Review Article

Archives of Clinical & Experimental Surgery

Hirudotherapy /Leech therapy: Applications and Indications in Surgery

Swaid Abdullah [1], Latief M. Dar [2], Adil Rashid[3], Anita Tewari [4]

Abstract

Hirudotherapy (HT) is the application of medicinal leeches (Hirudo medicinalis) for therapeutic use. It is one of the oldest remedies, being employed by various medicinal practitioners. HT involves the attachment of cultured leeches onto the affected areas. Leech therapy involves an initial bite, which is usually a painless bite, followed by the sucking of 5 and 15 ml of blood. Its major therapeutic benefits are not only due to blood sucked during the biting, but also from the various bioactive substances, such as Hirudin, calin, Hyaluronidase, and Histamine-like substances, to name a few.

HT has been employed in various disease conditions and surgical complications. It has been successfully used in plastic and reconstructive surgeries, cardiovascular complications, varicose veins, hemorrhoids and various joint ailments. Nowadays, it is also being utilized in gastrointestinal disorders, dermatology and gynecological abnormalities.

More recently, HT has found new applications in cancer therapy, hypersensitivity conditions, like asthma, male/female sterility and diabetes. Taking into consideration all the facts, HT efforts should be made in optimizing the success of medicinal leech therapy in clinical and private practice.

Key words: *Hirudotherapy, leech, annelid, saliva, bioactive, reconstructive surgery*

[1]Department of Veterinary Parasitology Govind Ballabh Pant University of Agriculture and Technology (GBPUAT) Pantnagar, Uttarakhand, India

[2,3]Division of Veterinary Pathology Faculty of Veterinary Sciences and Animal Husbandry Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K) Kashmir, India

[4]School of Public Health and Zoonoses Guru Angad Dev Veterinary and Animal Sciences University (GADVASU) Ludhiana, Punjab, India

Received: January 27, 2012
Accepted: April 02, 2012
Arch Clin Exp Surg 2012;1:172-180
DOI:10.5455/aces.20120402072447

Corresponding author
Swaid Abdullah, MVSc Scholar
Department of Veterinary Parasitology Govind Ballabh Pant University of Agriculture and Technology (GBPUAT) Pantnagar, Uttarakhand, India
swaidabdullah@gmail.com

Introduction

The treatment of disease conditions with medicinal leeches is termed as Hirudotherapy (HT), a Latin term [1]. In this non-invasive treatment methodology, medicinal leeches (Hirudo medicinalis) are used, hence the term "Hirudotherapy." The word 'leech' is supposed to be derived from an old English word for physician, laece [2,3]. HT takes the advantage of several biological properties of medicinal leeches. Among these, the earliest known fact was that leeches feed on the blood of their host (phlebotomy) and, during the course, release pain-killing (anesthetic) and blood-thinning substances (anticoagulants) along with their saliva. For centuries, leeches were the common tools of phy-

sicians, who were of the belief that diseases were the result of an imbalance of various humors and that the body can be stabilized by releasing blood [4,5]. Later on with due course of time, physicians employed these spineless blood-sucking animals as a remedy for a large number of diseases and deformities, from congested limbs to the treatment of various eye diseases. Modern leech therapy differs from the ancient therapy; nowadays, only the leeches, which are grown in farms and which have undergone strict quarantine, are employed for the therapy. Wild leeches are not used anymore and a leech is used for a single treatment [6,7]. Today, scientific studies concerning the active substances in the leeches have given us a better understanding of how these annelids work and have increased the field of applications of this ancient therapy.

Historical Perspective

Hirudotherapy (Leech therapy) is one of the oldest practices in medicine; it is known from the time of extreme antiquity and is still alive [8]. Leeches were used for treatment in Egypt as early as 1500 BC, where they were used to treat ailments, like nosebleeds and gout. Leech therapy was documented in Sanskrit writings from 1300 BC. Themison of Laodicea also reported HT in 50 BC [9]. Greek and Roman writings described leech therapy in 50 AD [10]. Therapeutic use of leeches has appeared in ancient Greece, China, India and Pre-Columbian America. Nicander of Colophon (200-130 BC) was probably the first medical practitioner to use leeches for therapeutic purposes [11]. Leech therapy is well documented in the works of Pliny, Galen, Themison and Avicenna [12].

Leeches were named Hirudo medicinalis by Linnaeus in 1758 [13]. During the seventeenth and eighteenth centuries, leech therapy played a pivotal role in therapeutic practices, which involved bloodletting and purification, a practice employed to treat many ailments, from gout to headaches [14].

The greatest use of leeches was in Europe during the 1800s. In 19th-century America, leeches were often a home remedy for gum disorders, hemorrhoids and large bruises [10].

The major event in the history of leech therapy was the discovery by J.B. Haycraft, a professor at King's College in Birmingham, that the throat and mouth of the leech contained a substance that prevented the blood from coagulating in 1884. This compound was later isolated from leech saliva by Jacoby and was named hirudin around 1904 [15]. Hirudin was employed in a blood transfusion in 1915 [16].

With the advent of antibiotics in the 20th century, however, the practice of leech therapy gradually lost favor [17]. Bloodletting using leeches and other methods enjoyed a revival in the early 19th century, particularly in France [2]. A specific area of leech therapy was soon to be determined by the surgeon Termier. He recommended the direct application of leeches in 1922. This technique was called "hirudinization of the blood." In 1935, scientist Bottenberg established the general indications for leech therapy [18].

Modern study of leech therapy began in the 1960s, when medicinal leech therapy achieved an international comeback, initially because of the spectacular results in plastic and reconstructive surgery for the treatment of postoperative venous congestion and graft rejections [10].

Since the 1980s, leech therapy has regained recognition in the medical literature after initial publications by Upton's group; Mahaffey's team in Europe also gave this treatment modality new impulse [14]. The use of medical leeching in modern microvascular surgery and tissue transfer began when two Slovenian surgeons used the parasites to assist with circulation after a tissue-flap transplantation.

In 1983, Henderson et a1. reported a case where leeches were used in the post-operative treatment of a scalp avulsion case [19].

In 1985, Harvard physician Joseph Upton used medicinal leeches to successfully reattach the ear of a five-year-old boy [20] Since then, leeches have been widely used to reduce venous congestion in fingers, toes, ears, and scalp reattachments, as well as to salvage vascularly compromised flaps, or muscle, skin, and fat tissue surgically removed from one part of the body to another, and replants, limbs or other body parts reattached after traumatic amputation.

In July 2004, the FDA approved leeches as a medical device in the area of plastic and reconstructive surgery [21]

Nowadays, HT is being employed in the surgical

field to treat venous congestions of microvascular replantations, reconstructive surgery and traumatology. In addition, leech therapy is used in neurology, dermatology, gynecology, and is giving good results in these areas as well [13].

Basic Biology of Leeches

The medicinal leech (Hiruda medicinalis) is a segmented annelid belonging to Phylum: Annelida, Class: Clitellata and Subclass: Hirudinea (Lamark, 1818) [2,13]. Leeches have two suckers, one on either extremity. The posterior suction cup helps it to move on dry surfaces and in attaching to its host; the anterior suction cup harbors a mouth and three sharp jaws. The bite looks like a Mercedes-Benz© symbol. Each of the three jaws has 100 teeth, for a total of 300 teeth [10].

Medicinal leeches inhabit clean, fresh waters. Leeches swim around freely in water by undulating movements. Leeches are poikilotherms and can survive in the temperature range of 0°C to 30°C; however, rapid temperature changes may stress these animals to death. They breathe water dissolved or atmospheric oxygen through their general body surface. Oxygen requirements are minimal and do not suffocate even in nearly completely closed containers. Harmful substances, like chlorine in water, even in low doses cause death of leeches. Leeches secrete a mucous layer over their body surface under unfavorable and stress conditions; it can thus act as a stress indicator of leeches. Leeches periodically shed their skin. Leeches are "protandrous" – first males then females lay eggs in cocoons 1-9 months post-copulation – about 4 cocoons each containing about 15 eggs and about 60 offspring per year. Young leeches feed on the blood of small water animals, like frogs and fish [22]. They become ready for medicinal use after they are several years old.

Leeches usually remain attached to the host for 30 minutes to 6 hours for feeding, and during the course they get engorged with blood. It can suck about 5 to 15 ml of blood, but the bite continues to ooze for 4 to 24 hours [10]. Leech saliva contains several bioactive substances, including anticoagulants, vasodilators and anesthetics. Its saliva is rich in a potent anticoagulant -Hirudin. The benefits of leech therapy are mainly because of its salivary anticoagulants, vasodilators and anesthetics as well as by its blood feeding (phlebotomy).

Mode of action of Hirudotherapy

Leech therapy involves an initial bite, which is usually painless (leech saliva contains a mild anesthetic), and an attachment period lasting 20 to 45 minutes, during which the leech sucks between 5 and 15 ml of blood. Its main therapeutic benefits are not derived from the blood removed during the biting (although this may provide dramatic relief at first), but from the anticoagulant and vasodilator contained in the leech saliva. These properties permit the wound to ooze up to 50 ml of blood for up to 48 hours. Leech bites usually bleed for an average of six hours [23,24].

Salivary glands of a medical leech contain more than 100 bioactive substances and the salivary gland secretion has anti-edematous, bacteriostatic, and analgesic effects; it possesses resolving activity, eliminates microcirculation disorders, restores the damaged vascular permeability of tissues and organs, eliminates hypoxia (oxygen starvation), reduces blood pressure, increases immune system activity, detoxifies the organism by antioxidant pathways, relieves it from the threatening complications, such as infarct and strokes, and improves the bioenergetic status of the organism [12]. The molecules existing in leech saliva and the most studied to date include:

- Hirudin: An active principle in the salivary gland secretion of leeches, which acts as a potent anticoagulant (blood thinner). It inhibits blood coagulation by binding to thrombin [11,12,25-27].
- Hyaluronidase (spreading factor): Facilitates the penetration and diffusion of pharmacologically active substances into the tissues, especially in joint pain and has antibiotic properties [11,12,16].
- Calin: Inhibits blood coagulation by blocking the binding of the Von Willebrand factor to collagen. It inhibits collagen-mediated platelet aggregation [11,12,28].
- Destabilase: Dissolves fibrin and has thrombolytic effects. [11,12,29].
- Hirustasin: Inhibits kallikrein, trypsin, chymotrypsin, and neutropholic cathepsin G [11,30,31].
- Bdellins: Anti-inflammatory effect and inhibits trypsin, plasmin and acrocin [11, 27].
- Chloromycetyn: Potent antibiotic [31].
- Tryptase inhibitor: Inhibits proteolytic en-

zymes of host mast cells [11].

- Eglins: Anti-inflammatory. They inhibit the activity of alpha-chymotrypsin, chymase, substilisin, elastase, and cathepsin G [11,12,27].
- Factor Xa inhibitor: Inhibits the activity of coagulation factor Xa (very important role during the treatment of Osteo-arthritis and Rheumatoid arthritis) [12,32,33].
- Anesthetic-like substances: Reduce pain during biting by a leech [6,34].
- Histamine-like substances: A vasodilator increases the inflow of blood at the bite site [12,27,35].
- Complement inhibitors: Replace natural complement inhibitors if they are deficient.
- Carboxypeptidase-A inhibitors: Increase the inflow of blood [36,37].
- Acetylcholine: Vasodilator [27,30,38,39].
- Collagenase: Reduces collagen [12,33].

Procedure of Hirudotherapy

The area to be exposed to leeches should be cleaned with sterile distilled water. Leeches are then placed on the desired area. The animals normally start feeding immediately, although in rare cases the skin can be punctured with a sterile needle, so that oozing blood will stimulate the leeches to feed. The leech is placed on a given spot of the skin using a 5 ml syringe [40]. For this purpose, the plunger of the syringe is removed. The leech is placed in the barrel of the syringe and the open proximal end of the syringe is placed on the area to be treated. When the leech starts feeding, the syringe is removed [40]. Feeding usually lasts for 45-120 minutes, and during this time the leech is monitored. The patient should be regularly monitored during the therapy for various clinical parameters and infections or allergic reactions [24,41]. After auto-detachment, the leeches are removed. Even after the detachment of the leech, blood continues to ooze from the attachment site of the leech for hours.

One to 5 leeches are used for each session of treatment, depending on the clinical case and purpose of the treatment [40]. The bite area is cleaned every 3-4 hours with a gauze sponge soaked in physiological saline, to remove any locally forming clots, and with a heparin-soaked (5,000 U/ml) gauze, to increase the time of blood oozing. Used leeches are not used again, even on the same patient [21]. The detached leech is killed in 70% ethyl alcohol and is disposed of in bags as biological waste [24,42].

Indications of Hirudotherapy

In the past, leeches were used for a variety of applications; their mechanism of action behind this therapy was obscure, and all that mattered was curing or relieving the problem. Today, the research studies concerning the active substances in leeches have given us a better understanding of how the therapy works and have increased their therapeutic use. HT may be applied to various diseases known nowadays, due to its anticoagulant, vasodilator, and thrombolytic, anti-inflammatory and anaesthetizing qualities [43]. Indications cited by medical practitioners of leech therapy are as follows:

- Inflammatory Reactions [5,35].
- Passive congestions and spastic conditions [5,44,45].
- Plastic and reconstructive surgery [20,21,45-49].
- Cardiovascular diseases [5,11,48,50-54].
- Hypertension [54,55].
- Varicose Veins. [54,56].
- Hemorrhoids [10].
- Arthrosis, osteoarthritis, periarthritis and rheumatoid arthritis [12,55].
- Thrombophlebitis, thrombosis and embolism [57].
- Hematomas [49].
- External ear and chronic ear infections [55].
- Eye diseases, including cataracts, glaucoma, traumatic injuries and inflammation [12].
- Dental problems, like gingivitis, paradontitis, gingival edema and stomatorrhagia [12,55].
- Vertebrogenic Pain Syndromes [12].
- GI tract – hepatitis, cholecystitis, pancreatitis, stomach ulcers [13,55].
- Chronic skin diseases, like scabies, psoriasis, eczematous dermatitis, and chronic ulcers [1,12,55].
- Respiratory disorders - Asthma, acute rhinopharyngitis and spasmodic coryza [11,16, 55].
- Gynecological disorders - male and female sterility, endometriosis, parametritis, mastitis, fibromastopathy [12,55].

Contraindications of Hirudotherapy

HT cannot be employed in all the patients. The patients should be firstly examined for their health status. HT is not given under the following conditions:

- Absolute hemophilia [12,13]
- Anemia [12,13]
- Leukemia [12,13]
- Hypotonia [12,13]
- Pregnancy [12,13]

Applications of Hirudotherapy in Human Surgery

Modern leech therapy utilizing Hirudo medicinalis is based on sound scientific principles and has resulted in important patient care enhancements. Leech therapy is most often used in the settings of localized venous congestion associated with flap reconstructions and surgical replantations. Hirudotherapy has also been used to treat soft tissue swelling and hematomas in trauma [20,21,45-49].

Arthritis:

The leech's saliva assists in the treatment of arthritis [12,55]. There are a number of substances and compounds in its saliva that help to reduce inflammation in a joint: some of these compounds are bdelins and eglins, acting as anti-inflammatories [11,12,27]. Apart from anti-inflammatory components, its saliva also has an anesthetic component that alleviates the pain felt in the joint and also contains a histamine-like substance that acts as a vasodilator [12,38,39]. Acetylcholine, another component of the leech's saliva, is also a vasodilator [38,39]. This is important for the treatment of arthritis because as the vessels dilate, it increases the flow of blood, thus removing the compounds from the site, thereby relieving pain and inflammation.

Skin flap:

Leeches are being employed in skin flap transplantations [19,45,46,48]. As soon as the leeches attach themselves to the skin flap site, they begin to suck blood. During this, they also release a component called hirudin from their saliva [11,12,25-27]. This component is very vital for the inhibition of platelet aggregation and coagulation cascade. If these two detrimental complications continue to supervene in a skin flap, there will be marked venous congestion, which slows down the healing process of the skin graft. When venous outflow of the skin flaps is inadequate the flap becomes cyanotic and congested. The venous congestion further compromises the arterial circulation unless it is alleviated. Because of the presence of hirudin and the Factor Xa inhibitor in the leech's saliva, these processes are inhibited. Presence of the vasodilator component in their saliva further reduces venous congestion, promoting good blood flow into the skin flap. After continuous medicinal leech therapy, the skin flap soon turns warm and pinkish, indicating an adequate blood supply to the flap.

Venous congestion:

Leech therapy has been proven to help patients suffering from venous diseases [5,44,45]. It can help reduce the pain and the swelling, due to varicose veins, and can help dissolve blood clots. However, leech therapy is not effective for diseases caused by insufficient valves and inadequate vessel dilation. The saliva of leeches is known to contain beneficial enzymes, which prevent blood from coagulating. Apart from this, there is also an enzyme that breaks up thrombi. These two properties function to make the blood thin, so that it flows freely in the veins. To further aid in this function, another enzyme acts as a vasodilator to allow better blood flow. A leech's saliva also has antibacterial properties [22], which helps individuals who have open sores complicated by venous disease. Leech therapy is best used in conjunction with compression stockings, weight management, diet, and exercise.

Vascular diseases:

Vascular disorders and diseases are now being cured by leech therapy [54-58]. Their saliva has over 100 bioactive substances that are very beneficial. One such component is hirudin, which acts as an anticoagulation agent [11,12,25-27]. Calin is another component that also inhibits blood coagulation [11,12,28]. A component that dissolves fibrin clots as well as inhibits the formation of thrombus is the destabilase [11,12,29]. Leech saliva also contains a Factor Xa inhibitor, and this compound restrains the coagulating effect of the coagulation Factor Xa [12,32,33]. It also has hyaluronidase that enhances the viscosity of the interstitial fluid [11,12,16]. For a vasodilating effect, it has acetylcholine and histamine-like substances as well as carboxypeptidase-A inhibitors [12,36-39]. These three can increase blood flow by dilating constricted vessels.

These are just some of the very useful components in leech saliva, which work in melieu to decrease the viscosity of the blood, so as to promote better flow. Blood that has a thick consistency makes it prone to clot formation as well as increases the blood pressure of an individual. These clots can travel to different parts of the body and can block a vessel, which could then cause a stroke or heart attack. Thick blood poses a risk that the distal extremities, especially the tips of the fingers and toes, will not receive adequate oxygenated blood and the nutrients they need. Therefore, the anticoagulation component in a leech's saliva is vital, as it naturally reduces viscosity of blood and works to inhibit platelet coagulation. As stated above, the saliva's vasodilating components dilate or widen the blood vessels, thereby promoting a better blood flow. With all these components working together, there will be a remarkable improvement in the vascular status of the patient.

Complications of Hirudotherapy

Excessive bleeding can occur with leech therapy; it can be controlled by applying direct pressure or topical thrombin [59]. Excessive blood loss may necessitate a blood transfusion, so patients should be informed of the possibility [60]. Allergic responses, including anaphylaxis, can also occur. Patients and their families should be alerted to watch out for and report allergy symptoms. Scarring may also occur, but is usually minimal.

The most serious complication of leech therapy is infection. The leech's digestive system contains Aeromonas hydrophila, a Gram-negative bacillus that enables the breakdown of ingested blood. Although most infections involving leech therapy are caused by *A. hydrophila*, infections with *Serratia marcescens*, *A. sobria*, and *Vibrio fluvialis* have been reported [60-62]. Infections can arise 2 to 11 days after therapy begins and can result in abscesses and cellulitis, which can progress in some cases to sepsis [61]. In a five-year retrospective study, Sartor and colleagues found that infections arose in 4.1% of patients who received leech therapy [63]. Prophylactic antibiotics are usually recommended: double coverage (two antibiotics) during therapy and single coverage (one antibiotic) for two weeks afterward [59]. An established infection is treated with antibiotics, such as third-generation cephalosporins, along with aminoglycosides, fluoroquinolones, tetracycline, or trimethoprim [61,62]. Because infection is a serious adverse effect of leech therapy, the patient and family should also be instructed to observe for and report early signs and symptoms.

Future Prospects of Hirudotherapy

Leech therapy has a long history, going from popular and well accepted, to falling out of favor and being thought of as an unscientific home remedy, to coming back into current medical practice with strong scientific support. Compared to other techniques of complementary and natural therapy, HT can be learned relatively quickly and can reduce the complications arising from the excessive use of synthetic drugs. Nowadays, research is being conducted in various fields to determine the therapeutic role of leeches in various disease conditions, like male and female sterility, diabetes, prostate diseases, asthma, lupus erythromatosis and many more. Recently, HT has been successfully employed for relieving symptomatic cancer pain [64]. In view of all the facts about HT, efforts should be made in optimizing the success of medicinal leech therapy in clinical and private practice.

Conflict of interest statement

The authors do not declare any conflict of interest or financial support in this study.

References

1. Whitaker IS, Rao J, Izadi D, Butler PE. Historical Article: Hirudo medicinalis: ancient origins of, and trends in the use of medicinal leeches throughout history. Br J Oral Maxillofac Surg 2004;42:133-137.
2. Davis A, Appel T. Bloodletting Instruments in the National Museum of History and Technology. Smithsonian Institution Press, Washington, D.C., 1979; 34-36.
3. Irish JC, Gullane PJ, Mulholland S, Neligan PC. Medicinal leech in head and neck reconstruction. J Otolaryngol 2000;29:327-332.
4. Bernard Aschner. Theories and Philosophies of Medicine. Institude of History of Medicine and Medical Research, New Delhi, 1973; 242-253.
5. Weinfeld AB, Yuksel E, Boutros S, Gura DH, Akyurek M, Friedman JD. Clinical and scientific considerations in leech therapy for the management of

acute venous congestion: an updated review. Ann Plast Surg 2000;45:207-212.
6. Godfrey K. Uses of leeches and leech saliva in clinical practice. Nurs Times 1997;93:62-63.
7. Cole D. Clinical hirudology: revival of an ancient art. N Z Med J 1985;98:28-29.
8. Ahmad T, Anwar M. Clinical importance of leech therapy. Indian Journal of Traditional Knowledge 2009;8:443-445.
9. Haycox CL, Odland PB, Coltrera MD, Raugi GJ. Indications and complications of medicinal leech therapy. J Am Acad Dermatol 1995;33:1053-1055.
10. Fort CW. Leech Therapy: Current Uses for an Old Treatment. Delaware Nurses Association (DNA) Reporter 2001;26:16-17
11. Eldor A, Orevi M, Rigbi M. The role of the leech in medical therapeutics. Blood Rev 1996;10:201-209.
12. Glyova O. Modern Hirudotherapy — A Review. (Biotherapeutics, Education and Research Foundation). The (BeTER) LeTTER 2005;2:1-3.
13. Mory RN, Mindell D, Bloom DA. The leech and the physician: biology, etymology, and medical practice with Hirudinea medicinalis. World J Surg 2000;24:878-883.
14. Munshi Y, Ara I, Rafique H, Ahmad Z. Leeching in the history--a review. Pak J Biol Sci 2008;11:1650-1653.
15. Fields WS. The history of leeching and hirudin. Haemostasis 1991;21 Suppl 1:3-10.
16. Adams SL. The medicinal leech. A page from the annelids of internal medicine. Ann Intern Med 1988;109:399-405.
17. Wells MD, Manktelow RT, Boyd JB, Bowen V. The medical leech: an old treatment revisited. Microsurgery 1993;14:183-186.
18. Sviridova L. Leech therapy in recent times. 8th International Conference on Biotherapy, Abstract no:23, November 11-14, 2010, Los Angeles, CA. [Abstract and Handouts, Available via http://www.bterfoundation.org/icb/abstract-book_icb-2010.pdf (Accessed 15 May 2012)].
19. Henderson HP, Matti B, Laing AG, Morelli S, Sully L. Avulsion of the scalp treated by microvascular repair: the use of leeches for post-operative decongestion. Br J Plast Surg 1983;36:235-239.
20. Mutimer KL, Banis JC, Upton J. Microsurgical reattachment of totally amputated ears. Plast Reconstr Surg 1987;79:535-541.
21. Whitaker IS, Izadi D, Oliver DW, Monteath G, Butler PE. Hirudo Medicinalis and the plastic surgeon. Br J Plast Surg 2004;57:348-353.
22. 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;47:70-73.
23. Ikizceli I, Avsarogullari L, Sözüer E, Yürümez Y, Akdur O. Bleeding due to a medicinal leech bite. Emerg Med J 2005;22:458-460.
24. Kowalczyk T. A low-tech approach to venous congestion. RN 2002;65:26-30.
25. Chang JY. The functional domain of hirudin, a thrombin-specific inhibitor. FEBS Lett 1983;164:307-313.
26. Bichler J, Fritz H. Hirudin, a new therapeutic tool? Ann Hematol 1991;63:67-76.
27. Seemüller U, Dodt J, Fink E, Fritz H. Proteinase inhibitors of the leech Hirudo medicinalis (hirudins, bdellins, eglins). In: Barrett AJ, Salvesen G (eds). Proteinase Inhibitors, Elsevier Science Ltd, New York, 1986: 337-359.
28. Munro R, Jones CP, Sawyer RT. Calin--a platelet adhesion inhibitor from the saliva of the medicinal leech. Blood Coagul Fibrinolysis 1991;2:179-184.
29. Zavalova LL, Baskova IP, Lukyanov SA, Sass AV, Snezhkov EV, Akopov SB, et al. Destabilase from the medicinal leech is a representative of a novel family of lysozymes. Biochim Biophys Acta 2000;1478:69-77.
30. Söllner C, Mentele R, Eckerskorn C, Fritz H, Sommerhoff CP. Isolation and characterization of hirustasin, an antistasin-type serine-proteinase inhibitor from the medical leech Hirudo medicinalis. Eur J Biochem 1994;219:937-943.
31. Sawyer RT. Leech Biology and Behavior: Volume II: Feeding Biology, Ecology, and Systematics. Oxford Science Publications, USA, 1986
32. Hofmann KJ, Nutt EM, Dunwiddie CT. Site-directed mutagenesis of the leech-derived factor Xa inhibitor antistasin. Probing of the reactive site.

Biochem J 1992;287 (Pt 3):943-949.
33. Rigbi M, Jackson CM, Latallo ZS. A specific inhibitor of factor Xa in the saliva of the medicinal leech Hirudo medicinalis. 14th International Congress of Biochemistry; Abstracts FR 037, p. 53, July 10-15, 1998; Prague.
34. Rigbi M, Levy H, Eldor A, Iraqi F, Teitelbaum M, Orevi M, et al. The saliva of the medicinal leech Hirudo medicinalis--II. Inhibition of platelet aggregation and of leukocyte activity and examination of reputed anaesthetic effects. Comp Biochem Physiol C 1987;88:95-98.
35. Kumar SA, Prakash SO. Analgesic and Anti-inflammatory activity of leech therapy in the management of arthritis. (International Research Journal Of Pharmacy) IRJP 2011;2:172-174.
36. Reverter D, Vendrell J, Canals F, Horstmann J, Avilés FX, Fritz H, et al. A carboxypeptidase inhibitor from the medical leech Hirudo medicinalis. Isolation, sequence analysis, cDNA cloning, recombinant expression, and characterization. J Biol Chem 1998;273:32927-32933.
37. Reverter D, Fernández-Catalán C, Baumgartner R, Pfänder R, Huber R, Bode W, et al. Structure of a novel leech carboxypeptidase inhibitor determined free in solution and in complex with human carboxypeptidase A2. Nat Struct Biol 2000;7:322-328.
38. Michalsen A, Roth M, Dobos G. Medicinal Leech Therapy. Thieme, Stuttgart, Germany, 2007.
39. Zaidi SM, Jameel SS, Zaman F, Jilani S, Sultana A, Khan SA. A systematic overview of the medicinal importance of sanguivorous leeches. Altern Med Rev 2011;16:59-65.
40. Mumcuoglu KY, Pidhorz C, Cohen R, Ofek A, Lipton HA: The use of the medicinal leech, Hirudo medicinalis, in the reconstructive plastic surgery. The Internet Journal of Plastic Surgery 2007;4(2).
41. Chepeha DB, Nussenbaum B, Bradford CR, Teknos TN. Leech therapy for patients with surgically unsalvageable venous obstruction after revascularized free tissue transfer. Arch Otolaryngol Head Neck Surg 2002;128:960-965.
42. Abdelgabar AM, Bhowmick BK. The return of the leech. Int J Clin Pract 2003;57:103-105.
43. de Chalain TM. Exploring the use of the medicinal leech: a clinical risk-benefit analysis. J Reconstr Microsurg 1996;12:165-172.
44. Conforti ML, Connor NP, Heisey DM, Hartig GK. Evaluation of performance characteristics of the medicinal leech (Hirudo medicinalis) for the treatment of venous congestion. Plast Reconstr Surg 2002;109:228-235.
45. Derganc M, Zdravic F. Venous congestion of flaps treated by application of leeches. Br J Plast Surg 1960;13:187-192.
46. Knobloch K, Gohritz A, Busch K, Spies M, Vogt PM. [Hirudo medicinalis-leech applications in plastic and reconstructive microsurgery--a literature review]. [Article in German]. Handchir Mikrochir Plast Chir 2007;39:103-107.
47. Mutimer KL, Banis JC, Upton J. Microsurgical reattachment of totally amputated ears. Plast Reconstr Surg 1987;79:535-541.
48. Kraemer BA, Korber KE, Aquino TI, Engleman A. Use of leeches in plastic and reconstructive surgery: a review. J Reconstr Microsurg 1988;4:381-386.
49. Riede F, Koenen W, Goerdt S, Ehmke H, Faulhaber J. Medicinal leeches for the treatment of venous congestion and hematoma after plastic reconstructive surgery. J Dtsch Dermatol Ges 2010;8:881-888.
50. Cherniack EP. Bugs as drugs, part two: worms, leeches, scorpions, snails, ticks, centipedes and spiders. Altern Med Rev 2011;16:50-58.
51. Ascenzi P, Amiconi G, Bode W, Bolognesi M, Coletta M, Menegatti E. Proteinase inhibitors from the European medicinal leech Hirudo medicinalis: structural, functional and biomedical aspects. Mol Aspects Med 1995;16:215-313.
52. Shah S. Why leeches influence my physical examination. Lancet 1998;352:2014-2015.
53. Orevi M, Rigbi M, Hy-Am E, Matzner Y, Eldor A. A potent inhibitor of platelet activating factor from the saliva of the leech Hirudo medicinalis. Prostaglandins 1992;43:483-495.
54. Ahmad T, Anwar M. Clinical importance of Leech therapy. Indian Journal of Traditional Knowledge 2009;8:443-445.
55. Gileva OS. Modern hirudotherapy: Experimental

background and clinical efficacy. 8th International Conference on Biotherapy, Abstract no:22, November 11-14, 2010, Los Angeles, CA. [Abstract and Handouts, Available via http://www.bter-foundation.org/icb/abstract-book_icb-2010.pdf (Accessed 15 May 2012)]

56. Niqar Z, Alam MA. Effect of taleeq (leech therapy) in dawali (varicose veins). Anc Sci Life 2011;30:84-91.
57. Pospelova ML, Barnaulov OD. Hirudotherapy in the treatment of bilateral internal carotid artery occlusion: Case report. Curr Top Neurol Psychiatr Relat Discip 2010;18:51-53.
58. Porshinsky BS, Saha S, Grossman MD, Beery Ii PR, Stawicki SP. Clinical uses of the medicinal leech: a practical review. J Postgrad Med 2011;57:65-71.
59. Whitaker IS, Elmiyeh B, Wright DJ. Hirudo medicinalis: the need for prophylactic antibiotics. Plast Reconstr Surg 2003;112:1185-1186.
60. Chepeha DB, Nussenbaum B, Bradford CR, Teknos TN. Leech therapy for patients with surgically unsalvageable venous obstruction after revascularized free tissue transfer. Arch Otolaryngol Head Neck Surg 2002;128:960-965.
61. Abdelgabar AM, Bhowmick BK. The return of the leech. Int J Clin Pract 2003;57:103-105.
62. Ardehali B, Hand K, Nduka C, Holmes A, Wood S. Delayed leech-borne infection with Aeromonas hydrophilia in escharotic flap wound. J Plast Reconstr Aesthet Surg 2006;59:94-95.
63. Sartor C, Limouzin-Perotti F, Legré R, Casanova D, Bongrand MC, Sambuc R, et al. Nosocomial Infections with Aeromonas hydrophila from Leeches. Clin Infect Dis 2002;35:E1-5.
64. Kalender ME, Comez G, Sevinc A, Dirier A, Camci C. Leech therapy for symptomatic relief of cancer pain. Pain Med 2010;11:443-445.