Medical Journal of Ahl al-Bayt University, Vol.3, No.1, Aug.2024

A Critical Review of Hirudo medicinalis and Historical Aspects of Leech Therapy

Luma Jasim Witwit [1 *], Liqaa Oday Ali [1], Ahmed Mohamed Abbas [1]

Department of Microbiology, College of Dentistry, University of Babylon[1].

ABSTRACT

Both the practice of bloodletting and the use of *Hirduo medicinalis* for medicinal purposes have roots in ancient Egypt and the dawn of civilization. The years have seen fluctuations in their popularity. *Hirudo medicinalis*, often known as medicinal leeches, are used therapeutically in hiruditherapy (HT), a traditional method of treating inflammatory illnesses and pain. Leeches therapy is currently receiving more attention in the supplementary medicine community. These are a class of segmented invertebrates that are employed by medical professionals in phlebotomy to treat a variety of illnesses. They are well recognized for being sanguinivorous blood feeders. Here, the affected areas are connected to cultured leeches, which bite without pain at first before draining blood.

Over the past few years, the four main German dealers have seen a steady growth in sales, which has resulted in an estimated 70,000 treatments (about 350,000 leeches sold per year, four to five of which are used for each treatment) that takes place in Germany. The majority of these therapeutic advantages are attributed to the saliva's numerous bioactive chemicals, including hyaluronidase, hirudin, calin, destabilase, apyrase, glin, and many others, rather than the blood that was drawn during the biting.

Reducing pain is the main objective of treatment for localized pain syndromes, especially for disorders involving the knee, such as osteoarthritis. Heart difficulties, varicose veins, hemorrhoids, gastrointestinal disorders, dermatitis, gynecological abnormalities, reconstructive surgery, and trauma situations such as the reattachment of amputated fingers, toes, and ears are among the ailments for which it is used. In order to keep blood flowing through clogged skin flaps, plastic surgery is also part of it. Recently, new applications for it have been found in the treatment of diabetes, hypersensitivity illnesses like asthma, cancer, and infertility in both men and women. Researchers are continuing to extract and synthesize a growing number of novel salivary chemicals for use in clinical and private practice due to the growing therapeutic potential of HT. Thus, by addressing two primary topics—a critical evaluation of *Hirudo medicinalis* and leech therapy—this review serves as an experiment to investigate the complex leech-man interaction.

Keywords: Hirduo medicinalis, Leech Therapy

Introduction

The segmented worm known as a leech (Hirudinea) belongs to the phylum Annelida (Yadav and Zhang, 2020). The Anglo-Saxon root "loece" (which meaning "to heal") is where the term "leech" originates. Egyptian artwork from 1500 BC show evidence of the use of leeches. It has been employed in medicine for "local depletion" (bloodletting) from the eras of classical Greece, Rome, and Arabia (Pourrahimi *et al*., 2020; Hyson, 2005).

Leeches fall into two categories based on how they feed. For example, vicious leeches feed on many different kinds of invertebrates. Sanguinivorous leeches are a different kind of leech; they are ectoparasites that consume the blood of vertebrates, together with humans. Leeches gather blood from their prey by biting their jaws and sucking; after completely engorged, they fall off on their own without the need to feed (Abdualkader *et al*., 2013). They can be found in aquatic habitats (ponds, streams, lakes, and the sea) as well as moist terrestrial ones (Shakouri and Wollina, 2021). Despite being widespread round the world, bloodsucking leeches are furthermost prevalent in Northern America, Europe, and Southeast Asia (Ghosh, 2019).

Leeches are sensitive to several stimuli such as light, heat, sound, touch, and vibrations in the water. many types of chemicals They are divided into multiple groups, such as "Brain sections," where each segment contains unique organs like testicles and ganglions for adhesion and crawling. Two sucker portions are used; the anterior one has three jaws, one of which contains many teeth. They typically bite the areas of the host that are the warmest. feeds itself by sucking its blood through regular contractions (Maria and Sergio, 2022). A leech needs 40 minutes or so to digest 10–15 milliliters of blood. for every meal Digestion involves a lot of enzymes. *Hydrophilia Aeromonas* and other mutual microorganisms One kind of *Pseudomonas* is *Pseudomonas hirudinia* (Gileva and Mumcuoglu, 3013; Herlin *et al*., 2016). Numerous studies have shown that leeches release a wide range of bioactive substances. Over twenty molecules are present (Abdullah *et al*., 2012; Das, 2014). While some of their methods of action have been established, others remain unidentified (Hildebrandt and, 2011; Singh, 2010). There are still plenty out there, ready to be discovered. These compounds do, in fact, possess analgesic, anticoagulant, anti-inflammatory, platelet inhibitory, and thrombin-regulating properties in addition to extracellular antibacterial and matrix-degradative ones. (Whitaker *et al*., 2005; Zaidi *et al*., 2011).

2. Morphological Features of Leeches

Leeches, or invertebrates, are colored in a variety of ways, including brown, dark green, and black. Their bodies could be striped in brown, orange, or red. A leech's front and back are covered in suckers. While the anterior sucker is used to extract blood, the posterior sucker is used for attachment and movement. It makes a characteristic Y-shaped incision in the skin when it bites, and each of its three jaws contains roughly 100 teeth in total. It uses its posterior sucker to crawl on land after swimming vertically and undulatingly in the water. It has two segmented tubular pumping hearts and no lungs; it breathes through its skin (Abdisa, 2018). Leech have no outer exoskeleton and a thin, flexible cuticle. Because it dries quickly, Water and it are intimately associated (Tilahun *et al*., 2020). The three triradiate-shaped jaws that make up the anterior sucker's mouth are in the center of the animal (Hyson, 2005). Porshinsky *et al*. (2011)

discovered that whereas leech bodies have 102 annuli, their internal structures are divided into 32 segments. According to (Ahirrao et al., 2017), the first four front segments are classified as head segments, the following 21 as midbody segments, and the final seven as tail sucker segments. By examining their sensory organs and annulation pattern, taxonomists are able to determine the genus and species of these worms' figure (1).

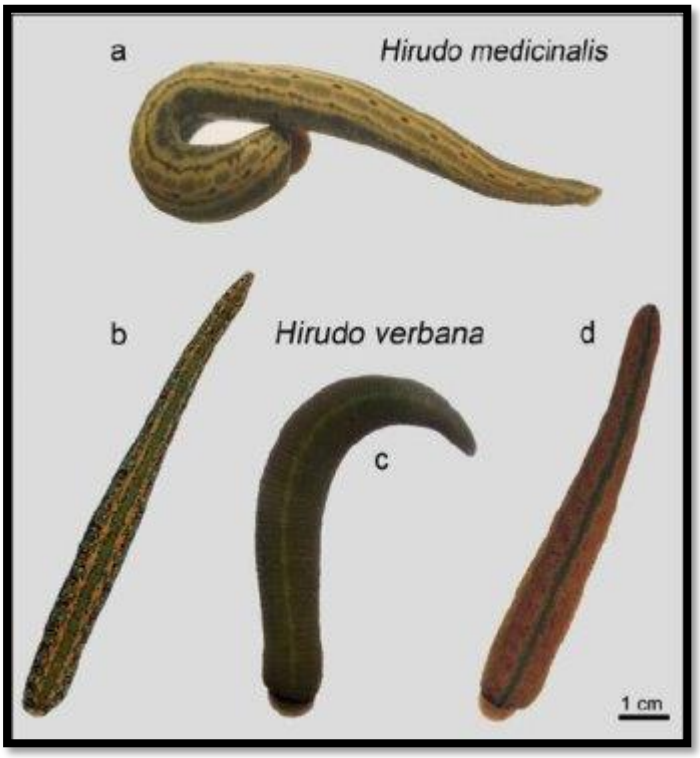

Figure (1): *Hirudo medicinalis*, *Hirudo verbena*, the darkly pigmented variety of *H. verbana* "var. nigra," and the mainly unpigmented variant *H. verbana* "var. monostriata" are the four adult living specimens of the European medicinal leech (A, B, C, D). (Kutschera, 2012)

3. Locality and Ecology of Leeches

A variety of environments, including damp land and water, are suitable for leeches to live in. Certain species can be found in freshwater, estuaries, rivers, ponds, lakes, and the ocean. Certain species of leeches have acquired larger nephridial vesicles (bladders) and more mucous glands to store and retain water, which allows them to survive dry spells in damp environments. Leeches also possess a high degree of physiological flexibility, which allows them to adapt to a variety of environmental stressors such as changes in temperature and oxygen scarcity. Because moisture is essential to terrestrial leeches' distribution and activity, they are frequently found in vast numbers in the woods and highlands of North America, Europe, and Southeast Asia. In the forests and highlands of North America, Europe, and Southeast Asia, leeches abound. Leeches are active all year long in areas that are perennially humid, like Malaysia. while in regions with rainy and dry seasons, they go through an inactive and a dormant period (Yule and Yong, 2004).

4. The Leech Life Cycle in General

There are three life phases for leeches: egg, juvenile, and adult. Leeches usually reach adulthood in a year or two. In a good habitat, an adult leech can live for 18 to 27 years, and if necessary, it can go up to a year without eating. Eggs are laid between 1 and 9 months after copulation, at which point a specific organ called the "clitellum" starts to secrete four cocoons. There are 15 eggs in each cocoon, yielding 60 offspring annually (Abdisa, 2018). Leeches are hermaphrodites, though to procreate, they require another leech (Porshinsky *et al*., 2011). According to Phillips *et al*. (2020), leeches have established the capacity to self-fertilize in the absence of suitable partners. The eggs are then buried in damp earth and covered with a cocoon formed of discharges that solidify into polymers when open to moisture and air. When young leeches hatch from their eggs, they spend the first few days of their lives eating the liquid protein found inside the cocoon. Leeches' food frequently on the body fluids of frogs afterwards developing from the cocoon because the latter's thin, delicate skin is easily punctured by the fangs of young leeches (Elliott, 2008) figure (2).

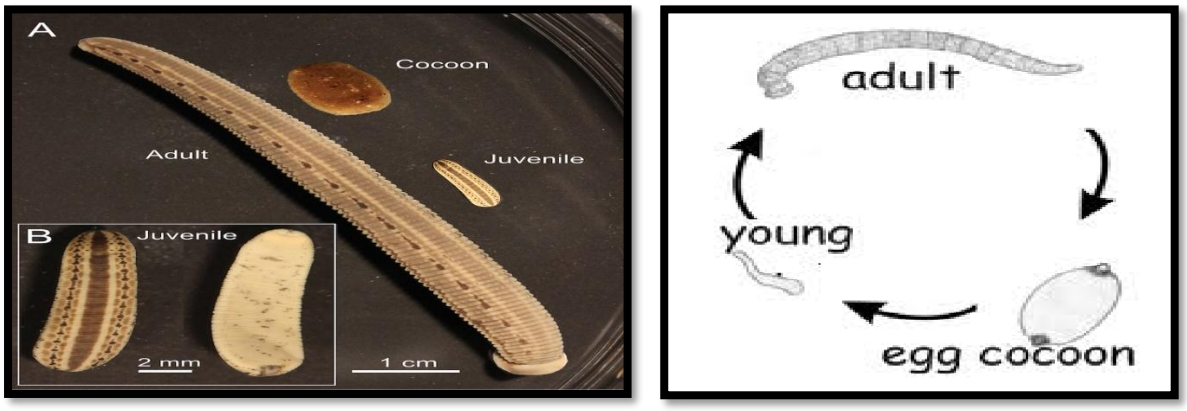

Figure (2): (A) Dorsal image of a cocoon with adult and juvenile H. medicinalis preserved in alcohol. (B) The inset displays, in dorsal and ventral views, the distinctive pigment pattern of a freshly hatched individual. (Kutschera and Roth, 2006; Fernadez and Matte 2010) .

5.Leech Physiological Functions

Leeches may identify suitable environments and approaching prey or hosts by using a range of sensory structures. The most distinctive are photoreceptors (the eyes), Mechanoreceptors—which detect vibrations or sounds—and chemoreceptors—which detect substances in the water and air. Leeches locate possible prey by using all three senses. Photoreceptors cannot capture high-resolution images, but they can detect light and darkness, as well as some movement. (Phillips *et al*., 2020).

Leech is subtle to a wide range of substances, light, touch, sound, and water waves. Through repeated contractions, they can bite and suck the blood. Leeches are sensitive to light, touch, sound, and waves of water, among other things. They may bite and draw blood by repeatedly contracting. A leech takes 40 minutes on average to digest 10 to 15 milliliters of blood at a time (Shakouri and Wollina, 2021). A leech simply foods, but in certain rare cases, it can eat more than ten times its own body weight in blood in a single blood meal from a suitable host (Lent *et al*., 1988). To conserve the blood, it is collected in a crop with ten pairs of diverticula. The concentrated material is retained in the crop for a few months after more

water and salt are released into the host's plasma (Zerbst-Boroffka and Wenning, 1986; Wenning, 1996). The crop contains Aeromonas spp., symbiotic bacteria associated with leeches that can postpone microbial or spontaneous food deterioration (Graf *et al*., 2006).

Harvest material is delivered to the intestine in little amounts and digested on a regular basis (Roters and Zebe, 1992). Inedible Leech is released from the anus; threat, intervention, a pharmacy, etc. 119 elements, chiefly derivatives of hem. The leech starts moving its jaws while also releasing saliva. The leech may empty the gland reservoirs during the duration of the feeding cycle (Hildebrandt and Lemke, 2011).

6.The Biology of Leech Feeding

Both sanguivorous and predatory leeches use their intestines to digest their food. Only months' worth of blood are stored inside the bodies of sanguivorous creatures. Hematophagous leeches go through a number of sluggish stages in the digestion of blood, allowing them to preserve the ingested blood for up to 18 months. In the leech's gut, there are symbiotic bacteria known as *Aeromonas* spp., including Rikinella-like species, *Aeromonas veronii*, and *A. hydrophila*. According to various studies (Indergand and Graf, 2000; Siddall, *et al*., 2011; Bomar, *et al*., 2011; Maltz and Graf, 2011; Maltz *et al*., 2014), symbionts like *A. veronii* secrete enzymes that aid in both the breakdown of the ingested blood's constituent parts and the production of antibiotics to stop blood putrefaction. Another alleged function avoid B complex deficiency, which frequently develops in animals that depend on blood nourishment (Sawyer,1986; Yule and Yong 2004).

7.Historical Review of Leeching

Both conventional and modern medicine rely heavily on medicinal leeches (Whitaker *et al*., 2004; Elliott and Kutschera, 2011). Around 1500 BC, a painting of an Egyptian tomb depicted leeches being employed for therapeutic purposes. This is the first legibly recorded instance of such use. In Europe, the popularity of hirudotherapy peaked in the 17th and 18th century AD, but in the Arab world, leeches were only employed for bloodletting (Munshi *et al*.,2008).

However, Nicander of Colophon's writings from the first century BC have survived to the present day (Papavramidou and Christopoulou-Aletra, 2009). Leech therapy has been practiced for centuries by Unani doctors who follow Galen's (130–201 AD) hypothesis, which was influenced by Hippocrates (460–370 BC), that ailments in the body are brought on by humeral imbalance. In his work as Canon of Medicine, Avicenna also advocated leech therapy, particularly for skin conditions (Lone *et al*., 2011).

Two Slovenian doctors pioneered leaching in modern microvascular surgery and tissue transfer, using the parasites to improve circulation following tissue-flap transplantation. In the case of a scalp avulsion, leeches were used for post-operative care, according to a 1983 report by Henderson *et al*. A five-year-old boy's ear was successfully reattached in 1985 by Harvard doctor Joseph Upon using medicinal leeches (Mutimer *et al*.,1987).

Leeches have since been widely used to salvage vascularly compromised flaps, or muscle, skin, and fat tissue surgically transferred from one part of the body to another, and replants,

limbs, or other body parts. Leeches have also been used to reduce venous congestion in reattached fingers, toes, ears, and scalp. amputation brought on by injury. Leeches of the *Hirudo medicinalis* species were in risk of going extinct due to the overuse of this substance in Europe. As a result, laws were developed to govern the leech trade. Therefore, when leech populations declined and alternative therapeutic options emerged, demand for leech therapy fell (Munshi *et al*., 2008).

The United States Food and Drug Adminstration clearance in 2004 to utilize *Hirudo medicinalis* for therapeutic purposes in plastic and reconstructive surgery and subsequent study on the composition of leech saliva rekindled interest in hirudotherapy (Mumcuoglu, 2014).

Despite being employed in traditional medicine since ancient times, medicinal leeches are now one of the alternative treatment methods of modern medicine thanks to scientific evidence of the efficacy of the active chemicals they secrete (Sig *et al*., 2017).

8.*Hirudo medicinalis*

According to Davis and Appel (1979) and Solijonov and Umarov (2022), the medicinal leech (*Hirudo medicinalis*) is a segmented annelid that is a member of the Phylum Annelida, which is a large phylum that contains 22.000 species. The environment, agriculture, and human health are all significantly impacted by this group of animals. Class: Clitellata, and Subclass: Hirudinea. Hematophagous animals, which consume the blood of their prey, it was discovered that the substances which are present in leech saliva play a great role in preventing thrombosis of blood and improving blood circulation have been observed to prevent blood clotting by secreting a variety of physiologically active substances, including the anticoagulants, in their salivary gland secretions (Babenko *et al*., 2020).

Therapists use leech therapy for a variety of ailments for decades since leeches first caught their notice. The majority of doctors favored the European medical leech species, *Hirudo medicinalis*, figure(3) popularly known as the healing leech, for various therapeutic uses, as opposed to *Hirudo decora*, an American species, can suck less blood since it just makes a small, superficial cut in its prey's skin (Whitaker *et al*., 2004). Many other species, including *Hirudinaria manillensis* (Electricwala *et al*., 1991), *Hirudo nipponia* (Kim and Kang, 1998), *Hirudo verbena*, *Hirudo orientalis* (Baskova *et al*., 2008), and *Haementeria depressa*, were also thought to be useful as medical tools.

Figure (3) *Hirudo medicinalis* (Wikipedia, 2023)

9. Security and Difficulties of Leeching

Contagion is the most common negative effect of leeching, affecting 2–36% of patients (Green and Shafritz, 2010). Aeromonas hydrophila, a Gram-positive rod that can result in pneumonia, muscle necrosis, flap failure, and even septicemia, is the agent. Pseudomonas, Vibrio, and Aeromonas species are complicated in these infections. Since A. hydrophila is resistant to penicillins and the first generation of cephalosporins, aminoglycosides and fluoroquinolones should be included in the cure regimen for such contagions (Srivastava and Sharma, 2010; Porshinsky *et al*., 2011). However, there are no reports of illnesses being spread by leech therapy, even though doctors who repeat leeching are counseled to utilize a leech once (Michalsen *et al*., 2007).

Numerous investigations have documented symptoms of localized hypersensitivity, including itching, blister formation, ulcerative necrosis, and even localized tissue destruction (flap death), which may be brought on by certain toxins found in leech saliva (Srivastava and Sharma, 2010). Additional post-leeching complications that have been reported include skin marks (scars) from the delayed healing of leech bites and blood loss from prolonged hemorrhage (Green and Shafritz, 2010).

10.Leech Works with Secreted Proteins

The impact mechanisms of leeches have been the subject of numerous scientific research to date. Of the almost 100 different of the proteins with varying molecular weights found in leech secretions, very few have been found to play a significant active role (Baskova *et al*., 2004), figure (4).

The first bite of leech therapy is followed by a bonding period of 20 to 45 minutes during which the leech feeds on blood. It was often thought that leeches feeding on their hosts' blood provided the primary therapeutic advantages, However, further studies have shown that the advantages were really caused by the bioactive substances found in leech saliva that were released into the host's bloodstream during sucking (Abdullah *et al*.,2012**).** Table (1) lists the bioactive substances present in leech saliva along with their intended uses. To make the effect mechanisms more understandable, they are separated into six sorts. However, because these mechanisms are interconnected, they should be assessed collectively. After biting, In order to avoid being detected, a leech must: increase blood flow; defend itself (antimicrobial activity); avoid detection (analgesic and anti-inflammatory actions); block adhesion, aggregation, and coagulation (inhibition of platelet activities, and anticoagulant impact); and (Montinari and Minelli, 2022).

Histochemical tests revealed that the gastrodermis, the epithelium lining the leech's digestive tract, Lacking lipases, glucolytic enzymes, endopeptidases, and other digestive enzymes. There is no physical differentiation between the absorptive and secretive portions of the gastrodermis (Jennings & van der Lande 1967; Sawyer 1986).

On the other hand, very active enzymes were found to be exopeptidases and basic and acidic phosphatases (Jennings & van der Lande 1967; Fischer 1970; van der Lande 1972). The

authors mentioned state that exopeptidases (arylamidases) are the only enzymes that can break down proteins in the intestines of leeches, where most of the digestion and absorption of food occurs. Because leeches lack endopeptidases, which initiate the digestive process in most other animals, leeches take longer to digest proteins. For *H. medicinalis*, however, different outcomes were seen. (Zebe et al. 1986; Roters & Zebe 1992b).

It was discovered that this species' stomach had three endopeptidases that belonged to the serine protease class. The leech makes these because they are very different from the serine proteases created in vertebrates. One of them is composed of trypsin-like proteases, the other two being composed of chymotrypsin-like proteases. Three endopeptidases from the serine protease class were found to be active in the gut of this species. Since these enzymes differ from the serine proteases present in vertebrates, it is the leech that produces them. Trypsin-like proteases make up one of them, while chymotrypsin-like proteases make up the other two (Zebe *et al*. 1986; Roters & Zebe 1992a; Baskova & Zavalova 2001)

Table (1) The Bioactive Compounds Found in Leech Saliva and Their Functions(Baskova and Zavalova 2001; Zaidi *et al*.,2011; Sig *et al*.,2017).

Modesof Action	**Substance**	**Target or Function**
	Bdellins	Inhibits trypsin, plasmin, and sperm acrosin
	Hirustasin	Inhibits tissue (but not plasma) kallikrein
	LDTI (leech-derived tryptase inhibitor)	Inhibits tryptase
	Eglins	Inhibits α-chymotrypsin, chymase, subtilisin, and the neutrophil

Analgesic and Anti-İnflammatory Effects		proteinases elastase and cathepsin GInhibitor.
	LCI (leech carboxypeptidase)	Inhibits Carboxypeptıdase A
	Complement C1 Inhibitor	It can bind to complement-fixing sites of antibodies (IgG and IgM)
	Guamerin from Hirudo nipponia	Inhibits Leukocyte-elastase.Specifically,
	Piguamerin from Hirudo nipponia	Inhibits kallikrien, and trypsin
Anticoagulant Effects	Hirudin	Inhibits thrombin
	Factor Xa Inhibitor	Inhibits Factor Xa
	Destabilase	Dissolves stabilized fibrin
	Gelin	Inhibits elastase, cathepsin G, and chymotrypsin
Extracellular Matrix Degradation	Hyaluronidase	Targets endoglucoronidic linkages of hyaluronic acid
	Collagenase	Dissolves the collagen particles
Anti-Platelet Effects	Apyrase	Targets epinephrine, platelet-activating factor (PAF), arachidonic acid, and adenosine 5' diphosphate.
	Calin	Inhibits collagen-induced platelet aggregation (directly) or von-Willebrand factor collagen binding (indirectly)
	Saratin	Inhibits the binding of α2 integrin subunit I domain to collagen
The Effects on Blood Flow	Acetylcholine	Targets blood vessels
	Histamine-like Substances	Targets blood vessels
Antimicrobial Effects	Destabilase	The bacterial cell wall's β1-4 bonds in the peptidoglycan layer

	Chloromycetin	Bacterial protein synthesis
	Theromacin	The bacterial membrane
	Theromyzin and Peptide B	

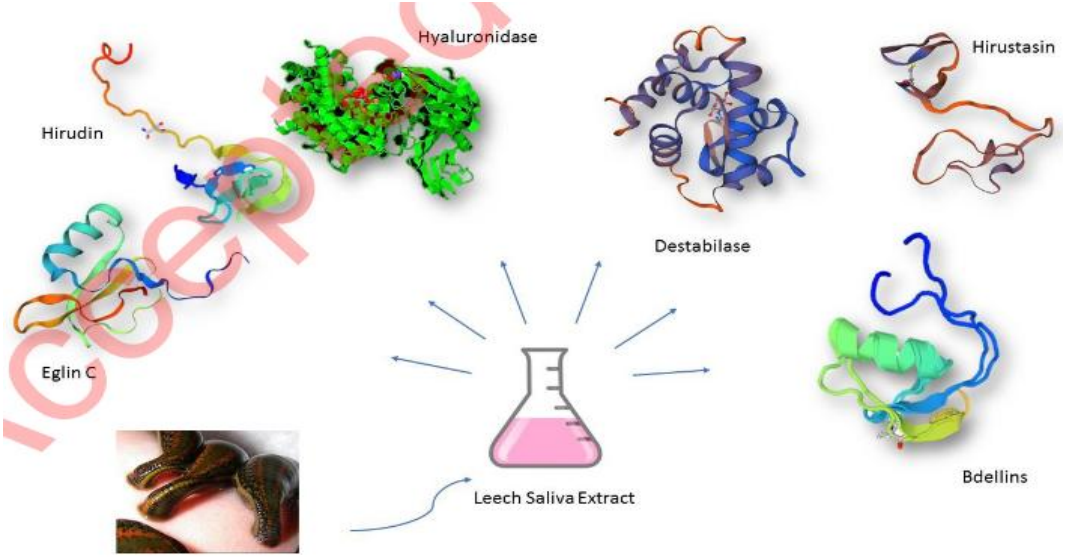

Figure (4): A variety of proteins and enzymes found in medical leech saliva have multiple functions that can help treat various illnesses (Shakouri and Wollina, 2021).

11. Clinical application of leech treatment in medicine

Leech therapy is used to treat most occurrences of localized venous congestion related to flap surgery replantation and reconstructions.

Arthritis

Saliva from leeches can be used to treat arthritis. Saliva contains certain substances and minerals that help to lessen joint inflammation. Among these are Bdellins and Eglin C, which function as anti-inflammatory substances. Its saliva also includes anesthetics, which reduce joint discomfort in addition to anti-inflammatory ingredients. It also has an ingredient that resembles histamine and functions as a vasodilator. Yet another vasodilator is acetylcholine found in leeches' saliva, is another component (Shakouri *et al*., 2018; Asutkar and Varshney, 2018).

Venous congestion

Leech therapy has been shown to benefit people with venous illnesses. It can aid in dissolving blood clots and lessen discomfort and edema caused by varicose veins. However, leech therapy is ineffective for conditions brought on by insufficient valve function and vessel dilatation (Singh, 2010).

vascular conditions

Leech therapy has been utilized recently to treat vascular diseases. More than 100 very advantageous bioactive substances have been found in leech saliva. Hirudin, which functions as an anticoagulant, is one of these ingredients. Calin is another element that prevents blood coagulation. One of the elements that breaks apart fibrin clots and prevents thrombus development is the destabilase25. Additionally, a Factor Xa inhibitor found in leech saliva inhibits the coagulation Factor's coagulating action. Hyaluronidase is another ingredient that increases the viscosity of the interstitial fluid. It comprises acetylcholine, histamine-like substances, and carboxypeptidase-A inhibitors, which have a vasodilating action. (Hu *et al*., 2020). Only a few extremely helpful substances, which function in the background to lower blood viscosity and improve flow, are present in leech saliva. Blood of a thick consistency has been shown to increase the risk of blood clot formation and elevate blood pressure. These coagulates can migrate to diverse areas of the body and obstruct a vessel, which can cause a stroke or heart spasm. A risk of obliterating the distal circulation arises from thick blood, namely from inadequate oxygenated blood supply and inadequate nutrition to the tips of the toes and fingers. Therefore, the anticoagulant found in leech saliva is significant, and when all of these factors work organized, the patient's vascular state will be significantly improved (Babenko *et al*., 2020).

Antibacterial properties

Thus far, it has been demonstrated that destabilase and chloromycetin are the only two important compounds with antibacterial activity. As stated earlier, destabilase has β-glycosidase activity that straight breaks the crucial β1-4 bonds in the peptidoglycan layer of the bacterial cell walls. This effect is clearly the same as that of lysozyme, which is consistently present in human saliva and lachrymal fluid. Additional research revealed that antimicrobial action depends on both non-enzymatic and enzymatic glycosidase activity (Grafskaia *et al*., 2019). The desaturated formula of destabilase even exhibits a dose-dependent bacteriostatic action on *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus*. Leech secretions include a strong antibiotic called chloromycetin, although little is known around this composite. Additionally, theromacin, theromyzin, and peptide B (Wilmer *et al*.,2019).

Pain control

Leeches are used to treat pain syndromes of various causes. Although it sometimes takes a while, the pain is relieved quickly. Leech therapy has been shown in reports to be helpful for severe cancer pain. Studies on osteoarthritis have argued that leech therapy can improve symptoms by having anti-inflammatory and analgesic properties (Wollina and Heinig, 2016).

Cancer-related leeches

Following surgery, a patient diagnosed with basal cell carcinoma received 9 months of leech therapy; successful completion of the flap's blood circulation was the main outcome. In a patient with intraoral cancer, medicinal leeches are useful in reducing venous congestion of a free forearm flap before rebuilding. The salivary gland secretions of the leech provide antimetastatic properties. The saliva of leeches contains a protein called antistasin that prevents lung cancer from colonizing. The secretions contain enzymes that are anti-proteolytic, platelet

aggregation inhibitors, and anticoagulants. Hyaluronidase is one of the other components that has anti-tumor activity. It is thought that by weakening the hyaluronic acid-CD44 interaction, pro-tumorigenic immune cell inhibition into the tumor stroma may partially explain the hyaluronidase anticancer activity (Ammar *et al*., 2015).

REFERENCES

Yadav, S., and Zhang, B. (2020). An uncommon cause of unilateral nasal bleeding. Grande Medical Journal. 2(1): 25-27.

Hyson, J.M. (2005). Leech therapy: a history. Journal of the History of Dentistry. 53(1): 25-27.

Pourrahimi, M.; Abdi, M., Ghods, R. (2020). Complications of leech therapy. Avicenna Journal of Phytomedicine. 10(3): 222-243.

Abdualkader, A.M.; Ghawi, A.M.; Alaam, M.; Awang, M., and Merzouk, A. (2013). Leech therapeutic applications. Indian Journal of Pharmaceutical Sciences,75(2): 127–137.

Shakouri, A., and Wollina, U. (2021). Time to change theory; medical leech from a molecularech salivary proteins playing a potential role in medicine. Advanced pharmaceutical bulletin. 11(2): 261-266.

Ghosh, S. (2019). Relevance of leech therapy in contemporary medicine: a mini- review. Quest International Journal of Medical and Health Sciences. 2(1): 8-12.

Maria RM, Sergio M. (2022). From ancient leech to direct thrombin inhibitors and beyond: new from old. Biomedicine & pharmacotherapy, 149.

Gileva OS, Mumcuoglu KY. Hirudotherapy. In: Grassberger M, Sherman RA, Gileva OS, Kim CMH, Mumcuoglu KY, editors. (2013). Biotherapy-history, principles and practice: A practical guide to the diagnosis and treatment of disease using living organisms. London: springer science & business media, 31-76.

Herlin C, Bertheuil N, Bekara F, Boissiere F, Sinna R, Chaput B. (2016). Leech therapy in flap salvage: systematic review and practical recommendations.;62(2):1-13.

Abdullah S, Dar LM, Rashid A, Tewari A. (2012). Hirudotherapy/leech therapy: applications and indications in surgery. Arch clin exp surg.;1(3):172-180.

Das BK. (2014). An overview on hirudotherapy/leech therapy. Ind res j pharm sci.;1(1):33-45.

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

Singh AP. (2010). Medicinal leech therapy (Hirudotherapy): A brief overview. Complement ther clin pract.;16(4):213-215.

Whitaker IS, Cheung CK, Chahal CAA, Karoo ROS, Gulati A, Foo ITH. By what mechanism do leeches help to salvage ischaemic tissues? A review. Br j oral maxillofac surg. 2005;43(2):155-160.

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

Abdisa, T. (2018). Therapeutic importance of leech and impact of leech in domestic animals. *MOJ Drug Design Development and Therapy*, 2(6):235–242.

Tilahun, T.; Babu, H., and Berhane, M. (2020). Leech in the rectum causing lower GI bleeding in a four-year-old child: a case report. *Ethiopian Journal of Health Sciences*. 30(6): 1055-1057.

Hyson, J.M. (2005). Leech therapy: a history. *Journal of the History of Dentistry.* 53(1): 25-27.

Porshinsky, B.S.; Saha, S.; Grossman, M.D.; Beery Ii, P.R., and Stawicki, S.P.A. (2011). Clinical uses of the medicinal leech: a practical review. *Journal of Postgraduate Medicine*. 57(1): 65-71.

Ahirrao, R.; Jadhav, J., and Pawar, S. (2017). A review on leech therapy. *Pharma Science Monitor* 8(1): 228-237.

Kutschera, U. (2012). The *Hirudo medicinalis* species complex. *The Science of Nature.* 99(5):4334

Yule, C.M.; and Yong, H.S.(2004). Freshwater Invertebrates of the Malaysian Region. Kaula Lampur: *Akademi Sains Malaysia*. ISBN: 983-41936-0-2.

Phillips, A.J.; Govedich, F.R., and Moser W.E. (2020). Leeches in the extreme: morphological, physiological, and behavioral adaptations to inhospitable habitats. International Journal for 130 Parasitology: *Parasites and Wildlife*. 12: 318-325.

Elliott, J.M. (2008). Population size, weight distribution and food in a persistent population of the rare medicinal leech, *Hirudo medicinalis*. *Freshwater Biology*. 53(8):1502–1512.

Kutschera, U., and Roth, M. (2006). Cocoon deposition and cluster formation in populations of the leech Hirudo verbana (Hirudinea: Hirudinidae). *Lauterbornia* 56: 5–8.

Fernandez, J., Olea, N., Tellez, V., and Matte, C. (2010). Structure and development of the egg of the glossiphoniid leech Theromyzon rude: reorganization of the fertilized egg during completion of the first meiotic division. *Developmental Biology.* 137 (1): 142–154

Lent, C.M.; Fliegner, K.H.; Freedman, E., and Dickinson M.H. (1988). Ingestive behavior and physiology of the medicinal leech. *Journal of Experimental Biology*. 137(1): 513-527.

Zerbst-Boroffka, I., and Wenning, A. (1986). Mechanisms of regulatory salt and water excretion in the leech, *Hirudo medicinalis* L. *Zoologische Beiträge*, 30: 359-377.

Wenning, A. (1996). Managing high salt loads: from neuron to urine in the leech. *Physiological Zoology*. 69(4): 719-745.

Graf, J.; Kikuchi, Y., and Rio, R.V.M. (2006). Leeches and their microbiota: naturally, simple symbiosis models. *Trends in Microbiology*. 14(8): 365-371.

Roters, F.J., and Zebe, E. (1992b). Proteinase of the medicinal leech, Hirudo medicinalis: purification and partial characterization of three enzymes from the digestive tract. Journal of Comparative *Biochemistry* & *Physiology*. B 102(3): 627–634.

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

Indergand, S., and Graf, J. (2000). Ingested blood contributes to the specificity of the symbiosis of Aeromonas veronii biovar sobria and Hirudo medicinalis, the medicinal leech. *Applied* and *Environmental Microbiology*. 66(11):4735-41.

Siddall, M.E.; Min, G.S.; Fontanella, F.M.; Phillips, A.J., and Watson, S.C. (2011). Bacterial symbiont and salivary peptide evolution in the context of leech phylogeny. *Parasitology*, 138(13): 1815–1827.

Bomar, L.; Maltz, M.; Colston, S., and Graf, J. (2011). Directed culturing of microorganisms using metatranscriptomics. *Microbiology* 2(2): e00012-11.

Maltz, M.A., and Graf, J. (2011). The Type II Secretion System Is Essential for Erythrocyte Lysis and Gut Colonization by the Leech Digestive Tract Symbiont *Aeromonas veronii*. *Applied and Environmental Microbiology*. 77(2): 597–603.

Maltz, M.A.; Bomar, L.; Lapierre, P.; Morrison, H.G.; McClure, E.A.; Sogin, M.L., and Graf, J. (2014). Metagenomic analysis of the medicinal leech gut microbiota. Front. *Microbiology*. 5, 151.

Sawyer, R.T. (1986). Leech Biology and Behaviour. Michigan: Clarendon Press; Feeding, biology, ecology and systematics. Vol. 2.

Whitaker, I. S.; Rao, J.; Izadi, D., and 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.

Elliott J.M., and Kutschera U. (2011). Medicinal Leeches: Historical use, Ecology, Genetics and Conservation, Freshwater *Reviews*. 4(1), 21-41.

Munshi, Y.; Ara, I.; Rafique, H., and Ahmad Z. (2008). Leaching in the history--a review. *Pakistan journal of biological sciences*. PJBS. 11(13): 1650–1653.

Papavramidou, N., and Christopoulou-Aletra, H. (2009). Medicinal use of leeches in the texts of ancient Greek, Roman and early Byzantine writers. *Internal Medicine Journal.* 39(9):624-7.

Lone, A.H.; Ahmad, T.; Anwar, M.; Habib, S.; Sofi, G., and Imam, H. (2011). Leech Therapy-A Holistic approach of treatment in Unani (Greeko-Arab) medicine. *Ancient Science of Life*. 31(1):31.

Mutimer, K.L.; Banis, J.C., and Upton, J. (1987). Microsurgical reattachment of totally amputated ears. *Plast Reconstr Surg*. 79(4): 535-541.

Mumcuoglu, K.Y. (2014). Recommendations for the use of leeches in reconstructive plastic surgery. *Evidence- Based Complementary and Alternative Medicine*. 2014, Article ID 205929, 7 pages.

Sig, A.K.; Guney, M.; Guclu, A.U., and Ozmen, E. (2017). Medicinal leech therapy—an overall perspective. I*ntegrative Medicine Research*. 6(4):337–43.

Davis, A., and Appel, T. (1979). Bloodletting Instruments in the National Museum of History and Technology. *Smithsonian Institution Press*, Washington, D.C. (41): 34-36.

Solijonov, K., and Umarov, F. U. (2022). Ecology of leeches and gastropods of the lower akbuura river, Fergana valley, Uzbekistan. *Bulletin of the Iraq Natural History Museum*. 17(2):229-250.

Babenko VV, Podgorny OV, Manuvera VA, Kasianov AS, Manolov AI, Grafskaia EN, et al. (2020). Draft genome sequences of Hirudo medicinalis and salivary transcriptome of three closely related medicinal leeches. BMC Genomics;21(1):331. doi: 10.1186/s12864-020-6748-0

Whitaker, I. S.; Rao, J.; Izadi, D., and 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.

Electricwala, A.; Sawyer, R.T.; Jones, C.P., and Atkinson, T. (1991). Isolation of thrombin inhibitor from the leech Hirudinaria manillensis. *Blood Coagulation* & *Fibrinolysi*. 2(1):83-9.

Kim, D.R., and Kang, K.W. (1998). Amino acid sequence of piguamerin, an antistasin-type protease inhibitor from the blood sucking leech Hirudo nipponia. *European journal of biochemistry*.254(3):692-7.

Baskova, I.P.; Kostrjukova, E.S.;Vlasova, M.A.; Kharitonova, O.V.;Levitskiy, S.A.; Zavalova, L.L.; Moshkovskii, S. A. and Lazarev, V. N .(2008). Proteins and peptides of the salivary gland secretion of medicinal leeches Hirudo verbana, H. medicinalis, and H. orientalis. Biochemistry (Mosc). 73(3):315-320.

Green, P.A., and Shafritz, A.B. (2010). Medicinal leech is used in microsurgery. Journal of *Hand Surgery*. 35(6):1019-21.

Srivastava, A., and Sharma, R. (2010). A brief review on applications of leech therapy. *Archives of Applied Science Research*. 2:271–4.

Porshinsky, B.S.; Saha, S.; Grossman, M.D.; Beery Ii, P.R., and Stawicki, S.P.A. (2011). Clinical uses of the medicinal leech: a practical review. *Journal of Postgraduate Medicine*. 57(1): 65-71.

Michalsen, A.; Roth, M.; Dobos, G., and Aurich, M. (2007). Medicinal Leech Therapy. *Stattgurt, Germany: Apple Wemding*;. Archives of facial plastic surgery: official publication for the American Academy of Facial Plastic and Reconstructive Surgery, Inc. and the International Federation of Facial Plastic Surgery Societies. 9(6):448-448.

Baskova, I.P.; Zavalova, L.L.,;Basanova, A.V.; Moshkovskii, S.A., and Zgoda, V.G.(2004). Protein profiling of the medicinal leech salivary gland secretion by proteomic analytical methods. *Biochemistry* (Moscow). 69(7):770-775.

Abdullah, S.; Dar, L.M.; Rashid, A., and Tewari, A. (2012). Hirudotherapy/leech therapy: applications and indications in surgery. *Archives of Clinical and Experimental Surgery*. 1(3):172-80.

Montinari, M.R., and Minelli, S.(2022). From ancient leech to direct thrombin inhibitors and beyond: New from old. *Biomedicine and Pharmacotherapy*.149: 112878.

Jennings, J.B., and van der Lande, V. (1967). Histochemical and bac- teriological studies on digestion in nine species of leech. *Biology Bulletin*. 133(1): 166–183.

Fischer, E. (1970). Lokalisation der Leucyl-aminopeptidase (LAP) beim Blutegel (*Hirudo medicinalis* L.) und beim Pferegel (*Haemopis sanguisuga* L.). *Acta Histochem*. 37(1): 170–175.

van der Lande, V. (1972). Observations on histochemical "amino- peptidase" reaction in the intestine of certain species of leech (Annelida: Hirudinea), with reference to Erpobdella octoculata (L.). *Comparative Biochemistry* & *Physiology*. A 41: 813– 824

Zebe, E.; Roters, F.J., and Kaiping, B. (1986). Metabolic changes in the medical leech Hirudo medicinalis following feeding. C*omparative Biochemistry and Physiology*. A 84(1): 49–55.

Roters, F.J., and Zebe, E. (1992b). Proteinase of the medicinal leech, Hirudo medicinalis: purification and partial characterization of three enzymes from the digestive tract. Journal of Comparative *Biochemistry* & *Physiology*. B 102(3): 627–634.

Baskova IP, Zavalova LL. (2001). Proteinase inhibitors from the medicinal leech Hirudo medicinalis. Biochemistry (Mosc);66(7):703-14. doi: 10.1023/a:1010223325313

Shakouri A, Adljouy N, Balkani S, Mohamadi M, Hamishehkar H, Abdolalizadeh J, et al. (2018). Effectiveness of topical gel of medical leech (Hirudo medicinalis) saliva extract on patients with knee osteoarthritis: a randomized clinical trial. Complement Ther Clin Pract; 31:352-9. doi: 10.1016/j.ctcp.2017.12.001

Asutkar SG, Varshney S. (2018). The cumulative anti-inflammatory effect of bloodletting by leech (Jallaukavacharana) in patients of arthritis (Aamvata) w.s.r. inflammatory markers-a research realm. Ayurline Int J Res Indian Med;2(5):1-17.

Singh AP. (2010). Medicinal leech therapy (hirudotherapy): a brief overview. Complement Ther Clin Pract;16(4):213-5. doi: 10.1016/j.ctcp.2009.11.005

Hu B, Xu L, Li Y, Bai X, Xing M, Cao Q, et al. (2020). A peptide inhibitor of macrophage migration in atherosclerosis purified from the leech Whitmania pigra. J Ethnopharmacol; 254:112723. doi: 10.1016/j.jep.2020.112723

Grafskaia EN, Nadezhdin KD, Talyzina IA, Polina NF, Podgorny OV, Pavlova ER, et al. (2019). Medicinal leech antimicrobial peptides lacking toxicity represent a promising alternative strategy to combat antibiotic- resistant pathogens. Eur J Med Chem; 180:143-53. doi: 10.1016/j.ejmech.2019.06.080

Wilmer A, Slater K, Yip J, Carr N, Grant J. (2013). The role of leech water sampling in choice of prophylactic antibiotics in medical leech therapy. Microsurgery;33(4):301-4. doi: 10.1002/micr.22087

Wollina U, Heinig B, Nowak A. (2016). Medical leech therapy (hirudotherapy). Dermatol Online J;7(1):91-6. doi: 10.7241/ourd.20161.24

Ammar AE, Hassona MH, Meckling GR, Chan LG, Chin MY, Abdualkader A, et al. (2015). Assessment of the antitumor activity of leech (huridinaria manillensis) saliva extract in prostate cancer. Cancer Res;75(15 Suppl):5130-. doi: 10.1158/1538-7445.am2015-5130