International Journal of Innovation Sciences and Research
Vol.4, No, 12, pp.535-545, December- 2015

Available online at
http://www.ijisr.com

REVIEW ARTICLE

HIRUDOTHERAPY-MODERN TWIST TO ANCIENT SCIENCE AND ITS RELEVANCE IN MAXILLOFACIAL REGION- A REVIEW ARTICLE

***[1]Dr. Tripthi, P. S. and [2]Dr. Ashalatha, M.**

[1]Department of Oral and Maxillofacial Surgery, A. B. Shetty Memorial Institute of Dental Sciences, Mangalore 575018, India
[2]Department of Dravyaguna, GAMC, Bangalore 560001, India

Accepted 15th November, 2015; Published Online 30th December, 2015

ABSTRACT

Hematophagous animals that feed on prey blood are known to overcome blood clotting by producing in their salivary gland secretion many biologically active compounds, especially the anticoagulants. Amongst the blood-sucking organisms, leech is a distinct example which possesses a highly-developed mechanism by which they prevents blood clotting. Hirudotherapy (HT) is the application of medicinal leeches (Hirudo medicinalis) for therapeutic use. Through centuries, leeches have attracted the attention of therapists who employed leech therapy for a wide range of diseases. . 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, haemorrhoids and various joint ailments. Presently it is also being utilized in gastrointestinal disorders, gynecological abnormalities and dermatology. More recently, HT has found new applications in cancer therapy, hypersensitivity conditions, like asthma, male/female sterility and diabetes. The current review summarizes the importance of leeches as a complementary source of medical therapy for a large number of ailments mostly highlighting its applications in maxillofacial region.

KEY WORDS: Hirudotherapy, Leech, Anticoagulant, Reconstructive surgery, Maxillofacial.

INTRODUCTION

The treatment of disease conditions with medicinal leeches is termed as Hirudotherapy (Koh and Kini, 2008). In this non-invasive treatment methodology, medicinal leeches (Hirudo medicinalis) are used[2]. HT takes the advantage of several biological properties of medicinalleeches. 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 (Faria *et al*., 1999; Whitaker *et al*., 2004). For centuries they were the common tools of physicians who believed that diseases were the result of an imbalance of various humors and that the body can be stabilized by releasing blood (Smith, 1833; Moore, 1952). Modern leech therapy differs from the ancient therapy; nowadays only the leeches which are grown in controlled sterile 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 (Electricwala *et al*., 1991; Kim and Kang, 1998). 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. Leeches can live in a variety of environments, including aquatic and moist terrestrial regions. Some species live in freshwater, estuaries, rivers, ponds, lakes, and sea.

Corresponding author: Dr. Tripthi, P. S.,
Department of Oral and Maxillofacial Surgery, A. B. Shetty Memorial Institute of Dental Sciences, Mangalore 575018, India.

Others are adapted with more mucous glands and larger nephridial vesicles (bladder) that retain and store extra water enabling leeches to tolerate the lack of water on damp land. Moreover, leeches have high physiological flexibility, which makes them able to withstand numerous environmental challenges, such as oxygen shortage and temperature fluctuations (Jung *et al*., 1995; Baskova *et al*., 2008; Chudzinski-Tavassi *et al*., 1998). Because moisture is a very essential factor affecting the terrestrial leech's behavior, they will stay active throughout the year in humid conditions while they go through an active and a dormant phase in wet and dry seasons (Yule and Yong, 2004).

Leech taxonomy and morphology

Leeches (*Euhirudinea*) were first named by Linnaeus in 1758 AD (Whitaker *et al*., 2004). They are related to the phylum *Annelida*, class *Clitellata*. In general, early studies classified leeches into 4 subclasses, 3 orders, 10 families, 16 subfamilies, 131 genera and more than 696 species (Sawyer, 1986). Recently, taxonomists identified more than 1000 leech species (Rouse *et al*., 2006). They vary in size among families and can reach up to 20 cm in length, in addition to some giant species, such as the Amazonian leech, *Haementaria ghilianii*, which is about 50 cm in length (Britanica, 2012). Typically, a leech has anterior and posterior suckers. Suckers are very essential during movement (inchworm-like locomotion) and for attachment to host surface (Britanica, 2012). Leeches breathe through the skin and they are considered as hermaphrodites, but always require another leech for fertilization (Britanica, 2012).

The biology of leech feeding

Based on feeding habits, leeches are divided into two major groups. The first group includes the predacious leeches, which are predators of many invertebrates. The second group, named the sanguivorous leeches are ecto parasites that feed on the blood of vertebrates including human (Yule and Yong, 2004). With the help of suckers and the biting jaws, leeches are able to absorb prey blood (Ascenzi *et al*., 1995). It is interesting to note that leeches generally suck 2-20 ml of blood within 10-30 min, then drop-off spontaneously after being completely engorged with no immediate desire of more feeding (Porshinsky *et al*., 2011; Michalsen *et al*., 2007).

Leeches, both sanguivorous and predacious, digest their food in their intestine. The sanguivorous species only store blood inside their body for months. Actually, the digestion process of blood in hematophagous leeches undergoes many slow stages allowing leeches to store the ingested blood for up to 18 months. Symbiotic bacteria named *Aeromonas* spp., located in the leech's gut, secrete enzymes that help not only in breaking down the components of the ingested blood, but also in producing antibiotics to prevent blood putrefaction after a long storage period in leech crop. Furthermore, another presumed role of these enzymes is to prevent B complex deficiency, which often occurs in blood nutrition-depending animals (Yule and Yong, 2004).

Historical Perspective

The importance of leech in clinical therapy can be simply represented from the Anglo-Saxon word for a physician "laece" indicating that both doctors and these annelids were etymologically related to each other since the beginning of civilization (Whitaker *et al*., 2004; Knobloch, 2011). The usage of leech for various medical applications can be traced back thousands of years ago. Before the Christian era (BC), medicinal leeching was mentioned in the 18thdynasty Pharaohs paintings (1500 BC). Talmud, Bible, and other Jewish manuscripts outlined the medical indications of leechin (Knobloch, 2011). The Greek poets, Nicader of Colophain (200-130 BC) mentioned leeches in his medical poems (Whitaker *et al*., 2004).

During the Christian era, the usage of bloodsucking action of leeches became so popular and was utilized in almost every region in the world. Greek physicians used leeches for bloodletting and for treating rheumatic pains, gout, all types of fever and hearing loss. The usage of leeches during that time depended upon the humor concept of Galen (130-201 AD), which was an inspiration from Hippocrates (460-370 BC) hypothesis about body fluids imbalance-related illnesses. Galen believed that illnesses alleviation can be achieved by restoring the balance between the body fluids when a leech withdraws blood from patients (Whitaker *et al*., 2004; Munshi *et al*., 2008).

Galen would prescribe bloodletting by leech for almost all illnesses such as simple inflammatory conditions, mental disorders and hemorrhoids (Upshaw and O'Leary, 2000). Moreover, Themission of Laodice, a Syrian doctor, outlined that removing blood from the patient will evacuate the evil spirits, which can cause diseases (Whitaker *et al*., 2004). In addition, leech practice was also documented in Islamic literature. For instance, Avicenna (980-1037 AD) delineated in his book "Canon of Medicine" that leech can suck blood from deep veins which cannot be reached by the conventional wet cupping (Whitaker *et al*., 2004; Munshi *et al*., 2008) and he recommended leeching for skin diseases (Srivastava and Sharma, 2010). In 12thcentury, Abd-el-latif al-Baghdadi mentioned in his texts the beneficial usage of leech application after surgical operations. Thereafter, Ibn Maseehi (1233-1286 AD) in his book "Umda Fi Jarahat" differentiated the medical leeches from the nonmedical (poisonous) ones according to their shape and colour (Munshi *et al*., 2008).

Later, in the middle ages, medics depended more on leech therapy, which was prescribed for a wide range of disorders including nervous system diseases (epilepsy, brain congestion), urinary, and reproductive organs diseases (nephritis, subacute ovaritis, sexually-transmitted diseases), inflammatory diseases (acute gastritis, laryngitis) and eye illnesses. Some French physicians prescribed leeches for the patient even before seeing him. Actually, the widespread indications of leeching might be attributed to the concept, which suggested that bloodletting by leech was less painful than using the lancet or the scarifier. Moreover, leech application is more suitable and manageable for hemorrhoids and vaginitis where the blade or the cupping glass is not tolerable by the patients (Whitaker *et al*., 2004; Munshi *et al*., 2008).

After reaching a popular peak in the early nineteenth century, leech trading became a lucrative business which encouraged more people to collect large numbers of leeches, which eventually caused them to become endangered species. Consequently, European and American authorities offered rewards for the invention of a new method to breed leeches. Meanwhile, leeches were employed to treat mental disorders, skin diseases, gout, headache, and whooping cough (Britanica, 2012). By the end of 19th century, leeching gradually fell into disrepute, and almost stopped by the early twentieth because hirudo therapy did not match the new requirements of the modern medical regulations and the great advancement in all medical fields (Whitaker *et al*., 2004).

During this era, bloodletting by leeches was still common in the treatment of epilepsy along with other traditional remedies, such as cauterisation and baths. Therapists used to apply leeches to the scalp in order to reduce cerebral congestion and brain blood supply, which were thought to be involved in the etiology of epilepsy (Sun, 2007). Even though, the scientific interest of leech continued as a result of Haycraft's researches that brought leeches back into the medical stream when he outlined for the first time the presence of an anticoagulant agent in leech saliva, which he called hirudin (Haycraft, 1883), which was later isolated and identified by Markwardt who demonstrated its antithrombin activity (Markwardt, 1970). Another physician wrote about the superb beneficial usage of leeches in the management of coronary thrombosis, and he exaggeratedly expressed his desire to be fully covered by leeches to benefit from its hirudin-containing saliva (Upshaw and O'Leary, 2000). In 1981, a foundation for leech breeding development and medicinal leech research was established by an American biologist, Roy T. Sawyer (Whitaker *et al*., 2004). Some surgeons have recently developed an artificial prototype of leeches termed as "mechanical leech," which can perform blood sucking for the treatment of venous congestion.

They claimed that this device is more acceptable by the patients and more accurate than the creatures themselves (Conforti *et al*., 2002; Hartig, 2003). After the recession period of leech therapy, it has resurged after the mid-20th century with new applications in many medical fields including surgical and reconstitution procedures, vascular diseases, arthritis, migraine (Munshi *et al*., 2008; Sun, 2007). This novel therapeutic utilization of leeches resulted in more interest in isolation and characterization of the active constituents of leech saliva (Gasic *et al*., 1983). In 2004, the Food and Drug Organization (FDA) approved leeches for medicinal purposes (Munshi *et al*., 2008). It was assumed that leech therapy depends mainly on two concepts. First, as the leech bites the skin of its prey, it injects the salivary gland secretion into the wound. Second, another part of these secretions will be mixed with the ingested blood to keep it in a liquid state (Penchenik, 2000; Baskova and Zavalova, 2001).

Basic Science of Hiruda Medicinalis

The medicinal leech Hiruda medicinalis is a segmented annelid belonging to Phylum: Annelida, Class: Clitellata and Subclass: Hirudinea (Lamark, 1818) (Faria *et al*., 1999; Rouse *et al*., 2006). These 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 0ºC to 30ºC; 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 these 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.

Hiruda medicinalis are "protandrous": first males then females lay eggs in cocoons 1-9 months post-copulation. They become ready for medicinal use after they are several years old. Leeches usually remain attached to the host for 30minutes to 6 hours for feeding, and during the course they get engorged with blood. It can suck about 5 to15 ml of blood, but the bite continues to ooze for 4 to24 hours (Chudzinski-Tavassi *et al*., 1998). 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 his may provide dramatic relief at first), but from the anti-coagulant 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 (Haycraft, 1883; Markwardt, 1970). 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 bio energetic status of the organism (Sawyer, 1986). The molecules existing in leech saliva and the most studied to date include:

1. Hirudin: An active principle in the salivary gland secretion of leeches, which acts as a potent anti coagulant (blood thinner). It inhibits blood coagulation by binding to thrombin (Yule and Yong, 2004; Sawyer, 1986; Conforti *et al*., 2002; Gasic *et al*., 1983).
2. Hyaluronidase (spreading factor): Facilitates the penetration and diffusion of pharmacologically active substances into the tissues, especially in joint pain and has antibiotic properties (Yule and Yong, 2004; Sawyer, 1986; Porshinsky *et al*., 2011).
3. Calin: Inhibits blood coagulation by blocking the binding of the Von Willebrand factor to collagen. It inhibits collagen-mediated platelet aggregation (Yule and Yong, 2004; Sawyer, 1986; Penchenik, 2000)
4. Destabilase: Dissolves fibrin and has thrombolytic effects. (Yule and Yong, 2004; Sawyer, 1986; Baskova *et al*., 2001)
5. Hirustasin: Inhibits kallikrein, trypsin, chymotrypsin,and neutropholic cathepsin G (Yule and Yong, 2004; Maton, 1993; USA, 2011).
6. Bdellins: Anti-inflammatory effect and inhibits trypsin, plasmin and acrocin (Yule and Yong, 2004; Gasic *et al*., 1983).
7. Chloromycetyn: Potent antibiotic (USA, 2011).
8. Tryptase inhibitor: Inhibits proteolytic enzymes of host mast cells (Yule and Yong, 2004).
9. Eglins: Anti-inflammatory. They inhibit the activity of alpha-chymotrypsin, chymase, substilisin, elastase, and cathepsin G (Yule and Yong, 2004; Sawyer, 1986; Gasic *et al*., 1983).
10. Factor Xa inhibitor: Inhibits the activity of coagulation factor Xa (very important role during the treatment of Osteo-arthritis and Rheumatoid arthritis) (Sawyer, 1986; Corral-Rodríguez *et al*., 2010; Baskova *et al*., 1997).
11. Anesthetic-like substances: Reduce pain during biting by a leech (Electricwala *et al*., 1991; Cherniack, 2011).
12. Histamine-like substances: A vasodilator increases the inflow of blood at the bite site (Sawyer, 1986; Gasic *et al*., 1983; Walsmann and Markwardt, 1985).
13. Complement inhibitors: Replace natural complement inhibitors if they are deficient.
14. Carboxypeptidase-A inhibitors: Increase the inflow of blood (Markwardt, 2002; Strube *et al*., 1993).
15. Acetylcholine: Vasodilator (Gasic *et al*., 1983; Maton, 1993; Hong and Kang, 1999; Salzet *et al*., 2000)

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 (Harsfalvi *et al*., 1995). Indications of leech therapy are as follows:

- Inflammatory Reactions (Moore and Professor, 1952; Walsmann and Markwardt, 1985).
- Passive congestions and spastic conditions (Moore and Professor, 1952; White *et al*., 2007; Katzung *et al*., 2009).
- Plastic and reconstructive surgery (Upshaw and O'Leary, 2000; Srivastava and Sharma, 2010; Katzung *et al*., 2009; Brankamp *et al*., 1991; Chopin *et al*., 2000; Murray *et al*., 2009; Swadesh *et al*., 1990).
- Cardiovascular diseases (Moore and Professor , 1952; Yule and Yong , 2004; Murray *et al*., 2009; Whitaker *et al*., 2005; Green and Shafritz, 2010; Koch *et al*., 2012; Henderson *et al*., 1983; Batchelor *et al*., 1984).
- Hypertension (Batchelor *et al*., 1984).
- Varicose Veins. (Batchelor *et al*., 1984; Mutimer and Banis, 1987).
- Hemorrhoids (Chudzinski-Tavassi *et al*., 1998).
- Arthrosis, osteoarthritis, periarthritis and rheumatoid arthritis (Sawyer, 1986; Mutimer *et al*., 1987).
- Thrombophlebitis, thrombosis and embolism (Jeng *et al*., 1994).
- Hematomas (Swadesh *et al*., 1990).
- External ear and chronic ear infections (Mutimer *et al*., 1987).
- Eye diseases, including cataracts, glaucoma,traumatic injuries and inflammation (Sawyer, 1986).
- Dental problems, like gingivitis, paradontitis, gingival edema and stomatorrhagia (Sawyer, 1986; Mutimer *et al*., 1987).
- Vertebrogenic Pain Syndromes (Sawyer, 1986).
- GI tract – hepatitis, cholecystitis, pancreatitis, stomach ulcers (Rouse *et al*., 2006; Mutimer *et al*., 1987).
- Chronic skin diseases, like scabies, psoriasis, eczematous dermatitis, and chronic ulcers (Koh and Kini , 2008; Sawyer, 1986; Mutimer *et al*., 1987).
- Respiratory disorders - Asthma, acute rhinopharyngitisand spasmodic coryza (Yule and Yong , 2004; Porshinsky *et al*., 2011; Mutimer *et al*., 1987).
- Gynecological disorders - male and female sterility, endometriosis.

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 (Sawyer, 1986; Rouse *et al*., 2006):

- Absolute hemophilia
- Anemia
- Leukemia
- Hypotonia
- Pregnancy

Modern Medical Applications Of Leech Therapy

Cardiovascular diseases

CVDs are a group of chronic abnormalities affecting the cardiovascular system including heart, veins and arteries (Maton, 1993). Among the incurable diseases, CVDs were considered the principal culprit of mortality, causing up to 30% of global deaths by the year 2008 (USA, 2011). The on-going incidence rate of morbidity and mortality caused by CVDs were the main reason behind intensive researches looking for potent medications with fewer side-effects (Corral-Rodríguez *et al*., 2010).

Leech therapy has established itself as an alternative remedy for the treatment of vascular disorders, since leech saliva can temporarily improve blood flow and ameliorate connective tissue hyperalgesia (Michalsen *et al*., 2007). By the year 1997, a novel antithrombotic and anticoagulant pharmaceutical preparation was released to the Russian markets under the trade name "Piyavit", which consisted of the medicinal leech saliva extract. The product was prescribed as thrombolytic and antiplatelet. Clinical studies revealed that it can reduce blood hypercoagulability with an antiinflammatory effect in patients with thrombophlebitis (Baskova *et al*., 1997). Likewise, patients with phlebitis who received topical leeching exhibited better walking ability, less pain and minor leg swelling, along with near-normal leg skin color (Cherniack, 2011). In such cases, medics usually apply 4-6 leeches directly to the affected area. Many therapists used leeches for the healing of hypertension, varicose veins, hemorrhoids, gonarthritis, and secondary ischemia-related dermatosis (Michalsen *et al*., 2007; Srivastava and Sharma, 2010).

The effectiveness of leech saliva in CVDs is the results of specific thrombin inhibitors, hirudin, which was first isolated from *H. Medicinalis* (Srivastava and Sharma, 2010; Sun, 2007; Haycraft, 1883) and was shown to possess a potent inhibitory effect on both free and clot-bound thrombin (Walsmann and Markwardt, 1986; Markwardt, 2002). Furthermore, other thrombin inhibitors were identified from different leech species. For instance, bufrudin was isolated from *H. manillensis* with a chemical structure closely similar to hirudin[6]. A tight-binding thrombin inhibitor named haemadin was identified from the whole body extract of the leech species *Haemadipsa sylvestris* (Strube *et al*., 1993). Another antithrombin named granulin-like was isolated from the leech species *H. Nipponia* (Hong and Kang, 1999). Finally, a human granulocyte and monocyte protein inhibitor known as theromin was characterized from the head extract of *Theromyzon tessulatum* leech species with an antithrombin activity (Salzet *et al*., 2000).

Noteworthy, hirudin is the only hematophagous animal-derived anticoagulant has been approved by FDA for clinical purposes. Many studies revealed that hirudin is more effective than heparin in preventing deep venous thrombosis (DVT) and ischemic events in patients with unstable angina. In contrast to the indirect thrombin inhibitors, heparin and low molecular weight heparins, hirudin has the advantage of exerting a direct inhibitory effect on thrombin without the need for endogenous cofactors (antithrombin III). Thus, hirudin became the drug of choice for patients with a disseminated intravascular

coagulation syndrome (antithrombin III deficiency). Hirudin can be used safely in patients with platelet abnormalities or heparin-induced thrombocytopenia because it has no immune effects on erythrocytes. Furthermore, and unlike heparins, hirudin has a promising prophylactic activity in patients who are at a high-risk of developing cardiovascular events because it can hinder thrombus growth due to its ability to block thrombin-fibrin binding. Consequently, it was reported that hirudin can reduce DVT, pulmonary embolism and the spread of venous thrombosis (Corral-Rodríguez *et al*., 2010; Markwardt, 2002). Hirudin discovery was the motive for developing many new promising anticoagulants using recombinant technology methods. For example, two analogs, lepirudin, and desirudin have been approved by FDA and are currently in use under the trade names, Refludan® and Iprivask®, respectively (Corral-Rodríguez *et al*., 2010). Precisely, desirudin is meanwhile in use for the prevention of DVT following hip or knee replacement surgery (Sohn *et al*., 2001).

On the other hand, leeches have developed other active compounds targeting different coagulation factors, such as antiplatelet, factor Xa (FXa) inhibitors, and fibrinolytic enzymes (Salzet, 2001). First, a potent antiplatelet named decorsin was identified from *Macrobdella decora* with a high affinity to glycoprotein IIb-IIIa receptors (Seymour *et al*., 1990). Second, a platelet adhesion and activation inhibitor named calin was isolated from the salivary secretion of the European leech *H. medicinalis* and it was believed to act by inhibiting collagen and von Willebrand factor (Harsfalvi *et al*., 1995). In addition, saratin from the leech *Haementeria ghilianii* has been described as a platelet aggregation inhibitor via blocking the binding of collagen to integrin $\alpha_2\beta_1$ and von Willebrand factor (White *et al*., 2007). From a pharmacological point of view, the activated platelet glycoprotein IIb-IIIa functions as a receptor for fibrinogen, vitronectin, von Willebrand factor and fibronectin. Therefore, the inhibitors of these surface receptors could be used as medications for the treatment of acute coronary syndrome disease (Katzung *et al*., 2009).

Furthermore, several inhibitors of factor Xa were identified from leech saliva extract such as ghilanten (Brankamp *et al*., 1991), lefaxin (Faria *et al*., 1999) and therostatin (Chopin *et al*., 2000) from *H. ghilianii*, *H. depressa* and *T. tessulatum*, respectively. It has been evidenced that FXa plays a key role in the human body hemostasis. Both extrinsic and intrinsic pathways of the coagulation process result in the activation of FXa, which mediates the conversion of prothrombin (FII) into thrombin (FIIa) (Murray *et al*., 2009). Moreover, hementin and hementerin were characterized from *H. Ghilianii* (Swadesh *et al*., 1990) and*H. Depressa* (Chudzinski-Tavassi *et al*., 1998) and reported as fibrinogenolytic enzymes. Interestingly, the cleavage of fibrinogen leads to early blockade of the coagulation cascade, which also makes fibrinogenolytic compounds very promising therapeutical tools (Chudzinski-Tavassi *et al*., 1998).

Cancer and metastasis

In 2008, cancer was responsible for about 13% of all global deaths. These alarming rates are expected to increase during the next two decades to reach up 13.2 million deaths by the year 2030 (Atlanta, 2011). This review was conducted taking into account that leech therapy is not established for cancer treatment as a cytotoxic agent by scientific reports. The review was carried out based on some studies, which were oriented towards using leech saliva and leech extract as antimetastatic agents rather than using it for treating the tumor itself.

Leech application as antimetastatic agent was inspired from a previously reported metastatic inhibitory activity of some anticoagulant such as warfarin and heparin. It was presumed that the extraordinary combination of many anticoagulants, protease inhibitors, and other components in leech saliva could be more powerful as an antimetastatic drug. It was outlined that the salivary gland extract from *H. ghilianii* and*Haementeria officinalis* inhibited the metastatic colonization of lung tumor cells, which were injected intravenously into the experimental animals. Later, an antimetastatic and anticoagulant protein named ghilanten was purified from the salivary gland secretion of the proboscis leech, *H. ghiliani*. It was reported that ghilanten could suppress metastasis of melanoma, breast cancer, lung cancer, and prostate cancer. Another research described a synthetic hirudin preparation as an efficacious metastasis inhibitor of a wide range of malignant tumor cells, such as pulmonary carcinoma, breast carcinoma, bladder carcinoma, colorectal carcinoma, soft-tissue sarcoma, leukemia, and lymphoma (Wallis *et al*., 1992; Gasic and Patent, 1986; Cardin and Sunkara, 1994).

The Mexican leech *Haementeria officinalis* was subjected to many studies, which eventually led to unveil the antimetastatic activity of its salivary gland secretion. It was observed that its saliva contains a 17-kDa protein, called antistasin, having the capability to prevent lung cancer colonization. They argued that the antimetastatic activity of the Mexican leech saliva was due to the existence of platelet aggregation inhibitors, anticoagulants, and the antiproteolytic enzymes (Tuszynski *et al*., 1987; Gasic *et al*., 1984). By the year 2010, other scientists delineated for the first time that a 2 month treatment by topical application of *H. medicinalis* can completely cure the local lumbar pain in patients with advanced stages of renal cancer and leiomyosarcoma (Kalender *et al*., 2010). Recently, it was evidenced that saliva extract from the tropical leech *H. manillensis*(Lesson, 1842) displayed an antiproliferative activity *in vitro* against small cell lung cancer (SW1271). Besides, leech saliva obtained therefrom exhibited a supra-additive synergistic activity with carboplatin (Merzouk *et al*., 2012).

Diabetes mellitus and its complications

Diabetes mellitus (DM) is a group of metabolic disorders resulting in an elevated blood glucose level, which eventually leads to clinical symptoms and complications (Masharani, 2010). Recently, DM has been considered as a global pandemic due to the progressive increasing rates of people suffering from diabetes, expecting to be a worldwide burden by 2030 with 366 million diabetic patients (Wild *et al*., 2004). A comprehensive search through the literature revealed that there are no documented scientific reports on leech therapy as an antihyperglycemic medication. On the other hand, leech application has been used traditionally for the treatment of DM complications (Susanto, 2011).

One of the most severe complications of DM is the cardiovascular ones due to coronary atherosclerosis, hyperglycemia, increased blood lipid levels, platelet adhesion disorders, coagulation factors, high blood pressure, oxidative stress, and inflammation. Diabetic patients are at a high-risk of myocardial infarction, which is the main death-causing reason in type 2 DM (Masharani, 2010). On the other hand, the presence of blood-affecting peptides and proteins in leech saliva can be of an important benefit for the relieving of these conditions. First of all, hirudin plays an essential role in preventing clotting process because of its ability to bind thrombin and consequently suppress thrombin-mediated conversion of fibrinogen into fibrin enabling it to be efficacious for the relieving of ischemic events (Corral-Rodríguez *et al*., 2010). Calin, isolated from *H. medicinalis*, has been proven to obstruct the formation of thrombi as described above (Harsfalvi *et al*., 1995). In addition, other coagulation factors-interfering peptides and proteins were isolated from other leech species as described above, could be of paramount benefits to diabetic patients.

The peripheral vascular complications in diabetic patients can lead to less blood flow to the distal parts of the body resulting in ischemic diseases of limbs like gangrene. The control of gangrene is very crucial to diabetic patients by lowering both blood pressure and lipidemia, along with increasing blood circulation in the peripheral blood vessels (Masharani, 2010). The wild leech species *Whitmania pigra* (Family: *Hirudinidae*) has been used by the traditional Chinese therapists to augment blood flow to the distal parts of the body and to alleviate coagulation disorders. It was reported that the aqueous and alcoholic extracts of the whole body of this leech species possessed a potent anticoagulant activity (Ding *et al*., 1994) From the leech *W. pigra*, a myoactive peptide called the leech excitatory peptide was isolated and reported to enhance the muscular contraction of penis and intestine (Whitaker *et al*., 2004).

By the year 2002, an official center for leech therapy was opened, which has been during a short period of time an international center for DM treatment by leeches. The founder of this center said that he would use four leeches in one session, and in many severe cases, more leeches can prevent amputatio[75]. Recently, it was reported that leech saliva from the tropical leech *H. manillensis* possessed an antihyperglycemic activity against alloxan-induced DM in rats with effective doses ranged from 250 to 500 µg/kg body weight (Unpublished data).

Reconstructive and microsurgery

Microsurgery is a type of surgical operations carried out using the microinstruments under the microscope aiming to anastomose small blood vessels, veins and arteries during the replantation of tissues or amputated digits (Knobloch, 2011). Arterial thrombosis is not common while venous occlusion is a serious threat in newly transplanted tissues and may lead to thrombus formation, stasis, and eventually tissue necrosis. Thus, physicians argued that relieving venous congestion is a vital step in order to mitigate this risk and to salvage these transplanted tissue. Consequently, not only the active blood drainage that results from the leech sucking action, but also from the passive oozing after leech detachment due to the presence of the long-acting anticoagulants in leech saliva motivated medics to use leech to alleviate venous congestion. The relieving effect is the accumulated result of the leech bite-induced blood oozing, which is a consequence of many factors, including bleeding wound, secreted bioactive enzyme, anticoagulants, and vasodilators. On the other hand, surgeons who practice plastic operations considered leeching as a promising remedy, since they observed that the Y-shaped wounds caused by leech bites usually heal without scars or complication. Nevertheless, no international protocols on leech therapy instructions have been established, some reported that leech application for a week is sufficient to get good results. All data on the application of the medicinal leech in microsurgery depend on case reports and case series with no controlled studies being published up to date (Knobloch, 2011; Koch *et al*., 2012; Green and Shafritz, 2010).

Leeching has been reported as a successful remedy to improve blood flow after microsurgery of a severely avulsed scalp (ripped away by an injury). The scalp was partially salvaged with normal hair growth in the whole injured areas (Henderson *et al*., 1983). By the year 1984, some physicians used leech therapy to treat seven patients with engorged (swollen) skin flaps. They applied leeches 2-4 times a day for 2-4 days. They reported that leeching prevented flap collapse with noticeable improvement in color and minor complication (Batchelor *et al*., 1984). Leeches were also used to decongest completely amputated ears (Mutimer *et al*., 1987). Others used a 4 day leeching course for the treatment of eight individuals who received replantation and revascularization operations after amputation injuries. It was outlined that four patients responded positively and gained normal circulation (Rouholamin and Harris, 1991).

Replantation of amputated facial tissues (nasal tips, lower lip, scalp and ears) with microvascular anastomosis achieved a great success and better cosmetic outcomes when venous drainage was augmented by leech application along with arterio-venous fistula and pinpricks. It was reported that more than half of the treated cases were completely salvaged (Jeng *et al*., 1994). Others outlined that bloodletting by leeches in combination with vascular endothelial growth factor may improve flap survival (Kubo *et al*., 2002). Furthermore, leech application was prescribed as a postoperative care in patients who underwent a surgical operation for replantation of the fingertip (Tsai *et al*., 1989). More recently, some medics outlined a successful application of leech to salvage an ischemic finger. At the 7th day of the treatment, the patient described sensation improvement and sensitivity to pinprick at the top of the finger (Durrant *et al*., 2006).

Many successful leech applications after resection and replantation procedures were documented. For example, a woman who suffered from basal cell carcinoma over the nose and underwent through surgical procedures exhibited a normal blood circulation, and a healthy flap after nine months of leech therapy (Michalsen *et al*., 2007). Leech therapy was successfully applied to avoid venous insufficiency in patients who received free perforator flaps for the medial sural artery which supplies the medial gastrocnemius muscle and the overlying skin (Kim *et al*., 2009). Recently, it was reported that leeching was used to treat six patients with venous congested microvascular free flaps in which venous efflux and surgical

operation could not be performed. They highlighted that a treatment regimen for a period of 4-14 days resulted in all flaps were safely salvaged (Koch *et al*., 2012).

Infectious diseases

The continuously increasing rates of infectious diseases led to a higher usage of the commercially available antibiotics, which resulted in a new challenging phenomenon known as resistance to antimicrobial agents. Therefore, scientists have set up new strategies to develop antimicrobial drugs with novel mechanisms of action and lower incidence of bacterial resistance (Irish *et al*., 2000). Many reviewers who investigated the therapeutic importance of the medicinal leech cited that leeching could be effective for the treatment of infection without mentioning more details or information about leech application protocols and the nature of the active component. For instance, some reported that leech therapy was used by traditional dentists as a remedy for dental infections such as periodontitis and alveolar abscesses (Kalender *et al*., 2010).

A protein named destabilase with a lysozyme-like activity had been isolated from the medicinal leech extract. It was reported that this protein had an antibacterial activity against some bacterial strains because it can destroy their cellular component (Bernard Aschner). Some researchers delineated that injecting lipopolysaccharides or making a surgical cut in the leech *T. tessulatum* resulted in a rapid release of neurosignaling and antimicrobial peptides (AMPs) that work synergistically to suppress the bacterial incursion and to activate the immune response of the attacked cells. Two AMPs, theromacin and theromyzin, were isolated from the body fluid of the leech *T. tessulatum*. It was found that both had an antibacterial activity against the Gram-positive bacterial strains, *Micrococcus luteus*. Moreover, it was reported that the nervous system of the European leech, *H. medicinalis*, could initiate an antimicrobial response after injury by signaling the synthesis of AMPs. Three different peptides with antibacterial activities were identified from this leech species. Hm-lumbricin and neuromacin were isolated from neurons and microglial cells while peptide B was found in leech body fluids.

Recently, some researchers patented the usage of the leech extract from many leech species of the family*Hirudinidae* as an antimicrobial agent with various applications. They argued that the purified extract obtained from any part of leech body, especially salivary glands, showed an antimicrobial activity against many Gram-negative/positive pathogens. They reported that leech extract had a high antibacterial activity against *Shewanella* and *Aerococcus viridans* while a lower activity was observed against *Escherichia coli*,*Salmonella typhi* and *Staphylococcus aureus*. They outlined that leech extract could be used in the treatment of bacteria-induced illnesses including arthritis, foodborne disorders, and nosocomial infections. They also highlighted a beneficial usage of the leech extract in cleaning products for hospital disinfection and the daily domestic cleaning (Irish *et al*., 2000). Finally, the salivary gland secretion obtained from the tropical leech *H. manillensis*was found to have a wide spectrum antibacterial activity against both Gram-positive (*S. aureus*) and Gram-negative (*Sal. typhi* and *E. coli*) bacterial strains.

Arthritis and analgesic

The painkiller effects of leech application were ascertained in many trials on patients with osteoarthritis who claimed that leeching was more relieving than topical diclofenac with no adverse effects. Likewise, some studies proved that hirudin can reduce synovial inflammation in arthritis patients by inhibiting DING protein, a derivative of synovial stimulatory protein acting as autoantigen in rheumatoid arthritis patients[87]. In another study, a group of women with osteoarthritis of the first carpometacarpal joint received a treatment course by 2-3 leeches locally. All treated individuals revealed less pain and disability improvement. The efficacy of leeching was observed after 1 week of therapy and lasted for at least 2 months.

Another clinical trial on patients with advanced osteoarthritis at the knee proved that leech therapy could effectively reduce the need for analgesic intake. It has been outlined that a double treatment regimen at a 4-week interval exhibited a longer term relieving and a better physical activity than a single treatment course. Moreover, the effectiveness of leech therapy in combination with the traditional Unani herbal formulation was also assessed. It was observed that patients who received the combined treatment displayed less pain and stiffness with better working ability. Other reports indicated leech therapy as an analgesic for iliosacral joints pain and cervicobrachialgia syndrome (Michalsen *et al*., 2007).

Audiology and ear abnormalities

It was reported that leeches and their salivary secretion were successfully used for the treatment of tinnitus, acute and chronic otitis. Leeching has been applied in sudden hearing loss. In such cases, the therapist used just two leeches; one behind the ear and the other one over the jaw in front of the ear, and the treatment was repeated 2-3 times at intervals of 3-4 days. Despite the unexplained reasons of tinnitus, leeches were proven to be of great benefits in the treatment of this disorder.

Dentistry

Although, the benefits of leeching in dentistry have not been established yet, many reports mentioned leech application in dental abnormalities (Srivastava and Sharma, 2010). Traumatic and postoperative macroglossia (tongue swelling) have been associated with life-threatening complications, especially, airways occlusion. The bloodletting by leeches was reported to be considerably successful in the management of severe postoperation macroglossia cases when the common treatment method was not satisfactory. Other case reports described the use of the medicinal leech in the treatment of sublingual hematoma and massive lingual hematoma. Others outlined the usage of leeches in gum diseases. For example, the direct application of 3-4 leeches can be a successful remedy for abscess and inflammation (Michalsen *et al*., 2007)

Skin disorders

Leeching has been practiced by traditional therapists for the treatment of skin disorders with no scientific studies supporting this utilization like in the viral skin infection named shingle disease (Michalsen *et al*., 2007).

Hirudotherapy in maxillofacial region

Leeches in modern facial cosmetology

Nowadays hirudotherapy is a venue in holistic healing of all our body systems. In a study by Xin Li *et al*. (Katzung *et al*., 2009) they state that the body detoxification, blood purification and oxygenation caused by hirudotherapy has rejuvenating effect on the entire body. Medicinal leeches' salivary glands contain Lipids along with Hirudin, Proteins, Serotonin, Hyaluronidase, Collagenase, Elastase which are active essential ingredients in the skincare (reconstruction of the cell membranes, skin cells and tissue). Lipids represent about 20% of the total weight of the salivary glands.

Together with lipids are natural steroid hormones such as cortisol, dehydroepiandrosterone (androstenolone), testosterone, progesterone and estradiol. The other largest group of compounds in the salivary glands is formed by phosphatidic acids and free fatty acids (important source of energy) as documented on medicinal leeches in Russia, O. Kamenev, A.Baranovski, U. Krashenyuk, Baskova and others]. They claim Hirudotherapy brings healthy look and glow on a face, improves skin elasticity, stops hair loss, significantly reduces cellulite, dissolves scar tissue & visibly diminished scars, eliminates spider veins, improves blood circulation. There is also a application for non-invasive face lift. Studies have attempted lift of the eyelids done without surgery with application of hirudotherapy. After hirudotherapy treatment the healthy body shows better attitude -- the feeling of well-being has been influenced by endorphins.

Facial reconstructive flap surgery

Review of the literature indicates that the survival of the compromised, venous-congested flap is improved by early intervention with the medicinal leech. H medicinalis injects salivary components that inhibit both platelet aggregation and the coagulation cascade. The flap is decongested initially as the leech extracts blood and is further decongested as the bite wound oozes after the leech detaches (Seymour *et al*., 1990). When a flap begins to fail, salvage of that flap demands early recognition of reversible processes, such as venous congestion. The surgeon must be familiar with the use of leeches and should consider their use early, since flaps demonstrate significantly decreased survival after 3 hours if venous congestion is not relieved. In the four cases presented, a standardized protocol facilitated early leech use and provided for the psychological preparation of the patient, availability of leeches, and an antibiotic prophylaxis regimen. The complications associated with leech use can be minimized with antibiotic therapy, wound care, and hematocrit monitoring. The use of the medicinal leech for salvage of the venous-congested flap is a safe, efficacious, economical, and well-tolerated intervention.

Revision of facial scars and keloid with hirudotherapy

Keloid usually grows beyond the borders of the original wound in claw-like growths and can develop after acne, body piercings, burns, laceration, surgical wounds etc. In a study by Arshid Iqbal *et al*., 2015 to assess the effect of leech therapy in resolving the keloid without surgical intervention and to avoid the scar formation and recurrence. The study has been conducted at Regional Research Institute of Unani Medicine, Srinagar, to evaluate the keloid resolving activity by the bioactive substances present in the leech saliva proved very effective by giving the Hirudotherapy to a young female patient with post traumatic keloid .The keloid was completely resolved and leaving the skin surface very smooth without scar formation .There was no recurrence of keloid even after one year of post leech therapy follow ups (Gasi, 1986).

Facial space infections

Leeches themselves may prove a direct source of antibiotics. Michel Salzet of the University of Science and Technology in Lille, France, has found infection-fighting peptides in leeches akin to those that have already been discovered in insects and other invertebrates. In leeches, these peptides are produced within 15 minutes of a bacterial infection. "These antimicrobial peptides diffuse quicker and easier than antibodies," he says, suggesting that such speed and potency might add up to a defence that can outbreed and outrun pathogens. "Antibacterial peptides from leeches may cure human diseases," Salzet says (Ding *et al*., 1994).

Facial haematomas

Treatment of unsightly facial haematomas and lingual haematomas with hirudotherapy have been advocated in past literatures (Sun, 2007).

Complications of Hirudotherapy

Infection is the most common complication of leeching and occurs in 2-36% of the patients (Green and Shafritz, 2010). Several bacterial strains have been encountered in these infections involving *Aeromonas* spp., *Pseudomonas* spp. and*Vibrio* spp. agent is the Gram-positive rod, *Aeromonas hydrophila*, which can cause pneumonia, mascular necrosis, flap failure and even septicaemia. Because *A. hydrophila* are resistant to penicillins and the first generation of cephalosporins, the treatment regimen of such infections should contain aminoglycosides, fluoroquinolones. On the other hand, there are no reports on the leech therapy-transmitted diseases, even though; physicians who practice leeching are advised to use a leech once (Michalsen *et al*., 2007). Many reports outlined local hypersensitivity conditions including itching, blister forming, ulcerative necrosis and even local tissue damage (flap death), which might result from the existence of some toxins in leech saliva (Srivastava and Sharma, 2010). Blood loss because of the prolonged hemorrhage and skin marks (scars) left by impaired healing of leech bites are also reported as post leeching complications (Koch *et al*., 2012).

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. Presently 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 (Atlanta, 2011). 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.

Conclusions

To conclude, leeching was a popular therapeutic practice throughout the ages for a wide range for diseases and it was applied as an unscientific home remedy by traditional therapists. Nowadys, leech came back to the contemporary medicine with fewer applications, which were proven and supported by a huge number of scientific studies and case reports. Leech therapy in the field of plastic and reconstructive surgery is expected to be of paramount importance due to the ease of leech application and reduced side-effects. Hence, more efforts should be undertaken to optimize this utilization. More investigations are required also to assess leech efficacy and safety in the treatment of DM and cancer.

Conflict of interest statement

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

REFERENCES

Ascenzi, P., Amiconi, G., Bode, W., Bolognesi, M., Coletta, M. and Menegatti, E. 1995. Proteinase inhibitors from the European medicinal leech *Hirudo medicinalis*: Structural, functional and biomedical aspects. *Mol Aspects Med,* 16:215–313.

Atlanta: American Cancer Society Inc; 2011. [Cited on 2011 Oct 9]. ACS. Global cancer facts and figures. Available from:http://www.cancer.org/Research/CancerFactsFigures/GlobalCancerFactsFigures/global-facts-figures-2nd-ed .

Baskova, I.P. and Zavalova, L.L. 2001. Proteinase inhibitors from the medicinal leech *Hirudo medicinalis*. Biochemistry (Mosc) 66:703–14.

Baskova, I.P., Korostelev, A.N., Chirkova, L.D., Zavalova, L.L., Basanova, A.V. and Doutremepuich, C. 1997. Piyavit from the medicinal leech is a new orally active anticoagulating and antithrombotic drug. *Clin. Appl. Thromb. Hemost.,* 3:40–5.

Baskova, I.P., Kostrjukova, E.S., Vlasova, M.A., Kharitonova, O.V., Levitskiy, S.A., Zavalova, L.L., *et al*. 2008. Proteins and peptides of the salivary gland secretion of medicinal leeches *Hirudo verbana, H. medicinalis*, and *H. orientalis*. Biochemistry (Mosc), 73:315–20.

Batchelor, A.G., Davison, P. and Sully, L. 1984. The salvage of congested skin flaps by the application of leeches. *Br. J. Plast Surg.,* 37:358–60.

Bernard Aschner, 1973. Theories and Philosophies of Medicine. Institude of History of Medicine and Medical Research, New Delhi, 242-253.

Blankenship, D.T., Brankamp, R.G., Manley, G.D. and Cardin, A.D. 1990. Amino acid sequence of ghilanten: Anticoagulant-antimetastatic principle of the South American leech, *Haementeria ghilianii*. *Biochem. Biophys. Res. Commun.,* 166:1384–9.

Brankamp, R.G., Manley, G.G., Blankenship, D.T., Bowlin, T.L. and Cardin, A.D. 1991. Studies on the anticoagulant, antimetastatic and heparin-binding properties of ghilanten-related inhibitors. Blood Coagul Fibrinolysis, 2:161–6.

Britanica, 2012. Leech: Encyclopedia Britanica. 2012. [Cited on 2012 Mar 31]. Available from:http://www.britannica.com/EBchecked/topic/334632/leech .

Cardin, A.D. and Sunkara, S.P. 1994. Munich, Germany: European Patent Office, issued March 2; Ghilanten antimetastatic principle from the south American leech *Haementeria ghilianii*. European Patent No. EP 0404055.

Cherniack, E.P. 2011. Bugs as drugs, part two: Worms, leeches, scorpions, snails, ticks, centipedes, and spiders. *Altern Med Rev.,*16:50–8.

Chopin, V., Salzet, M., Baert, J.l., Vandenbulcke, F., Sautiére, P.E., Kerckaert, J.P., *et al*. 2000. Therostasin, a novel clotting factor Xa inhibitor from the rhynchobdellid leech, *Theromyzon tessulatum*. *J.Biol.Chem.,*275:32701–7.

Chudzinski-Tavassi, A.M., Kelen, E.M., de Paula Rosa, A.P., Loyau, S., Sampaio, C.A., Bon, C., *et al*. 1998. Fibrino (geno) lytic properties of purified hementerin, a metalloproteinase from the leech *Haementeria depressa*. *Thromb Haemost.,* 80:155–60.

Conforti, M.L., Connor, N.P., Heisey, D.M., Vanderby, R., Kunz, D. and Hartig, G.K. 2002. Development of a mechanical device to replace medicinal leech (*Hirudo medicinalis*) for treatment of venous congestion. *J. Rehabil. Res. Dev.,* 39:497–504.

Corral-Rodríguez, M.A., Macedo-Ribeiro, S., Pereira, P.J., Fuentes-Prior, P. 2010. Leech-derived thrombin inhibitors: From structures to mechanisms to clinical applications. *J. Med Chem.,* 53:3847–61.

Davis, A. and Appel, T. 1979. Bloodletting Instruments in theNational Museum of History and Technology.Smithsonian Institution Press, Washington, D.C., 34-36.

Ding, J.X., Ou, X.C. and Zhang, Q.H. 1994. Comparison among 5 different extracting methods of *Whitmania pigra*whitman. Zhongguo Zhong Xi Yi Jie He Za Zhi., 14:165–6. 134.

Durrant, C., Townley, W.A., Ramkumar, S. and Khoo, C.T. 2006. Forgotten digital tourniquet: Salvage of an ischaemic finger by application of medicinal leeches. *Ann. R. Coll. Surg. Engl.,* 88:462–4.

Electricwala, A., Sawyer, R.T., Jones, C.P. and Atkinson, T. 1991. Isolation of thrombin inhibitor from the leech*Hirudinaria manillensis*. Blood Coagul Fibrinolysis, 2:83–9.

Faria, F., Kelen, E.M., Sampaio, C.A., Bon, C., Duval, N. and Chudzinski-Tavassi, A.M. 1999. A new factor Xa inhibitor (lefaxin) from the *Haementeria depressa* leech. Thromb Haemost. 82:1469–73.

Gasic, G.J. 1986. U.S. Patent No. 4,588,587. Washington, DC: U.S. Patent and Trademark Office, issued May 13; 1986. Method of treatment to inhibit metastasis.

Gasic, G.J., Iwakawa, A., Gasic, T.B., Viner, E.D. and Milas, L. 1984. Leech salivary gland extract from *Haementeria officinalis*, a potent inhibitor of cyclophosphamide- and radiation-induced artificial metastasis enhancement. *Cancer Res.,* 44:5670–6.

Gasic, G.J., Viner, E.D., Budzynski, A.Z. and Gasic, G.P. 1983. Inhibition of lung tumor colonization by leech salivary gland extracts from *Haementeria ghilianii*. *Cancer Res.,* 43:1633–5.

Green, P.A. and Shafritz, A.B. 2010. Medicinal leech use in microsurgery. *J. Hand Surg. Am.,* 35:1019–21.

Harsfalvi, J., Stassen, J.M., Hoylaerts, M.F., Van Houtte, E., Sawyer, R.T., Vermylen, J., *et al*. 1995. Calin from *Hirudo medicinalis*, an inhibitor of von Willebrand factor binding to collagen under static and flow conditions. *Blood,* 85:705–11.

Hartig, G.K., Connor, N.P., Warner, T.F., Heisey, D.M., Sarmadi, M. and Conforti, M.L. 2003. Testing a device to replace the leech for treating venous congestion. *Arch. Facial. Plast. Surg.,* 5:70–7.

Haycraft, J.B. 1883. On the action of a secretion obtained from the medicinal leech on the coagulation of the blood. *Proc. R. Soc. Lond*., 36:478–87.

Henderson, H.P., Matti, B., Laing, A.G., Morelli, S. and Sully, L. 1983. Avulsion of the scalp treated by microvascular repair: The use of leeches for post-operative decongestion. *Br. J. Plast Surg.,* 36:235–9.

Hong, S.J. and Kang, K.W. 1999. Purification of granulin-like polypeptide from the blood-sucking leech, *Hirudo nipponia*. Protein Expr Purif., 16:340–6.

Irish, J.C., Gullane, P.J., Mulholland, S. and Neligan, P.C. 2000. Medicinal leech in head and neck reconstruction.J Otolaryngol., 29:327-332.

Jeng, S.F., Wei, F.C. and Noordhoff, M.S. 1994. Replantation of amputated facial tissues with microvascular anastomosis. *Microsurgery,* 15:327–33.

Jung, H.I., Kim, S.I., Ha, K.S., Joe, C.O. and Kang, K.W. 1995. Isolation and characterization of guamerin, a new human leukocyte elastase inhibitor from *Hirudo nipponia*. *J Biol Chem.,* 270:13879–84.

Kalender, M.E., Comez, G., Sevinc, A., Dirier, A. and Camci, C. 2010. Leech therapy for symptomatic relief of cancer pain. *Pain Med.,* 11:443–5.

Katzung, B.G., Masters, S.B. and Trevor, A.J. 2009. 11th ed. China: McGraw Hill Companies; Basic and Clinical Pharmacology.

Kim, D.R. and Kang, K.W. 1998. Amino acid sequence of piguamerin, an antistasin-type protease inhibitor from the blood sucking leech *Hirudo nipponia*. *Eur. J. Biochem.,* 254:692–7

Kim, E.S., Hwang, J.H., Kim, K.S. and Lee, S.Y. 2009. Plantar reconstruction using the medial sural artery perforator free flap. *Ann. Plast. Surg.,* 62:679–84.

Knobloch, K. 2011. Leeches in microsurgery – An evidence-based approach. In: Kini RM, Clemetson KJ, Markland FS, McLane MA, Morita T, editors. Toxins and Hemostasis. Netherlands: *Springer Science,* pp. 735–45.

Koch, C.A., Olsen, S.M. and Moore, E.J. 2012. Use of the medicinal leech for salvage of venous congested microvascular free flaps of the head and neck. *Am. J. Otolaryngol.,* 33:26–30

Koh, C.Y. and Kini, R.M. 2008. Anticoagulants from hematophagous animals. *Expert Rev Hematol.,* 1:135–9

Kubo, T., Yano, K. and Hosokawa, K. 2002. Management of flaps with compromised venous outflow in head and neck microsurgical reconstruction. *Microsurgery,* 22:391–5.

Markwardt, F. 1970. Hirudin as an inhibitor of thrombin. *Methods Enzymol.,* 19:924–32.

Markwardt, F. 2002. Historical perspective of the development of thrombin inhibitors. Pathophysiol Haemost Thromb., 32(Suppl 3):15–22.

Masharani, U. 2010. Diabetes mellitus and hypoglycemia. In: Mchphee SJ, Papadakis MA, editors. Current Medical Treatment and Diagnosis. 49th ed. USA: McGraw Hill Companies, pp. 1079–122.

Maton, A. 1993. New Jersey: Prentice Hall; 1993. Human Biology and Health.

Merzouk, A., Ghawi, A., Abdualkader, A.M., Abdullahi, A. and Alaama, M. 2012. Anticancer effects of medical Malaysian leech saliva extract (LSE) *Pharm Anal Acta S.* 15:2–6.

Michalsen, A., Roth, M., Dobos, G. and Aurich, M. 2007. Stattgurt, Germany: Apple Wemding, Medicinal Leech Therapy.

Mineo, M., Jolley, T. and Rodriguez, G. 2004. Leech therapy in penile replantation: A case of recurrent penile self-amputation. *Urology,* 63:981–3.

Moore, J.P. 1952. Professor A.E. Verrill's fresh-water leeches-A tribute and a critique. *Acad Nat Sci Phila.,* 245:1–16.

Munshi, Y., Ara, I., Rafique, H. and Ahmad, Z. 2008. Leeching in the history – A review. *Pak. J. Biol. Sci.,* 11:1650–3.

Murray, R.K., Bender, D.A., Botham, K.M., Kennelly, P.J., Rodwell, V.W. and Weil, P.A. 2009. 28th ed. China: McGraw Hill Companies; Harper's Illustrated Biochemistry.

Mutimer, K.L. and Banis, J.C. 1987. Upton J. Microsurgical reattachment of totally amputated ears. *Plast Reconstr Surg.,* 79:535–41.

Pantuck, A.J., Lobis, M.R., Ciocca, R. and Weiss, R.E. 1996. Penile replantation using the leech *Hirudo medicinalis*. *Urology,* 48:953–6

Penchenik, J. 2000. 4th ed. Boston: McGraw-Hill Companies; Biology of Invertebrates.

Porshinsky, B.S., Saha, S., Grossman, M.D., Beery Ii, P.R. and Stawicki, S.P. 2011. Clinical uses of the medicinal leech: A practical review. *J. Postgrad. Med,* 57:65–71.

Rouholamin, E. and Harris, D. 1991. 'Reach for a leech': Leeches and microsurgery. Injury, 22:159–60.

Rouse, G. and Pleijel, F. 2006. Enfield, USA: Science Publishers; Reproductive Biology and Phylogeny of *Annelida*.

Salzet, M. 2001. Anticoagulants and inhibitors of platelet aggregation derived from leeches. FEBS Lett. 492:187–92.

Salzet, M., Chopin, V., Baert, J., Matias, I. and Malecha, J. Theromin, a novel leech thrombin inhibitor. *J. Biol. Chem.,* 275:30774–80.

Sawyer, RT. 1986. Leech Biology and Behaviour. Vol. 2. Michigan: Clarendon Press; Feeding, biology, ecology and systematics.

Seymour, J.L., Henzel, W.J., Nevins, B., Stults, J.T. and Lazarus, R.A. 1990. Decorsin. A potent glycoprotein IIb-IIIa antagonist and platelet aggregation inhibitor from the leech *Macrobdella decora*. *J. Biol. Chem.,* 265:10143–7.

Smith, D.B. 1833. On the medicinal leeches. *J Phila Coll Pharm.,* 4:265–71.

Sohn, J.H., Kang, H.A., Rao, K.J., Kim, C.H., Choi, E.S., Chung, B.H., *et al*. 2001. Current status of the anticoagulant hirudin: Its biotechnological production and clinical practice. *Appl. Microbiol. Biotechnol.,* 57:606–13.

Srivastava, A. and Sharma, R. 2010. A brief review on applications of leech therapy. *Arch Appl. Sci. Res*., 2:271–4.

Strube, K.H., Kröger, B., Bialojan, S., Otte, M. and Dodt, J. 1993. Isolation, sequence analysis, and cloning of haemadin. An anticoagulant peptide from the Indian leech. *J. Biol. Chem.,* 268:8590–5.

Sun, H. 2007. From leeching to anaphrodisiacs: Treatments of epilepsy in the nineteenth century. *Univ. Toronto. Med J.,* 84:107–10.

Susanto, S. 2008. Indonesia: The Jakarta Post, Bantul., [Cited on 2011 Oct 9]. Leeches used to treat diabetes patients. Available from: http://www.thejakartapost.com/news/2008/04/30/leeches-used-treat-diabetes-patients.html .

Swadesh, J.K., Huang, I.Y. and Budzynski, A.Z. 1990. Purification and characterization of hementin, a fibrinogenolytic protease from the leech *Haementeria ghilianii*. *J. Chromatogr.,* 502:359–69.

Tsai, T.M., McCabe, S.J. and Maki, Y. 1989. A technique for replantation of the finger tip. *Microsurgery,* 10:1–4.

Tuszynski, G.P., Gasic, T.B. and Gasic, G.J. 1987. Isolation and characterization of antistasin. An inhibitor of metastasis and coagulation. *J. Biol. Chem.,* 262:9718–23.

Upshaw, J. and O'Leary, J.P. 2000. The medicinal leech: Past and present. *Am. Surg.,* 66:313–4.

USA, World Health Organization, 2011. [Cited on 2011 Oct 9]. WHO. Cardiovascular diseases (CVDs) Available from: http://www.who.int/mediacentre/factsheets/fs317/en .

Wallis, R.B., Fidler, I.J. and Esumi, N. Munich, Germany: European Patent Office, issued September 16; 1992. Hirudin for the inhibition of cancer metastasis. European Patent No. EP 0503829.

Walsmann, P. and Markwardt, F. 1985. On the isolation of the thrombin inhibitor hirudin. Thromb Res., 40:563–9.

Whitaker, I.S., Cheung, C.K., Chahal, C.A., Karoo, R.O., Gulati, A. and Foo, I.T. 2005. By what mechanism do leeches help to salvage ischaemic tissues? A review. *Br. J. Oral. Maxillofac Surg.,* 43:155–60.

Whitaker, I.S., Rao, J., Izadi, D. and Butler, P.E. 2004. Historical article: *Hirudo medicinalis*: Ancient origins of, and trends in the use of medicinal leeches throughout history. *Br J Oral Maxillofac Surg.,* 42:133–7.

Whitaker, I.S., Rao, J., Izadi, D. and Butler, P.E. 2004. Historical Article: Hirudo medicinalis: ancient origins of, andtrends in the use of medicinal leeches throughouthistory. *Br. J. Oral Maxillofac Surg.,* 42:133-137.

White, T.C., Berny, M.A., Robinson, D.K., Yin, H., DeGrado, W.F., Hanson, S.R., *et al*. 2007. The leech product saratin is a potent inhibitor of platelet integrin alpha2beta1 and von Willebrand factor binding to collagen. *FEBS J.,* 274:1481–91.

Wild, S., Roglic, G., Green, A., Sicree, R. and King, H. 2004. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. *Diabetes Care,* 27:1047–53

Yule, C.M. and Yong, H.S. 2004. Kaula Lampur: Akademi Sains Malaysia, Freshwater Invertebrates of the Malaysian Region.

****