

Hirudotherapy-A Cure in Vampire's kiss:A Review

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Abstract:

Leeching is an art dating back to atleast to ancient Egypt where Pharaohs' tombs were decorated with leech like images. Surgeons have used the leech (*Hirudo medicinalis*) for more than 2,500 years. They were used because of their excellent decongestive properties to treat venous congestion in otherwise unsalvable skin flaps and replants, to treat different cardiovascular diseases and various other systemic diseases. But leeching had lost its favor during World war II due to the invention of anticoagulants. The achievements of modern biology and biochemistry show that the multiple curative effects of the leech are determined by its saliva and so in the last 10 -15yrs there has been a resurgence of interest in the leech therapy among physicians.

Key Words: Hirudotherapy, leech therapy, Bloodletting, Leech saliva, Leeching.

INTRODUCTION:-

Bloodletting is an ancient art in which blood was drawn out from the body or diseased area in the hope that removing impure blood would heal the body of its illness and disease [1-3]. It was practiced according to the humoral theory, which proposed that the four humors blood, phlegm, black and yellow bile in the human body should be in balance for good health. An imbalance in this proportion of these humors has been believed to be the cause of ill health [4]. The first record concerning bloodletting by cutting veins and venesection, were found in the Hippocrates collection in 5th century [5].

Venesection and arteriotomy done with lancet, scarifier or fleam were thought to abate disease by the general effect of bloodletting on the body. Usage of leeches in this procedure was considered to be less painful and more dependable in removing a fixed amount of blood. So leeches eventually become a more popular alternative to the mechanical instrument of bloodletting[1]. The word leech is likely derived from the old English word for physician "laece" [6]. Homers use of the term leech is somewhat enigmatic since the Greek word "ieteroi" can be translated as "leeches" in its archaic & obsolete sense of physician or surgeon. Even admitting the possibility of a double entendre in the use of the term, it is noteworthy that from medieval times through the age of enlightenment, the term leech was used to designate English physicians; moreover, even Anglo-saxon medical writing often referred to as "leech books"[2].

According to Sanskrit writings, Shri.Dhanvantari, the "God of vaidyas" brought the knowledge of ayurveda and surgery to the earth. He has Shankh, Chakra, Amruta and Jalouka in his hands. Jalouka means Leech, in one of Lord Dhanvantari's hands represents as a para surgical instrument used in all the detoxifying procedures of

Ayurveda (Panchkarma), for sucking out the 'impure' blood harmlessly. Sushrut, an ancient Indian surgeon, known as father of surgery, taught and practiced his art on the banks of the Ganges in the city of Varanasi. In his book "Sushruta Samhita", he has devoted an entire chapter named "Jalokavacharniya Adhayay" describing about scientific method of leech uses and its applications.

The practice of leeching or hirudotherapy can be traced to ancient India and Greece, and continued well into the 18th and 19th centuries in Germany, Europe especially more in Russia and North America. In modern times, the practice of leeching is much rarer and has been replaced by other contemporary uses of leeches, such as the reattachment of body parts and reconstructive and plastic surgeries. Hirudotherapy is the best alternative in treating illness and may even surpasses pharmacological treatments. Because of its healing effect in the human body, this traditional method of curing disease has come back into life.

HISTORY:

Leeches are the segmented worms first named by Linnaeus in 1758 [7,8]. Leech therapy has been administered since ancient times. According to archaeologist, the existence of bloodletting tools of the Stone Age is evident, but the first use of leeches in this process is unknown. Leech therapy in Greek medicine can be found in the poem Alexipharmicia by Nicander Colophon (200 – 130BC) [2]. He may have been the first person to use leech medicinally. Paintings of medicinal leeches have been found in pharaoh tombs (1500BC) [5,9]. Hirudotherapy has also been reported by Themison of Laodicea in the year 50BC [10]. In the XVI book of Iliad, Homer described physician usage of leeches to treat the battle wounds of Odysseus, Agamemnon and Eurypides. Roman physician Galen (129-199AD) put forward the consideration of humoral concept to restore

healthy balance of four humors using leeches to eliminate the obnoxious substance in body. Due to the influence of Galen writings, Antyllos (150AD) one of the greatest surgeons of antiquity described about venesection, arteriotomy, cupping and the application of leeches for a variety of diseases. Persian physician Avicenna (980-1037) referred that leeching kept lovers from sinking into madness. Ambroise Paré (1510-1590), the most celebrated surgeon of Renaissance and author of *Ouvres* had discussed about treatment of gunshot wounds, ligation of arteries after amputation and entire chapter devoted to leeching [2]. Leech therapy played an important role during the 17th & 18th centuries. It was used for medicinal “bloodletting” and “purification”- a practice believed to cure a variety of ailments from gout to headaches [5,11,12]. It gained popularity among practitioners of phlebotomy due to its ability to achieve more gradual rate of blood loss. Francois Joseph Broussais (1722- 1832) an ex-army surgeon in Napoleon’s army caused a boom in leech industry. Under his influence, Napoleon imported millions of leeches from Hungary to treat battle wounds of soldiers. Leeching was extensively use in military medicine not only in France but also in England[1,2,13]. Sir George Bollingal, the British army surgeon (1832) in his “outliners of military surgery” recommended repeated leeching for gunshot wounds especially those of the joints[2].

Leeching had been in usage in Russia too. After World War II, the use of living leeches generally lost favor because of its powerful competitor heparin in 1938, but scientific interest in hirudotherapy continued. Haycraft first noted the anti-thrombic properties of leech saliva in early 1880’s and Jacoby discovered the anti-coagulant factor in leech saliva and named it hirudin in 1904 [14]. In mid 1950 Fritz Markwardt, inaugurated modern research on the anticoagulant substances in leech [2]. Re-entry of hirudotherapy occurred in 1970’s & 1980’s and it was used as an adjunct to plastic reconstruction and trauma surgeries[5,11,12]. French microsurgions began using leeches to assist with distal digital replantation involving arterial repairs[15].

Today the medicinal leeches are often used to treat venous congestion in setting of microvascular replantation, reconstructive surgeries and traumatology[16,17]. Leech therapy had begun to spread its ray of light in the field of dentistry.

SPECIES:

Leeches are what we call annelids, belong to the subclass HIRUDINEA. There are three types of leeches, those in fresh water, marine and terrestrial. Most of them live in fresh water, but some prefer to live in low foliage or in rain forests. Leeches are also found in dry forest with bit of moisture. Leeches that suck blood are called Haemophagic leeches. They attach themselves to the host until they become full, simply fall off and start to digest the ingested blood.

Roughly there are 600 species of leeches have been reported till date, of which only 15 are of medicinal value. These leeches are known as medicinal leeches, of which *Hirudo medicinalis* is the most popular and common

species used. *Hirudo provincialis* and *hirudo affincinalis* where used in Europe. Because of the unavailability of *hirudo medicinalis* in India the species used traditionally for therapeutic purpose is *hirudinaria granulose*[18]. The other species used for therapeutic purpose *macrobdella decora*, *H.michaelseni*, *H.nipponia*, *H.verbena* and *H. orientalis*.

CHARACTERISTIC FEATURE OF LEECH:-

The leech is very specialized oligochaetes both anatomically and behaviorally [9,19]. It can grow upto 12cm approx. in length, with its resting length being about one third of its maximal length [13]. It consists of 102 annuli (each of which usually consists of five segments). Leeches are hermaphroditic with the male and female systems opening independently of each other. The leech crawls using a large posterior sucker [8]. Posteriorly, the leech has three jaws arranged in a triradiate configuration that attach to and bite through human skin [13]. Leeches work by attaching themselves to the prey by means of their 2 suckers, located at either ends of their bodies. One of these suckers surrounds the leech’s mouth, which contains 3 sets of jaws that bite through the prey’s skin, making a Y-shaped incision and a smaller anterior sucker that is utilized for feeding.

The feeding behavior of the medicinal leech is controlled by the neuro transmitter serotonin which is abundant in the largest neural cell, the Retzius cell [20,21]. The act of feeding is stimulated by the proximity of mammalian range temperature and by the sodium and arginine in blood [21]. Leech finds the right spot by a high sensitive chemical, heat and touch receptor. If the leech tastes blood, glucose or sweat, senses the temperature between 35°C and 40°C and pulsating movements indicating the proximity of an artery, it knows how to find a good feeding site. The host is frequently unaware of this attack due to the natural anesthetic substance secreted in the leech saliva.

Mechanism of leech therapy:

Leech therapy has two phases. Active bloodletting which occurs during leech biting and passive bleeding from the leech wound after attachment, which can last for 24-48hrs. This is due to presence of anticoagulant substance hirudin in their saliva. It is known to act at different points in the coagulation cascade, thereby preventing blood from clotting by inhibiting conversion of fibrinogen to fibrin. It is also known to inhibit platelet aggregation, which further contributes to the process and also possess antiseptic qualities [22]. The actual volume of blood drawn by a single leech is minimal, approximately 2 ml to 20 ml per feeding [11,23,24]. Following extraction of this small volume of blood, the leech usually becomes satiated within 10 to 30 min, detaches from the host, and will not re-feed unless purged by incision of the posterior crop [8,23,24,25]. Leech saliva also contains several other bio-active substances including prostaglandins, vasodilators, anesthetics and proteins like calin, apyrase, hyaluronidase, Eglin, destabliase, and collaginase, which have different properties responsible for carrying out the desired medical effect [18].

Bioactive ingredients of the leech body and saliva:

There are more than 100 biological active substances present in the leech saliva. The basis of modern concepts of hirudotherapy has on the human depends on combination of its multiple effects resulting in blood circulation- enhancing, blood and lymph drainage, anticoagulation effect, antithrombotic, thrombolytic, hypotensive, regenerating, anti-inflammatory, bacteriostatic, analgesic effect, increases resistance to infection, improvement of an endocellular exchange, positive influence on metabolic activity, pain relief, segmental effects, immunostimulating and immunomodulating action produced by the following substances present in the leech saliva .

Hirudin: It's a heparin like substance and is the most potent known natural inhibitor of thrombin. Due to its affinity for thrombin, inhibits almost all the physiological actions of thrombin. It may be a useful alternative anticoagulant, in case of patient's sensitized to heparin or with hereditary or acquired antithrombin III deficiency [26].

Hyaluronidase: It is a spreading or diffusing substance that modifies the permeability of connective tissue through the hydrolysis of endoglucoronidic linkages of hyaluroic acid [27].

Hirustasin binds specifically to tissue kallikrein. Antistasin and ghilanten are potent and specific inhibitor of the blood coagulation factor Xa.

Calin: It suppresses collagen induced platelet aggregation as well as adhesion of platelets to collagen coated micro carrier beads. It also interferes with Von-Willebrand factor collagen binding [28], which is one of the events for thrombus formation at sites of damaged endothelium.

Destabilase: It possesses glycosidase activity. Destabilase is the first invertebrate lysozyme with both enzymatic and non-enzymatic antibacterial action[29]. Thrombolysis occurs by the selective hydrolysis of isopeptide bonds of stabilized fibrin. Injected intravenously, partially purified destabilase exhibited thrombolytic properties[30].

Apyrase: Adenosine 5'- diphosphate diphosphohydrolase is a nonspecific inhibitors of platelet aggregation by nature of its action on adenosine 5' diphosphate, arachidonic acid, platelet – activity factor (PAF) and epinephrine [31,32].

Eglin: Eglins (elastase –cathepsin G Leech inhibitors) are small protein having strong inhibitory activity against chymotrypsin and subtilisin like serine proteinase activity on non-cationic substances[33,34].

Bdellin: Plasmin inhibitor to control bleeding. Systemically administrated, they are rapidly excreted in urine[33].

Decorsin: It acts as an antagonist of platelet glycoprotein II b-III a and as potent inhibitor of platelet aggregation[35].

Guamerin: Elastase inhibitors.

Piguamerin: Serine protease inhibitor of plasma kallikrein.

Gelin: Potent thrombin inhibitor analogous to eglin.

Gamma- Glutamyl transpeptidase: this enzyme are similar to those of bovine gamma-glutamyl transpeptidase[36].

Platelet activating factor (PAF) and an **Ornithine- Rich Peptide- PAFA** is a more effective antithrombotic agent than heparin.

Protease, triglyceridase, cholesterol esterase, Leech Prostanoids, Lipase.

Manipulation and Application of leech:-**Storage of leeches:**

The leeches were stored in de-chlorinated water at 5-6°C, which has to be changed twice a week. Under these conditions, leeches survive up to two years without feeding. One to two gram heavy leeches were used for all treatments. They were kept at room temperature for 1/2 – 1 hr before being transferred to the skin of the patient.

Preparation of the patient:

The area to be exposed to leeches should be cleaned with sterile distilled water. Nothing having pungent smell should be applied as it repels the leech [5]. If leeches are applied in the oral cavity for caries, dental plaque and tartar removal, oral rinsing with antiseptic solution should be done prior to the therapy.

APPLICATION OF LEECH:

The standard method for applying a leech involves loading one into an empty barrel of syringe without a plunger and pressing the open proximal end over the area to be treated [37]. The animals normally started feeding immediately; although in rare cases the skin has to be punctured with a sterile needle so that oozing blood would stimulate the leeches to feed, or a drop of Doppler gel application to the desired region facilitate attachment [38]. In case of oral cavity the mucosa should be massaged to increase the blood flow prior to the application. When the leech started feeding the syringe was removed. Feeding will last for 45-120 minutes, and during this time the leech should be monitored.

During intraoral leeching, path to the oropharynx is blocked with gauze to prevent leech migration into the more distal aerodigestive tract, and the perioperative tracheotomy is left in position to protect the airway [38]. Leech migration was an issue, where these “wandering leeches” are being swallowed by patients or finding their way into other body orifices [8]. Granzow et al described the use of an anchoring suture on leeches and to the dressing to immobilize the disobedient leeches and prevent movement after feeding sessions[39]. Davila et al examined the immediate impact of suture placement on leech survival and the results demonstrated no differences in leech survival or mobility[40]. After auto-detachment, they should be removed and disposed of as biohazardous after sacrificing in 70% alcohol [18]. If a leech does not detach, this may indicate arterial insufficiency, and the leech should be removed with 5% topical cocaine, which will paralyze the leech. The leech must not be forcibly detached, and alcohol must not be applied while the leech is still attached [6]. One to 5 leeches were used for each session of treatment.

Post-op maintainence:

The bite area should be cleaned every 3-4 hours with a gauze sponge soaked in saline to remove surface clot and with a heparin (5,000 U/ml) soaked gauze, to increase the time of blood oozing. During feeding, leeches ingest approximately 5 ml of blood. Due to the anticoagulant- and vasodilator-containing saliva, the wound oozes up to 50 ml of blood within 24-48 hrs[37,41].

Complications of hirudotherapy:

One of the main complications developed after the application of the leech therapy is infection. The gut of leeches is colonized by endosymbiotic bacteria, mostly *Aeromonas spp* which is responsible for digestion of ingested blood [9]. It is impossible to obtain *Aeromonas* free leeches[42]. This micro-organism, *Aeromonas hydrophila* has been implicated in various human infections such as allergic reactions and bacterial infections[43], peritonitis[44], wound infection [45], cellulitis, gastroenteritis, endocarditis, osteomyelitis, myonecrosis, sepsis[46], pneumonia and gastroenteritis. Because of the intimate contact between the leech and the patient, infections associated with hirudotherapy occur in 2.4-20% of patients who do not receive antibiotic prophylaxis[9,47]. Appropriate antibiotic prophylaxis should be administered to the patient who needs leech therapy. All isolated organism were 100% susceptible to third generation cephalosporins ciprofloxacin, cefotaxime, ceftazidime, gentamicin [9]. A further study conducted for the surface bacterial flora of medical leeches' shows presence of *Pseudomonas spp.* and other NFGNR, susceptibility to aminoglycosides, quinolones, tetracycline and trimethoprim sulphamethoxazole [48]. In order to evaluate the possibility of rendering leeches safe for use on patients, *H. medicinalis* were fed artificially with a NaCl or 2 g/L arginine solution (used as a phagostimulant) supplemented with ciprofloxacin (100 mg/L). Treated leeches survived for up to 4 months[49]. Ciprofloxacin treated leeches gives 100% infectious free therapy results.

It is necessary to conduct blood test before hirudotherapy. This is dictated by the fact that three hours after hirudotherapy, leukocytosis may occur. So this may adversely affect the patient's condition and in case of leukemia may lead to death.

Excess blood loss – it is necessary to monitor the amount of blood removed, since a drop in red blood cell counts can occur in rare cases of prolonged bleeding. Large transfusion is required were common[38,50]. De Chalan found an average transfusion requirement of 4.4U of packed red blood cells during leech therapy. As many as 14U for free flaps and 17U for replants have been reported, but most of the cases don't require [51].

Stabilization of leeches is essential to prevent loss of leeches in body orifices and spaces[1,52]. Allergic reactions such as itching followed by burning and blister formation and ulcerative necrosis may occur due to leech bite toxin or antigens in leech saliva [1,22,41,53].

Hirudotherapy is contraindicated in non viable tissues. Conditions including various blood borne infections like HIV and hepatitis[54,55], blood disorders like hemophilia, thrombocytopenia, anemia and leukaemia, children below 7yrs, malignant diseases, blood stream infections[56], in immunocompromised patients can cause sepsis[57], acute infectious diseases[58], myonecrosis[59,60,61], individual intolerance, pronounced hypotension, pregnancy, hypotonic was also added in the count.

Use of leeches:

Hirudotherapy is mainly used in trauma and plastic and reconstructive surgery to salvage tissue flaps and skin grafts whose viability is threatened by venous congestion, unsalvageable skin flaps, post surgical and traumatic haematomas and tissues replants [62-65].

In Europe especially in Russia, leeches are used to treat dentistry related problems such as acute and chronic inflammatory dystrophic and neurological lesions of the oral mucosa and periodontium (ulcero-erosive Ruber lichen planus, leukoplakia after cryotherapy, recurrent aphthous ulceration, cheilitis glandularis, glossalgia), - Alveolitis, Abscess, Gingivitis, Periodontitis, Trigeminal neuralgia, Neuralgia of the glossopharyngeal nerve, Melkersson-Rosenthal syndrome, facial furuncle and carbuncle[4]. Physicians are discovering other conditions where the practice may have a role to play which include osteoarthritis, different cardio vascular disease such as arterial hypertension, angina pectoris, myocardial infarction, postinfarctional cardiosclerosis, inflammatory and traumatic processes of the uveal tract (keratitis, chorioretinitis, periorbital haematoma, subretinal haemorrhage), glaucoma and cataract, acute and chronic otitis, sinusitis, laryngitis, dermatitis and dermatosis (eczema, psoriasis, paronychia, scleroderma), inflammatory processes of the urogenital tract, dysfunction and injuries of the genital organs, in post operative rehabilitation of urological patients and in respiratory diseases such as bronchitis and bronchial asthma.

Leeches in dentistry:

In dentistry too, there are evidences on successful use of leech in treatment of various dental & associated oral and maxillofacial diseased conditions[66]. In 1817, Thomas bell treated a case of an oroantral fistula with facial swelling by application of six leeches to that part of the face [66,67]. Chapin A Haris in 1839 recommended the application of leeches to the gums for drainage of abscessed tooth, giving the patient immediate relief from the pain. Leeches were placed along the edges of the incision in the transitional fold for 5-10 minutes and continued for 3-5 days [66].

In 1854 Spencer Bate successfully treated a large cavity in maxillary central incisor with swelling with "a leech to the gum". Several days later, he reported the "tooth firmer, with less swelling"[66].

Alveolitis or dry socket was treated with curettage and irrigation with antiseptic solution followed by leech therapy. Leech was placed in the edge of extracted site or in transitional fold near extracted region for 5 minutes. This procedure was done daily or every other day for 3-5 sessions. Hirudotherapy reduced the recovery period of the patients for 2-3 days.

In 1822, Philip Crampton applied leeches directly to the inflamed tonsils. The leech was introduced into the mouth, and its head directed by a probe was brought to be in contact with the inflamed tonsil. In less than 5 minutes it was been gorged with blood and fell on the tongue and was withdrawn. Hyperemia and inflammation reduced leading to faster pain relief [68].

The medicinal leeches were used to prevent new blisters from forming in pemphigus patients [67]. The hirudotherapy was also used in treating ruber lichen planus with dermal and mucosal lesions. Positive results were seen after 1-2 courses such as the decrease of papul's number, the healing of erosive areas of the skin and mucosa, the diminish of edema, erythema and perifocal inflammation, induration and eczematization of the derma, disappearance of the pruritus and xeroderma, recovery of salivation. Hirudotherapy can also be used as an adjuvant to root canal treatment as referred from <http://arizonaleechtherapy.com/hirudotherapy-for-dental-issues/> (accessed on 1st July 2016).

CONCLUSION:-

In the present day scenario, hirudotherapy are being successfully employed for only a few conditions, notably in the field of reconstructive or microsurgery. The anticoagulant properties of hirudin, contained in leech saliva may lead to wider therapeutic applications in the prevention and treatment of various diseases. However, its application in dentistry is yet unexplored in many countries including ours and this should motivate further research on its use in treatment of various dental disorders.

REFERENCES

- Adams SL. The medicinal leech. A page from the annals of internal medicine. *Ann of Intern Med* 1988 Sep (1); 109(5):399-405.
- Giacometti L. Leeching in the twentieth century. *The American Journal of Cardiology* 1987 Nov; 60 (13):1128-31.
- Abel L. Bloodletting, Barber-surgeon's shaving and bleeding bowls. *JAMA* 1970 Nov;214 (5): 900-1.
- Sviridova L. Leech therapy in recent times, Eighth International Conference on biotherapy, Abstract no.23, Nov 11-14, 2010, Los Angeles, CA.
- Munshi Y, Ara I, Rafique H and Ahmed Z. Leeching in the history-a review: *Pak J Biol Sci* 2008 Jul; 11(13): 1650-53.
- Irish JC, Gullane PJ, Mulholland S, Neligan PC. Medicinal leech in head and neck reconstruction. *J Otolaryngol* 2000 Oct; 29(5): 327-32.
- Lineaweaver WC, O'Hara M, Stridde B, Valauri FA, et al. Clinical leech use in a microsurgical unit: The San Francisco experience. *Blood Coagul Fibrinolysis* 1991 Feb; 2(1):189-92.
- Mory RN, Mindell D, Bloom DA. The leech and the physician: Biology, etymology, and medical practice with *Hirudinea medicinalis*. *World J Surg* 2000 Jan; 24 (7):878-83.
- Eroglu C, Hokelek M, Guneren E, Esen S, et al. Bacterial flora of *Hirudo medicinalis* and their antibiotic sensitivities in the Middle Black Sea Region, Turkey. *Ann Plast Surg* 2001 Jul; 47 (1):70-3.
- Cole D. Clinical hirudology: Revival of an ancient art. *NZ Med J* 1985 Jan 23; 98(771):28-9.
- Durrant C, Townley WA, Ramkumar S, Khoo CT. Forgotten digital tourniquet: Salvage of an ischaemic finger by application of medicinal leeches. *Ann R Coll Surg Engl* 2006 Sep; 88 (5):4624.
- Mozos-Pirez BL, Font-Jimenez I. Leeches in the intensive care unit: Nursing care. *Enferm Clin* 2007 Jul-Aug; 17(4): 211-4.
- Valauri FA. The use of medicinal leeches in microsurgery. *Blood Coagul Fibrinolysis* 1991 Feb; 2(1): 185-7.
- Fields WS. The history of leeching and hirudin. *Haemostasis* 1991; 21(suppl 1):3-10.
- Foucher G, Henderson HR, Maneaud M, Merle M, et al. Distal digital replantation: One of the best indications for microsurgery. *Int J Microsurg* 1981; 3:265-70.
- Weinfeld AB, Yuksel E, Boutros S, Gura DH, et al. Clinical and scientific consideration in leech therapy for the management of acute venous congestion: An update review. *Ann Plast Surg* 2000 Aug; 45(2): 207-12.
- Pantuck AJ, Lobis MR, Ciocca R, Weiss RE. Penile replantation using the leech *Hirudo medicinalis*. *Urology* 1996 Dec; 48(6):953-6.
- Zaidi SM, Jameel SS, Zaman F, Jilani S, et al. A systematic overview of the medicinal importance of Sanguivorous Leeches. *Altern Med Rev* 2011 Mar; 16(1):59-65.
- Wade JW, Brabham RF, Allen RJ. Medicinal leeches: Once again at the forefront of medicine. *South Med J* 1990 Oct; 83(10): 1168-73.
- Lent CM, Dickinson MH. The neurobiology of feeding in leeches. *Sci Am* 1988 Jun; 258(6):98-103.
- Lent C. New medical and scientific uses of the leech. *Nature* 1986 Oct 9-15; 323(6088):494.
- Salzet M. Anticoagulants & inhibitors of platelet aggregation derived from leeches. *FEBS Lett.*2001; 492: 187- 192.
- Lee NJ, Peckitt NS. Treatment of a sublingual hematoma with medicinal leeches: Report of case. *J Oral Maxillofac Surg* 1996 Jan; 54 (1):101-3.
- Smeets IM, Engelberts I. The use of leeches in a case of post-operative life-threatening macroglossia. *J Laryngol Otol* 1995 May; 109(5):442-4.
- West BR, Nichter LS, Halpern DE. Emergent reuse leech therapy: A better method. *Plast Reconstr Surg* 1994; 93:1095-8.
- Bichler J, Fritz H, Hirudin, a new therapeutic tool. *Ann Hematol* 1991 Aug; 63(2): 67-76.
- Linker A, Meyer K, Hoffman P. The production of hyaluronate oligosaccharides by leech hyaluronidase & alkali. *J Bio Chem* 1960 Apr; 235:924-7
- Harsfalvi J, Stassen JM, Hoylaerts MF, et al. Calin from *Hirudo medicinalis*, an inhibitor of Von Willebrand factor binding to collagen under static and flow conditions. *Blood* 1995 Feb; 85(3): 705-11.
- Zavalova LL, Yudina TG, Artamonova II, Baskova IP. Antibacterial non-glycosidase activity of invertebrate destabilase- lysozyme and of its helical amphipathic peptides. *Chemotherapy* 2006; 52:158-160.
- Baskova IP, Nikonov GI. Destabilase, the novel epsilon-(gamma-Glu)-Lys isopeptidase with thrombolytic activity. *Blood Coagul Fibrinolysis* 1991 Feb; 2(1): 167- 172.
- Rigbi M, Orevi M, Eldor A. Platelet aggregation and coagulation inhibitors in leech saliva and their roles in leech therapy. *Seminar in thrombosis & Hemostasis*. 1996; 22: 273-278.
- 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.
- Seemuller U, Dodt J, Fink E, Fritz H. Proteinase inhibitors of the leech *Hirudo medicinalis* (hirudins, bdellins, eglins) In: Baretta AJ, Salvesen G, eds. *Proteinase* New York, NY: Elsevier Science Ltd; 1986:337-359.
- Ascenzi P, Aducci P, Amiconi G, et al. Binding of the recombinant proteinase inhibitor eglinc from leech *Hirudo medicinalis* to serine (pro)enzymes: a comparative thermodynamic study. *J Mol Recognit* 1991; 4: 113-119.
- 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 Jan; 265(17): 10143-7.
- Friedrich T, Kroger B, Koerwer R, et al. An isopeptide bond splitting enzyme from *Hirudo medicinalis* similar to gammaglutamyl transpeptidase. *Eur J Biochem* 1998 Sep 1; 256 (2): 297-302.
- Utley DS, Koch RJ, Goode RL. The failing flap in plastic and reconstructive surgery: role of the medicinal leech. *Laryngoscope* 1998 Aug; 108(8pt 1):1129-35.
- 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: 960-65.
- Granzow JW, Armstrong MB, Panthaki ZJ. A simple method for the control of medicinal leeches. *J Reconstr Microsurg* 2004 Aug; 20(6): 461-2.
- Davila VJ, Ian C, Hoppe et al. The effect of anchoring sutures on medicinal leech mortality. *Eplasty* 2009; 9:e29:278-81.
- Heldt TJ. Allergy to leeches. *Henry Ford Hosp Med Bull* 1961 Dec; 9: 498-519.
- Snower DP, Ruef C, Kuritza AP, Edberg SC. *Aeromonas hydrophila* infection associated with the use of medicinal leeches. *J Clin Microbiol* 1989; 27:1421-2.
- Wells MD, Manktelow RT, Boyd JB, Bowen V. The medical leech: an old treatment revisited. *Microsurgery*.1993; 14(3): 183- 186.

- [44]. Munoz P, Fernandez-Baca V, Pelaez T, Sanchez R, et al. *Aeromonas* peritonitis. *Clin Infect Dis* 1994; 18:32-37.
- [45]. Blatz DJ. Open fracture of the tibia and fibula complicated by infection with *Aeromonas hydrophila*. A case report. *J Bone Joint Surg Am* 1979; 61A:790-1.
- [46]. Janda JM, Guthertz LS, Kokka RP, Shimada T. *Aeromonas* species in septicemia: laboratory characteristics and clinical observations. *Clin Infect Dis* 1994 Jul; 19(1): 77-83.
- [47]. Ardehali B, Hand K, Nduka C, Holmes A, et al. Delayed leech-borne infection with *Aeromonas hydrophila* in escharotic flap wound. *J Plast Reconstr Aesthet Surg* 2006; 59(1):94-5.
- [48]. Nonomura H, Kato N, Ohno Y, Itokazu M. Indigenous bacterial flora of medicinal leeches and their susceptibilities to 15 antimicrobial agents. *J Med Microbiol* 1996; 45:490-3.
- [49]. Mumcuoglu KY, Huberman L, Cohen R, Temper V, et al. Elimination of symbiotic *Aeromonas* spp from the intestinal tract of the medicinal leech, *Hirudo medicinalis* using ciprofloxacin feeding. *Clin Microbiol Infect* 2010 Jun; 16(6):563-7.
- [50]. Soucacos PN, Beris AE, Malizos KN, Xenakis TA, et al. Successful treatment of venous congestion in free skin flaps using medicinal leeches. *Microsurgery* 1994; 15: 496-501.
- [51]. DeChalian TMB. Exploring the use of the medicinal leech: a clinical risk-benefit analysis. *J Reconstr Microsurg* 1996; 12:165-172.
- [52]. Walton RL, Beahm EK, Brown RE, et al. Microsurgical replantation of the lip: a multi-institutional experience. *Plast Reconstr Surg* 1998; 102:358-68.
- [53]. Godfrey K. Uses of leeches and leech saliva in clinical practice. *Nurs Times* 1997 Feb 26-Mar 4; 93(9):62-63.
- [54]. Wilken GB, Appleton CC. The persistence of hepatitis B antigen in the bloodmeal of the potential medicinal leech, *Asiatiscobdella buntonensis*. *S Afr Med J* 1993; 83:193-5.
- [55]. HIV infection transmissible also by leeches. *Fortschr Med* 1993; 111:13.
- [56]. Sartor C, Limouzain-Perotti F, Legre R. Nosocomial infections with *Aeromonas hydrophila* from leeches. *Clin Infect Dis* 2002; 35(1):e1-e5.
- [57]. Haycox C, Odland PB, Coltrera MD, Raugi GJ. Indications and complications of medicinal leech therapy. *J Am Acad Dermatol*. 1995; 33(6):1053-55.
- [58]. Varghese MR, Farr RW, Wax MK, Chafin BJ, Owens RM. *Vibrio fluvialis* wound infection associated with medicinal leech therapy. *Clin Infect Dis* 1996 Apr; 22(4):709-10.
- [59]. Mercer NSG, Beere DM, Bornemisza AJ, Thomas P: Medical leeches as sources of wound infections. *Br Med J (Clin Res ED)* 1987 ;294:937.
- [60]. Lineaweaver WC, Furnas H, Follansbee S, et al. Postprandial *Aeromonas hydrophila* cultures and antibiotic levels of enteric aspirates from medicinal leeches applied to patients receiving antibiotics. *Ann Plast Surg* 1992;29 :245.
- [61]. Lineaweaver WC, Hill MK, Buncke GM, Follansbee S. *Aeromonas hydrophila* infections following use of medicinal leeches in replantation and flap surgery. *Ann Plast Surg* 1992 Sep;29(3):238
- [62]. Hidalgo DA, Disa JJ, Cordeiro PG, Hu Q. A review of 716 consecutive free flaps for oncologic surgical defects: refinement in donor-site selection and technique. *Plast Reconstr Surg* 1998; 102(3) :722-732.
- [63]. Chalian AA, Anderson TD, Weinstein GS, Weber RS. Internal jugular vein versus external jugular vein anastomosis: implications for successful free tissue transfer. *Head Neck*. 2001 Jan; 23 (6):475-78.
- [64]. Hidalgo DA, Jones CS. The role of emergent exploration in free-tissue transfer: a review of 150 consecutive cases. *Plast Reconstr Surg* 1990 Sep; 86(3):492-498; discussion 499-501.
- [65]. Henderson HP, Matti B, Laing AG et al . Avulsion of the scalp treated by microvascular repair: the use of leeches for post-operative decongestion. *Br J Plast Surg* 1983; 36(2): 236-37.
- [66]. Hyson JM. *Journal of the History of Dentistry* 2005; 53 (1): 25-27.
- [67]. Srivastava A, Sharma R. A brief review on applications of leech therapy. *Arch Appl Sci Res* 2010; 2(2): 271-74.
- [68]. Menage MJ, Wright G. Use of leeches in a case of severe periorbital haematoma. *Br J of Ophthalmol* 1991 Dec; 75(12):755-56.