

An Overview on Hirudotherapy/Leech therapy

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ABSTRACT:

Hirudo medicinalis (Medicinal leeches) therapeutically used up in Hirudotherapy (HT) in conventional treatment of pain and inflammatory diseases. There is now renewed interest in leeches therapy in the field of complementary medicine. These, being a class of segmented invertebrates, best known for their blood-feeding habits (sanguivorous) and used in phlebotomy to treat various ailments, by medicinal practitioners. Here, cultured leeches are attached onto the affected areas proceeding with an initial bite (painless), followed by the sucking of blood. Sales of the four principal German traders have increased continuously throughout the past few years and led to an estimated 70,000 treatments (350,000 leeches sold/ year, four to five used for each single treatment) yearly in Germany (Roth M, unpublished data). The majority of these treatment benefits are not due to blood sucked during the biting, rather the various bioactive ingredients of the saliva, such as Hyaluronidase, Hirudin, Calin, Destabilase, Apyrase, Eglin and many others.

Treatments aim at pain reduction in regional pain syndromes, mostly for knee osteoarthritis or various joint ailments. Application extends in plastic surgery to maintain blood flow in congested skin flaps, reconstructive surgeries, cardiovascular complications, varicose veins, hemorrhoids, gastrointestinal disorders, dermatology, gynecological abnormalities and traumatic conditions such as re-attachment of severed extremities, finger, toes and ear. Recently, it has found new applications in cancer therapy, hypersensitivity conditions, like asthma, male/female sterility and diabetes. With increasing therapeutic potential of HT, researchers are continuing as many new salivary compounds to isolated and synthesized for clinical and private practice.

Keywords: Hirudotherapy, leech, annelid, saliva, bioactive, hirudin, blood-thinning.

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INTRODUCTION:

Since past, leeches have been historically documented in our literature and are recognized as both, a parasite and a therapeutic agent. This species survives well in temperate climates and muddy freshwater pools having weed growth serve as their natural habitat. They are sanguivorous or blood-sucking annelid worms which have gained popularity not only in the field of medical science, but also in dentistry. Their use had been practiced over the past in almost every region of the world. Different species of medicinal leeches are popular, among which, the most commonly known is the *Hirudo medicinalis* (Figure 1), a fresh water hermaphrodite, also known as the European Medical Leech. Other *Hirudo* species sometimes used as medicinal leeches are *H. orientalis*, *H. troctina*, *H. verbana*, *H. manillensis* or the Asian Medical Leech as *Hirudinaria granulosa*. Beside these *Macrobdella decora* or the North American Medical Leech is also used up.¹

It possesses a unique ability to remove blood by using their proboscis to puncture through the skin and simultaneously release an anaesthetic to eliminate pain of the bite inflicted. Salivary components are released which prevent the blood from clotting and allow maintenance of the blood-thinning substances (anticoagulants) procedure. Sucked blood is stored in lateral diverticula of the crop and, as the blood passes down the pharynx, it is mixed with a glandular secretion that prevents its coagulation. A leech ingests several times its own weight of blood at a single meal that may suffice for several months.²

Since antiquity, physicians, who were of the belief that diseases were the result of an imbalance of various humors inferred that the body can be stabilized by releasing blood with the help of leech.³ With span 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. Only the leeches, which are grown in farms and which have undergone strict quarantine, are employed for the therapy. A leech is used for a single treatment and wild leeches are not used anymore.^{4,5}



Fig1. *Hirudo medicinalis*

HISTORY:

Leeches were used for bleeding by Syrian physicians as early as 100 BC. Historically, the leeches were placed or directed to small areas such as the mouth, ear or other areas using a “leech tube” (ear infections, sinusitis). Leech therapy was documented in Sanskrit writings from 1300 BC. Themison of Laodicea also reported HT in 50 BC.⁶ Therapeutic uses 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.⁷ Leech therapy is well documented in the works of Pliny, Galen, Themison and Avicenna.⁸

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 1904.⁹ Hirudin was employed in a blood transfusion in 1915.

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 tissue-flap transplantation. In 1983, Henderson et al. reported a case where leeches were used in the post-operative treatment of a scalp avulsion case.¹⁰

In 1985, Harvard physician Joseph Upton used medicinal leeches to successfully reattach the ear of a five-year-old boy.¹¹ 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.¹²

Description of leeches:

Leeches once fed are satiated for 30-50 days. Once stomach contents are depleted, leeches can survive for months on its own body substance. Leeches are “protandrous” –first male then female and lay eggs in cocoons (1-9) months post copulation – about 4 cocoons each containing about 15 eggs-about 60 off-springs per year. Medicinal leech (*Hiruda medicinalis*) is a segmented annelid belonging to **Phylum**: Annelida, **Class**: Clitellata and **Subclass**: Hirudinea (Lamark, 1818).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. Each of the three jaws has 100 teeth, for a total of 300 teeth.¹⁴

Medicinally importance leeches inhabit clean, fresh waters. They 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. Young leeches feed on the blood of small water animals, like frogs and fish. They become ready for medicinal use after they are several years old.¹⁵

Secrets of Salivary Glands of Medical Leech:

It contains more than 100 bioactive substances (few are known in terms of chemical structure and mechanism) including bacteriostatic, analgesic, resolving actions, has anti-oedematous, improves immune system activity, it eliminates microcirculation disorders, restores permeability of tissues and organs, eliminates hypoxia, reduces BP, detoxifies the organism.

Benefits of Leech Saliva:

The molecules existing in leech saliva and the most studied to date include:^{7, 8, 16}

Bioactive Ingredients	Benefits
Hyaluronidase (spreading factor):	For penetration and diffusion of pharmacologically active substances into the tissues, especially in joint pain and has antibiotic properties.
Hirudin (a potent anticoagulant):	Enables the blood to flow for some time without clotting. It inhibits blood coagulation by binding to thrombin.
Calin:	Inhibits blood coagulation by blocking the binding of the Von Willebrand factor to collagen and can last upto 12 hours.
Destabilase:	Dissolves fibrin and has thrombolytic effects.
Bdellins:	Anti-inflammatory effect and inhibits trypsin, plasmin and acrocin.
Acetylcholine:	A vasodilator.
Histamine-like substances:	A vasodilator increases the inflow of blood at the bite site.
Hirustasin:	Inhibits kallikrein, trypsin, chymotrypsin, and neutrophilic cathepsin G.
Eglins:	Anti-inflammatory. They inhibit the activity of alpha-chymotrypsin, chymase, subtilisin, elastase, and cathepsin G.
Factor Xa inhibitor:	Inhibits the activity of coagulation factor Xa (very important role during the treatment of Osteo-arthritis and

	Rheumatoid arthritis).
Carboxypeptidase-A inhibitors:	Increase the inflow of blood.
Collagenase:	Reduces collagen.
Complement inhibitors:	Replace natural complement inhibitors if they are deficient.

Hirudin (a potent anticoagulant):

A thrombin inhibitor similar to hirudin, known as bufrudin, has been isolated from the leech *Hirudo manillensis*, which differs in its structural and immunological properties. Application of hirudin or derivatives may be indicated for prophylaxis and treatment of postoperative venous thrombosis, especially in cardiac surgery; enhancement of fibrinolytic therapy and/or angioplasty to prevent reocclusion; and plastic surgery. Hirudin may be a useful alternative anticoagulant, particularly in patients sensitized to heparin or in patients with hereditary or acquired antithrombin III deficiency. Due to its great demand, various recombinant systems have been developed, using bacteria, yeasts, and higher eukaryotes to obtain significant quantities of biologically active hirudin.¹⁷

Recombinant hirudins are currently undergoing clinical trials in deep venous thrombosis and acute coronary syndromes and as an adjuvant to thrombolysis in myocardial infarction. Since the common antithrombotic agents used in therapy (heparin, aspirin, coumarin derivatives) do not always have the desired effectiveness or cause complications,¹⁸ hirudin and its analogs show promise as novel therapeutic agents and are excreted in unchanged form in the urine.¹⁹

Hyaluronidase (spreading factor):

Hyaluronidase from the leech, therefore, is the most specific enzyme known for identification of hyaluronic acid. It reduces the viscosity and renders the tissues more readily permeable to injected fluids,²⁰ increasing the speed of absorption. This promotes resorption of excess fluids and extravasated blood in the tissues and increases the effectiveness of local anesthesia. Hence, hyaluronidase from leech saliva helps increase the spread of all salivary secretions. Currently, it is being examined in *ex vivo* studies for drug delivery through human skin. It has also been investigated as an additive to chemotherapeutic drugs for augmentation of the anticancer effect.²¹

Calin:

A pharmacological preparation of calin was tested in vitro and in vivo for activity in a thrombosis model in hamsters. Calin specifically and dose dependently (IC₅₀ 6.5 to 13 µg/mL) inhibited platelet aggregation induced by collagen.²²

Apyrase:

(adenosine 5'-diphosphate diphosphohydrolase) is a nonspecific inhibitor of platelet aggregation by virtue of its action on adenosine 5' diphosphate, arachidonic acid, platelet-activating factor (PAF), and epinephrine. Two apyrases (isoenzymes) have been isolated from the saliva of medicinal leeches.^{23,24}

Decorsin:

Decorsin is a protein isolated from American medicinal leech *Macrobdella decora*. It acts as an antagonist of platelet glycoprotein II b-III a and is a potent inhibitor of platelet aggregation.²⁵

Guamerin:

A new human leukocyte elastase inhibitor has been extracted and purified from a Korean native leech, *Hirudo nipponia*. The complete amino acid sequence of guamerin reveals a cysteine-rich polypeptide of 57 amino acid residues that shows no similarity to any known elastase inhibitors, but has 51-percent sequence homology with hirustasin.²⁶

Piguamerin:

Piguamerin is a serine protease inhibitor of plasma kallikrein that has been screened and purified from the Korean leech, *Hirudo nipponia*. The peptide potently inhibits plasma and tissue kallikrein and trypsin.²⁷

Gelin:

Gelin is a potent thrombin inhibitor analogous to eglin, and is isolated from the saliva of the *Hirudinaria manillensis*, a leech belonging to the same family as *Hirudo medicinalis*. Like eglin, gelin inhibits elastase, cathepsin G, and chymotrypsin, but has little or no activity on plasmin, thrombin, pepsin, or trypsin.²⁷

Action and procedure 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.^{28, 29}

Area to be exposed to leeches should be cleaned with sterile distilled water and then placed on the desired area. They 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.³⁰ 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. 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. 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. 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.¹² The detached leech is killed in 70% ethyl alcohol and is disposed of in bags as biological waste.²⁹

Leech bites sometimes leave small blood spots (ecchymoses) which sometimes develop into keloids in some individuals. Most of these spots disappear within 2-3 weeks.

Indication & Utility of Leech therapy: ^{8,31}

- It is used effectively in the management of non- healing ulcerative lesions like Diabetic ulcer, Leprotic wound etc. as it helps to improve the local blood circulation. Hence, healing is promoted.
- It relieves vascular congestion. So, can be effectively used in conditions like long standing varicose ulcers, filariasis, post-operation and skin grafting lesions.
- It is used in Arthritis, Sprain or spasm to relieve the pain, inflammation & discomfort symptomatically.
- Used in Abscess, Cellulites, Thrombophlebitis and Varicose Veins
- Useful in third degree thrombosed prolapsed Piles.
- Useful in Atherosclerosis of the limb as it improves circulation.
- Jaloka siddha oil is used locally to treat hyper pigmentation.
- Paradontosis and other teeth diseases.
- Male sterility.
- Skin diseased – neurodermatitis, psoriasis, herpes, eczema.
- Hirudo-relexotherapy used by placing the leeches strategically on the reflexogenic points as a “life needle” .
- Gynaecological disorders – female sterility, endometriosis, fibromastopathy.
- Plastic and reconstructive surgery.
- Eye diseases, including cataracts, glaucoma, traumatic injuries and inflammation.
- GI tract – hepatitis, cholecystitis, pancreatitis, stomach ulcers.
- Respiratory disorders - Asthma, acute rhinopharyngitis and spasmodic coryza.
- Research is going on to study the efficacy of Leech in the management of MI (where it is used around the pericardium) & stroke as it has got an anti- coagulant chemical called Hirudin which resembles drugs like Heparin & Strepto- kinase.

Leeches may not bite if:

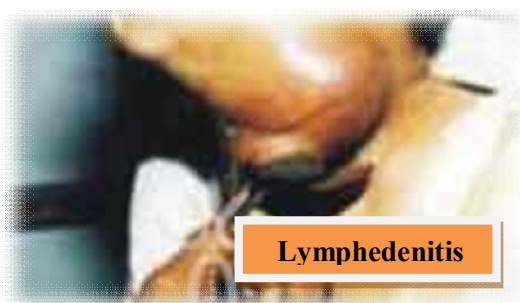
- The skin is cold.
- Older humans.
- Smokers.

- Perfumed skin.

(In these cases wash area thoroughly and sometimes a pin prick may help for attachment).

Contraindications of Hirudotherapy: ^{8,32}

- Anaemia.
- In extreme ages i.e. in children and old age.
- Weak patients.
- Allergic patients.
- In extreme hot or cold climate.
- Diseases like haemophilia.
- Pregnancy.



Identification of Non- poisonous Leech: ³⁹

The Non- poisonous or 'Nirvish Jalokas' can be safely identified by their

1. Yellowish- Manhshila-like colour,
2. Swift activity or
3. Glitter like or copper like colour,
4. Wide lotus- bud like mouth.

(Jalokas: - 'Jal' i.e. water, the 'Leeches' are known as 'Jaloka'.)

Application in Humans:

Venous congestion: It been proven to help patients suffering from venous diseases which reduces the pain and the swelling, due to varicose veins, and can help dissolve blood clots. It is not effective for diseases caused by insufficient valves and inadequate vessel dilation. The saliva of leeches contains 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, 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.^{40,41} If venous congestion is not corrected (either surgically or via some other means), the developed stasis within the vasculature of the tissue will cause the replanted region to necrose respectively.

Skin flap: They are being employed in skin flap transplantations. As soon as the leeches attach themselves to the skin flap site, they begin to suck blood. They release hirudin from their saliva which is very vital for the inhibition of platelet aggregation and coagulation cascade. If these two 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 respectively.^{42,43}

Arthritis: Saliva of leech assists in the treatment of arthritis as it consists of number of substances and compounds in its saliva that helps to reduce inflammation in a joint. Compounds are bdelins and eglins, acting as anti-inflammatory. Beside these, 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. Acetylcholine, another component of the leech's saliva, is also a vasodilator. 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.^{44,45}

Complications:

It being an innovative approach in medical science, still its uses is accompanied by various complications, the most common being prolonged bleeding. Other reported complications are allergic reactions and bacterial infections.³³ The bacteria aeromonas hydrophilia present in gut of leech, can cause pneumonia, septicemia or gastroenteritis. Allergic reactions such as itching followed by burning and blister formation and ulcerative necrosis due to toxins present in leech saliva have also been reported after leech therapy.³⁴ Transmission of certain infections from one subject to the other is another probable complication of leech therapy. Hence, it is mandatory to rule out the selected cases for certain conditions by performing a series of required hematological or serological

investigations. Few such conditions include various blood borne infections like HIV and hepatitis, blood disorders like hemophilia, thrombocytopenia and conditions like pregnancy and anemia.³⁴

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.³⁵ In a five-year retrospective study, Sartor and colleagues found that infections arose in 4.1% of patients who received leech therapy.³⁶ Prophylactic antibiotics are usually recommended: double coverage (two antibiotics) during therapy and single coverage (one antibiotic) for two weeks afterward.³⁷ An established infection is treated with antibiotics, such as third-generation cephalosporins, along with aminoglycosides, fluoroquinolones, tetracycline, or trimethoprim.³⁵

Summary and Outlook:

Leeches nowadays are used successfully, ranging from the field of reconstructive or microsurgery, to salvage tissue flaps and skin grafts whose viability is threatened by venous congestion. The anticoagulant properties of hirudin of leech, lead to a wider therapeutic applications in the prevention and treatment of thromboembolic diseases. Its application in dentistry is yet unexplored and this should motivate further research on its use in treatment of various dental disorders. At the same time optimal care should be taken when applying leeches, because their use can be associated with serious complications.³⁸ In comparison to other techniques of complementary and natural therapy, HT is relatively quick and can reduce the complications arising from the excessive use of synthetic drugs.

Further using recombinant DNA technology, scientists are exploring the potential of other therapeutically active compounds. Pharmaceutical companies are seeking to expand the leech salivary components for various uses. Identification of the neurite-stimulating activity of the salivary components provides new therapeutic agents for the treatment of neurodegenerative diseases.

Conflict of interest statement:

The author declares that there are no conflicts of interest.

REFERENCES:

1. A.P Singh. Complementary Therapies in Clinical Practice (2009), doi:10.1016/j.ctcp.2009.11.005
2. Bhatia ML. Hirudinaria ("e Indian Cattle Leech). Lucknow, India: Indian Zoological Memoirs; 1941.
3. Bernard Aschner. Theories and Philosophies of Medicine. Institute of History of Medicine and Medical Research, New Delhi, 1973; 242-253.
4. Godfrey K. Uses of leeches and leech saliva in clinical practice. Nurs Times 1997; 93:62-63.
5. Cole D. Clinical hirudology: revival of an ancient art. N Z Med J 1985; 98:28-29.
6. Haycox CL, Odland PB, Coltrera MD, Raugi GJ. Indications and complications of medicinal leech therapy. J Am Acad Dermatol 1995; 33:1053-1055.
7. Eldor A, Orevi M, Rigbi M. The role of the leech in medical therapeutics. Blood Rev 1996; 10:201-209.
8. Glyova O. Modern Hirudotherapy — A Review. (Biotherapeutics, Education and Research Foundation). The (BeTER) LeTTER 2005; 2:1-3.
9. Fields WS. The history of leeching and hirudin. Haemostasis 1991; 21 Suppl 1:3-10.
10. 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.
11. Mutimer KL, Banis JC, Upton J. Microsurgical reattachment of totally amputated ears. Plast Reconstr Surg 1987; 79:535-541.
12. Whitaker IS, Izadi D, Oliver DW, Monteath G, Butler PE. Hirudo Medicinalis and the plastic surgeon. Br J Plast Surg 2004; 57:348-35
13. Davis A, Appel T. Bloodletting Instruments in the National Museum of History and Technology. Smithsonian Institution Press, Washington, D.C., 1979; 34-36.
14. Fort CW. Leech Therapy: Current Uses for an Old Treatment. Delaware Nurses Association (DNA) Reporter 2001; 26:16-17
15. 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
16. Söllner C, Mentele R, Eckerskorn C, Fritz H, Sommerhoff CP. Isolation and characterization of hirustasin, an antistatin-type serine-proteinase inhibitor from the medical leech Hirudo medicinalis. Eur J Biochem 1994; 219:937-943.
17. Field WS. The history of leeching and hirudin. Haemostasis 1991; 21:3-10.
18. Becker RC. thrombin antagonists and antiplatelet agents. Am J Cardiol 1992; 69: 39A-51A.
19. Cannon CP, McCabe CH, Henry TD, et al. A pilot trial of recombinant desulfatohirudin compared with heparin in conjunction with tissue-type plasminogen activator and aspirin for acute myocardial infarction: results of the thrombolysis in Myocardial Infarction (TIMI) 5 trial. J Am Coll Cardiol 1994; 23:993-1003.
20. Menzel EJ, Farr C. Hyaluronidase and its substrate hyaluronan: biochemistry, biological activities and therapeutic uses. Cancer Lett 1998; 131:3-11.
21. Frost G, Csoka T, Stern R. The hyaluronidases: a chemical, biological and clinical overview. Trends Glycosci Glycotechnol 1996;8:419-434

22. Deckmyn H, Stassen JM, Vreys I, et al. Calin from *Hirudo medicinalis*, an inhibitor of platelet adhesion to collagen, prevents platelet-rich thrombosis in hamsters. *Blood* 1995; 85:712-719.
23. Rigbi M, Orevi M, Eldor A. Platelet aggregation and coagulation inhibitors in leech saliva and their roles in leech therapy. *Semin "romb Hemost* 1996; 22:273-278.
24. Rigbi M, Levy H, Iraqi F, et al. The saliva of the medicinal leech *Hirudo medicinalis*-I. Biochemical characterization of the high molecular weight fraction. *Comp Biochem Physiol B* 1987; 87:567-573.
25. Seymour JL, Henzel WJ, Nevins B, et al. Decorsin. A potent glycoprotein IIb-IIIa antagonist and platelet aggregation inhibitor from the leech *Macrobdella decora*. *J Biol Chem* 1990; 265:10143-10147.
26. Jung HI, Kim SI, Ha KS, et al. Isolation and characterization of Guamerin, a new human leucocyte elastase inhibitor from *Hirudo nipponia*. *J Biol Chem* 1995; 270:13879-13884.
27. Kim DR, Kang KW. Amino acid sequence of piguamerin, an antistasin-type protease inhibitor from the blood sucking leech *Hirudo nipponia*. *Eur J Biochem* 1998; 254:692-697.
28. Ikizceli I, Avsarogullari L, Sözüer E, Yürümez Y, Akdur O. Bleeding due to a medicinal leech bite. *Emerg Med J* 2005; 22:458-460.
29. Kowalczyk T. A low-tech approach to venous congestion. *RN* 2002; 65:26-30.
30. 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).
31. Niqar Z, Alam MA. Effect of taleeq (leech therapy) in dawali (varicose veins). *Anc Sci Life* 2011;30:84-91
32. 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
33. M D Wells , R T Manktelow, J B Boyd , V Bowen. *Microsurgery*, 1993; 14 (3): 183–186.
34. Michel Salzet. *Federation of European Biochemical Societies*, 2001; 492: 187- 192.
35. Abdelgabar AM, Bhowmick BK. The return of the leech. *Int J Clin Pract* 2003; 57:103-105.
36. 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.
37. Whitaker IS, Elmiyeh B, Wright DJ. *Hirudo medicinalis*: the need for prophylactic antibiotics. *Plast Reconstr Surg* 2003; 112:1185-1186.
38. A M Abdelgabar, B K Bhowmick. *Int J Clin Pract*. 2003; 57(2):103-5.
39. An article by Dr.Amar Dwivedi, M.S. (Ayu.) Associate professor, Shalya Tantra Dept. Dr.D.Y.Patil Ayurved College and Hospital, Nerul, Navi Mumbai
40. 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.
41. Derganc M, Zdravic F. Venous congestion of flaps treated by application of leeches. *Br J Plast Surg* 1960;13:187-192
42. Derganc M, Zdravic F. Venous congestion of flaps treated by application of leeches. *Br J Plast Surg* 1960; 13:187-192.

43. 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.
44. 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.bterfoundation.org/icb/abstract-book_icb-2010.pdf (Accessed 15 May 2012)]
45. 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