DH, Friedman JD. Clinical and scientific considerations in leech therapy for management of acute venous congestion- a review. *Ann Plast Surg*. 2000;45:207–12.
- 106- Heinz, P., Tvrđý, P., Pink, R., Dvořák, Z., & Michl, P. (2020). HIRUDOTHERAPY IN RECONSTRUCTIVE SURGERY: CASE-REPORTS AND REVIEW. *Acta chirurgiaplasticae*, 62(3-4), 95–102.

- 107- Arusan, S., Bayar, B., Gödekmerdan, A., & Sağlam, N. (2013). Olgu Sunumu: Mikro Cerrahiye Yardımcı Bir Metot, Hirudoterapi [A case report: hirudotherapy as a treatment modality in the microsurgery]. *Türkiye parazitolojii dergisi*, 37(2), 154–156.
- 108- Pantuck, A. J., Lobis, M. R., Ciocca, R., & Weiss, R. E. (1996). Penile replantation using the leech *Hirudo medicinalis*. *Urology*, 48(6), 953–956.
- 109- Mineo, M., Jolley, T., & Rodriguez, G. (2004). Leech therapy in penile replantation: a case of recurrent penile self-amputation. *Urology*, 63(5), 981–983.
- 110- Bhanganada, K., Chayavatana, T., Pongnumkul, C., Tonmukayakul, A., Sakolsatayadorn, P., Komaratat, K., & Wilde, H. (1983). Surgical management of an epidemic of penile amputations in Siam. *American journal of surgery*, 146(3), 376–382.
- 111- Durrant, C., Townley, W. A., Ramkumar, S., & Khoo, C. T. (2006). Forgotten digital tourniquet: salvage of an ischaemic finger by application of medicinal leeches. *Annals of the Royal College of Surgeons of England*, 88(5), 462–464.
- 112- McCrary B. F. (2007). Hyperbaric oxygen (HBO2) treatment for a failing facial flap. *Postgraduate medical journal*, 83(975), e1.
- 113- Lozano, D. D., Stephenson, L. L., & Zamboni, W. A. (1999). Effect of hyperbaric oxygen and medicinal leeching on survival of axial skin flaps subjected to total venous occlusion. *Plastic and reconstructive surgery*, 104(4), 1029–1032.
- 114- Tuncali, D., Terzioglu, A., Cigsar, B., & Aslan, G. (2004). The value of medical leeches in the treatment of class IIC ring avulsion injuries: report of 2 cases. *The Journal of hand surgery*, 29(5), 943–946.
- 115- de Chalain, T., & Jones, G. (1995). Replantation of the avulsed pinna: 100 percent survival with a single arterial anastomosis and substitution of leeches for a venous anastomosis. *Plastic and reconstructive surgery*, 95(7), 1275–1279.
- 116- Nair, H., Ahmad, N. W., Lee, H. L., Ahmad, N., Othamn, S., Mokhtar, N., & Chong, S. (2020). Hirudotherapy in Wound Healing. *The international journal of lower extremity wounds*, 1534734620948299. Advance online publication.
- 117- Zaidi SM. Unani treatment and leech therapy saved the diabetic foot of a patient from amputation. *Int Wound J*. 2016;13:263–4.
- 118- Smeets MG, Engelberts I. The use of leeches in a case of post-operative life threatening macroglossia. *The Journal of Laryngology and Otology*. 1995;109:442–44.
- 119- Lee NJ, Peckitt NS. Treatment of a sublingual hematoma with medicinal leeches: Report of case. *J Oral Maxillofac Surg* 1996;54:101–3.
- 120- Saah, D., Braverman, I., Elidan, J., & Nageris, B. (1993). Traumatic macroglossia. *The Annals of otology, rhinology, and laryngology*, 102(9), 729–730.
- 121- Baskova I, Nikonov GI. Destabilase, the Novel Epsilon- (Gamma-Glu)-Lys Isopeptide with thrombolytic activity. *Blood Coagul Fibrinolysis*. 1991;2:167–72.
- 122- Menage MJ, Wright G. Use of leeches in case of severe periorbital haematoma. *Br J Ophthalmol*. 1991;75(12):755–6.
- 123- Byrne PJ, Bernstein PE. The use of medicinal leeches to treat macroglossia secondary to blunt trauma. *Otolaryngol Head Neck Surg*. 2001;12:649–50.
- 124- Ramzan M, Droog W, Sleswijk V, van Roessel EW, Meyenaar IA. Leech got your tongue? Haematoma of the tongue treated with medicinal leeches: a case report. *Neth J Crit Care*. 2010;14(4):268–70.
- 125- Isgar B, Turner AG. Large scrotal haematoma treated with medicinal leeches. *Br J Urol*. 1989;64(5):549–50.
- 126- Goessel C, Steffen-Wilcke K, Miller K. Leech therapy for massive scrotal haematoma following percutaneous transluminal angioplasty. *J Urol*. 1997;158(2):545.
- 127- Heckmann JG, Dütsch M, Neundörfer B, Hartung U. Leech therapy in the treatment of median nerve compression due to forearm haematoma. *J Neurol Neurosurg Psychiatry*. 2005;76(10):1465.
- 128- Philip J, Armitage DW, Phillips KR, Parr NJ. Leech therapy for penoscrotal oedema in patients with hormone-refractory prostate carcinoma. *BJU Int*. 2003;91:579–80.
- 129- Makin CA, Edwards L. Application of leeches to reduce swelling after surgery. *Br J Urol*. 1987;59:189.
- 130- Cooper, E. L., & Mologne, N. (2016). Exploiting leech saliva to treat osteoarthritis: A provocative perspective. *Journal of traditional and complementary medicine*, 7(3), 367–369.
- 131- Wang, H., Zhang, J., & Chen, L. (2018). The efficacy and safety of medical leech therapy for osteoarthritis of the knee: A meta-analysis of randomized controlled trials. *International journal of surgery (London, England)*, 54(Pt A), 53–61.
- 132- Bäcker, M., Lütke, R., Afra, D., Cesur, O., Langhorst, J., Fink, M., Bachmann, J., Dobos, G. J., & Michalsen, A. (2011). Effectiveness of leech therapy in chronic lateral epicondylitis: a randomized controlled trial. *The Clinical journal of pain*, 27(5), 442–447.
- 133- Glickman-Simon, R., & Ehrlich, A. (2012). Leeches, creatine, xylitol, spinal manipulation, acupuncture. *Explore (New York, N.Y.)*, 8(3), 206–209.
- 134- Koeppen, D., Aurich, M., & Rampp, T. (2014). Medicinal leech therapy in pain syndromes: a narrative review. *Wiener medizinische Wochenschrift (1946)*, 164(5-6), 95–102.

- 135- Michalsen A, Deuse U, Esch T, Dobos G, Moebus S. Effect of leeches therapy (*Hirudo medicinalis*) in painful osteoarthritis of the knee: a pilot study. *Ann Rheum Dis*. 2001;60:986.
- 136- Michalsen A, Klotz S, Ludtke R, Moebus S, Spahn G, Dobos GJ. Effectiveness of leech therapy in osteoarthritis of knee: a randomized controlled clinical trial. *Ann. Int. Med*. 2003;139:724–30.
- 137- Andereya S, Stanzel S, Maus U, Müller-Rath R, Mumme T, Siebert CH, Stock F, Schneider U. Assessment of leech therapy for knee osteoarthritis. A randomized study. *Acta Orthop*. 2008;79(2):235–43.
- 138- Roos, E. M., & Toksvig-Larsen, S. (2003). Knee injury and Osteoarthritis Outcome Score (KOOS) - validation and comparison to the WOMAC in total knee replacement. *Health and quality of life outcomes*, 1, 17.
- 139- Stange, R., Moser, C., Hopfenmueller, W., Mansmann, U., Buehring, M., & Uehleke, B. (2012). Randomised controlled trial with medical leeches for osteoarthritis of the knee. *Complementary therapies in medicine*, 20(1-2), 1–7.
- 140- Lauche, R., Cramer, H., Langhorst, J., & Dobos, G. (2014). A systematic review and meta-analysis of medical leech therapy for osteoarthritis of the knee. *The Clinical journal of pain*, 30(1), 63–72.
- 141- Abbas Zaidi SMA, Jamil SS, Sultana A, Zaman F, Fuzail M. Safety and efficacy of leeching therapy for symptomatic knee osteoarthritis using Indian medicinal leech. *Indian J Tradit Knowl*. 2009;8(3):437–42.
- 142- Rai PK, Singh AK, Singh OP, Rai NP, Dwivedi AK. Efficacy of leech therapy in the management of osteoarthritis (Sandhivata). *Ayu*. 2011;32(2):213–7.
- 143- Shiffa M, Siddiqui MA, Sultana A, Zaman F, Fahamiya N, Akhtare MU. Comparative clinical evaluation of leech therapy in the treatment of knee osteoarthritis. *Eur J Integr Med*. 2013;5(3):261–269.
- 144- Michalsen A, Lütke R, Cesur O, Afra D, Musial F, Bäcker M, Fink M, Dobos GJ. Effectiveness of leech therapy in women with symptomatic arthrosis of the first carpometacarpal joint: a randomized controlled trial. *Pain*. 2008;137:452–9.
- 145- Hudak PL, Amadio PC, Bombardier C. Development of an upper extremity outcome measure: the DASH (disabilities of the arm, shoulder and hand). The Upper Extremity Collobarative Group (UECG). *Am J Ind Med*. 1996;29:602–8.
- 146- Kalender ME, Comez G, Sevinc A, Dirier A, Camci C. Leech therapy for symptomatic relief of cancer pain. *Pain Med*. 2010;11(3):443–5.
- 147- Rasi A, Faghihi A, Jalali MA, Zamanian A, Ghaffarpour G. Leech therapy for epidermoid cysts and review of the literature. *Adv Biomed Res*. 2014;3:112.
- 148- Hamidzadeh, N., Azizi, A., Zarshenas, M. M., & Ranjbar, S. (2017). Leech therapy in treatment of cutaneous leishmaniasis: a case report. *Journal of integrative medicine*, 15(5), 407–410.
- 149- Rasi, A., Faghihi, A., Jalali, M. A., Zamanian, A., & Ghaffarpour, G. (2014). Leech therapy for epidermoid cysts and review of the literature. *Advanced biomedical research*, 3, 112.
- 150- de Chalain TM. Exploring the use of the Medicinal Leech: A clinical risk-benefit. *J Reconst Microsurg*. 1996;12(3):165–72.
- 151- Thakur I., Reddy B.H.S., Patil S., Rajendra K. Hirudotherapy in dentistry. *Int J Oral Health Sci*. 2016;6:65–69.
- 152- Hyson JM. Leech Therapy: A History. *Journal of the History of Dentistry*. 2005;53(1):25–27.
- 153- Schenker, M., Murray, A., & Kay, S. P. (2006). Leech therapy in the treatment of median nerve compression due to forearm haematoma. *Journal of neurology, neurosurgery, and psychiatry*, 77(6), 799–800.
- 154- Philip, J., Armitage, D. W., Phillips, K. R., & Parr, N. J. (2003). Leech therapy for penoscrotal oedema in patients with hormone-refractory prostate carcinoma. *BJU international*, 91(6), 579–580.
- 155- Cherniack E.P. Bugs as drugs, part two: worms, leeches, scorpions, snails, ticks, centipedes, and spiders. *Altern Med Rev*. 2011;16:50–58.
- 156- Emtiazi, M., Jokar, S. Z., & Sadeghmanesh, R. (2016). Nasal Polyp in Iranian Traditional Medicine & Conventional Medicine. *Iranian journal of medical sciences*, 41(3 Suppl), S44.
- 157- Nagler, M., & Wuillemin, W. A. (2011). Leeching as a substitute for phlebotomy. *British journal of haematology*, 153(4), 420.
- 158- Huang H, Lei R, Li Y, Huang Q, Gao N, Zou W. *Hirudo* (Leech) for proliferative vitreous retinopathy: A protocol for systemic review and meta-analysis. *Medicine (Baltimore)*. 2021 Jan 22;100(3):e24412.
- 159- Price R. (1822). Case of Retention of Urine, Produced by Spasmodic Stricture, Effectually Relieved by Leeching. *The London medical and physical journal*, 48(286), 482–483.
- 160- Kılıç, M., & Ak, R. (2019). Orbital Cellulitis Due To Leech Therapy. *Bulletin of emergency and trauma*, 7(3), 335–336.
- 161- Levine, S. M., Frangos, S. G., Hanna, B., Colen, K., & Levine, J. P. (2010). *Aeromonas* septicemia after medicinal leech use following replantation of severed digits. *American journal of critical care : an official publication, American Association of Critical-Care Nurses*, 19(5), 469–471.

- 162- Whitlock MR, O'Hare PM, Sanders R, et al. The medicinal leech and its use in plastic surgery: a possible cause for infection. *Br J Plast Surg*. 1983;36:240–44.
- 163- Eroglu C, Hokelek M, Guneren E, Esen S, Pekbay A, Uysal OA. Bacterial flora of *Hirudo medicinalis* and their antibiotic sensitivities in the Middle Black Sea Region, Turkey. *Ann Plast Surg* 2001;47:70-3.
- 164- Ardehali, B., Hand, K., Nduka, C., Holmes, A., & Wood, S. (2006). Delayed leech-borne infection with *Aeromonas hydrophilia* in escharotic flap wound. *Journal of plastic, reconstructive & aesthetic surgery : JPRAS*, 59(1), 94–95.
- 165- Mory RN, Mindell D, Bloom DA. The leech and the physician: Biology, etymology, and medical practice with *Hirudinea medicinalis*. *World J Surg* 2000;24:878-83. 51.
- 166- Varghese MR, Farr RW, Wax MK, Chafin BJ, Owens RM. *Vibrio fluvialis* wound infection associated with medicinal leech therapy. *Clin Infect Dis* 1996;22:709-10.
- 167- Sevimli, N., Karadag, R., & Karadag, A. S. (2021). Periorbital ecchymosis and subconjunctival hemorrhage due to leech therapy for headache. *Arquivos brasileiros de oftalmologia*, 84(2), 183–185.
- 168- Çakmak, T., Çaltekin, İ., Gökçen, E., Savrun, A., & Yaşar, E. (2018). Kounis syndrome due to hirudotherapy (leech therapy) in emergency department; a case report. *Turkish journal of emergency medicine*, 18(2), 85–87.
- 169- Etemadi, J., Ardalan, M. R., Motavali, R., Tubbs, R. S., & Shoja, M. M. (2008). Thrombotic microangiopathy as a complication of medicinal leech therapy. *Southern medical journal*, 101(8), 845–847.
- 170- Daye, M., Işık, B., & Kılınc, F. (2021). Lichen Planus Due to Hirudotherapy. *Hirudoterapiye Bağlı Liken Planus. Türkiye parazitoloji dergisi*, 45(2), 149–152.
- 171- Temiz, S. A., Özer, İ., Ataseven, A., Dursun, R., & Fındık, S. (2019). Cutaneous Pseudolymphoma Due to Hirudotherapy. *Türkiye parazitoloji dergisi*, 43(1), 50–52.
- 172- Curcio, J., & Lloyd, C. M. (2020). Leech Me Alone! Atraumatic Hemarthrosis after Hirudotherapy. *Cureus*, 12(2), e6915.
- 173- Wilken, G. B., & Appleton, C. C. (1993). The persistence of hepatitis B antigen in the bloodmeal of the potential medicinal leech, *Asiaticobdella buntionensis*. *South African medical journal = Suid-Afrikaanse tydskrif vir geneeskunde*, 83(3), 193–195.
- 174- Porshinski BS, Saha S, Grossman MD, Beery P, Stawicki S. Clinical uses of the medicinal leech: a practical review. *J Postgrad Med*. 2011;57:65–71.