Leech Therapeutic Applications

A. M. ABDUALKADER*, A. M. GHAWI[1], M. ALAAMA, M. AWANG AND A. MERZOUK[2]
Departments of Pharmaceutical Chemistry, and [1]Basic Medical Science, Faculty of Pharmacy, International Islamic University Malaysia, Jalan Istana, 25200 Kuantan, Pahang, Malaysia, [2]Biopep Solutions Inc., 235-11590 Cambie Road, Richmond, BC V6X 3Z5, Canada

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Hematophagous animals including leeches have been known to possess biologically active compounds in their secretions, especially in their saliva. The blood-sucking annelids, leeches have been used for therapeutic purposes since the beginning of civilization. Ancient Egyptian, Indian, Greek and Arab physicians used leeches for a wide range of diseases starting from the conventional use for bleeding to systemic ailments, such as skin diseases, nervous system abnormalities, urinary and reproductive system problems, inflammation, and dental problems. Recently, extensive researches on leech saliva unveiled the presence of a variety of bioactive peptides and proteins involving antithrombin (hirudin, bufrudin), antiplatelet (calin, saratin), factor Xa inhibitors (lefaxin), antibacterial (theromacin, theromyzin) and others. Consequently, leech has made a comeback as a new remedy for many chronic and life-threatening abnormalities, such as cardiovascular problems, cancer, metastasis, and infectious diseases. In the 20th century, leech therapy has established itself in plastic and microsurgery as a protective tool against venous congestion and served to salvage the replanted digits and flaps. Many clinics for plastic surgery all over the world started to use leeches for cosmetic purposes. Despite the efficacious properties of leech therapy, the safety, and complications of leeching are still controversial.

Key words: Bloodletting, cancer, cardiovascular diseases, diabetes mellitus, hirudin, leech, microsurgery

Hematophagous animals that feed on prey blood have been known to overcome blood clotting by secreting in their salivary gland secretion a multitude of biologically active compounds, especially the anticoagulants[1]. Amongst the blood-sucking organisms, leech is a distinct example of an invertebrate, which possesses a highly-developed mechanism by which they prevents blood clotting[2]. Through centuries, leeches have attracted the attention of therapists who employed leech therapy for a wide range of diseases. For various therapeutic purposes, the European medicinal leech species, *Hirudo medicinalis,* also known as the healing leech was preferred by the majority of physicians compared to the American species, *Hirudo decora*, which can suck less blood due to a smaller and superficial incision on its prey skin[3-5]. In addition, many other species were also considered as medical tools, such as *Hirudinaria manillensis*[6], *Hirudo nipponia*[7,8], *Hirudo verbena*, *Hirudo orientalis*[9], and *Haementeria depressa*[2,10].

The current review summarizes the importance of leeches as a complementary source of medical therapy for a large number of ailments, including cardiovascular diseases (CVDs), plastic surgery, cancer and metastasis, diabetes mellitus (DM), and its complication and infectious disorders.

***Address for correspondence**
E-mail: armak1983@gmail.com

Leech locality and ecology:

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. 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. Because moisture is a very essential factor affecting the terrestrial leech's distribution and behavior, they can be found in a large number in the forests and highlands of North America, Europe, and South-East Asia. In permanently humid regions, such as Malaysia, leeches will stay active throughout the year while they go through an active and a dormant phase in territories with wet and dry seasons[11].

Leech taxonomy and morphology:

Leeches (*Euhirudinea*) were first named by Linnaeus in 1758 AD[3]. 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[12]. Recently, taxonomists identified more than 1000 leech species[13]. Leech size varies 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[14]. A classic leech body consists of many segments divided as two preoral, nonmetameric segments, and 32 postoral metameres (somites). Somites are subdivided into 2-16 external annuli, and the annulation pattern can be considered as a diagnostic feature for leech genus and species. Sensory structures, such as eyes, oculiform spots, papillae and sensilla are also used by taxonomists to identify genus, and species. Typically, a leech has anterior and posterior suckers. Some leeches related to the order *Rhynchobdellida* have a large anterior sucker with a small jaw-less mouth and protrusible muscular proboscis. Others from the order *Arhynchobdellida* possess a simple anterior sucker with a wide mouth, which may or may not have jaws such as in *hirudinids* and *erpobdellids*, respectively. Suckers are very essential during movement (inchworm-like locomotion) and for attachment to host surface[11]. Leeches breathe through the skin and they are considered as hermaphrodites, but always require another leech for fertilization[14].

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 ectoparasites that feed on the blood of vertebrates including human[11]. With the help of suckers and the biting jaws, leeches are able to absorb prey blood[15]. 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[16,17].

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[11,12].

HISTORICAL REVIEW OF LEECHING

The importance of leech in clinical therapy can be simply represented from the Anglo-Saxon word of physician "laece" indicating that both doctors and these annelids were etymologically related to each other since the beginning of civilisation[3,18]. 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 18th dynasty Pharaohs paintings (1500 BC). Talmud, Bible, and other Jewish manuscripts outlined the medical indications of leeching[18]. The Greek poets, Nicader of Colophain (200-130 BC) mentioned leeches in his medical poems[3].

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[3,19]. Galen would prescribe bloodletting by leech for almost all illnesses such as simple inflammatory conditions, mental disorders and hemorrhoids[20]. Moreover, Themission of Laodice, a Syrian doctor, outlined that removing blood from the patient will evacuate the evil spirits, which can cause diseases[3].

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[3,19] and he recommended leeching for skin diseases[21]. In 12th century, Abd-el-latif al-Baghdadi mentioned in his texts the beneficial usage of leech application after surgical operations[21]. 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[19].

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[3,19]. 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[3].

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[3]. Meanwhile, leeches were employed to treat mental disorders, skin diseases, gout, headache, and whooping cough[14].

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[3]. 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[22]. 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[23], which was later isolated and identified by Markwardt who demonstrated its antithrombin activity[24]. 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[20].

In 1981, a foundation for leech breeding development and medicinal leech research was established by an American biologist, Roy T. Sawyer[3]. 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[25,26].

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[19,21]. This novel therapeutic utilization of leeches resulted in more interest in isolation and characterization of the active constituents of leech saliva[27]. In 2004, the Food and Drug Organization (FDA) approved leeches for medicinal purposes[19]. 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[28,29].

MODERN APPLICATIONS OF LEECHING

Cardiovascular diseases:

CVDs are a group of chronic abnormalities affecting the cardiovascular system including heart, veins and arteries[30]. Among the incurable diseases, CVDs were considered the principal culprit of mortality, causing up to 30% of global deaths by the year 2008[31]. 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[32].

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[17]. 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[33]. 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[34]. 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[17,21].

The effectiveness of leech saliva in CVDs is the results of specific thrombin inhibitors, hirudin, which was first isolated from *H. medicinalis*[23-25] and was shown to possess a potent inhibitory effect on both free and clot-bound thrombin[35,36]. 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*[37]. Another antithrombin named granulin-like was isolated from the leech species *H. nipponia*[38]. 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[39].

Noteworthy, hirudin is the only hematophagous animal-derived anticoagulant has been approved by FDA for clinical purposes[32]. Many studies revealed that hirudin is more effective than heparin in preventing deep venous thrombosis (DVT) and ischemic events in patients with unstable angina[32]. 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[36]. 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[32,36].

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[32]. Precisely, desirudin is meanwhile in use for the prevention of DVT following hip or knee replacement surgery[40].

On the other hand, leeches have developed other active compounds targeting different coagulation factors, such as antiplatelet, factor Xa (FXa) inhibitors, and fibrinolytic enzymes[41]. First, a potent antiplatelet named decorsin was identified from *Macrobdella decora* with a high affinity to glycoprotein IIb-IIIa receptors[42]. 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[43]. 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[44]. 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[45].

Furthermore, several inhibitors of factor Xa were identified from leech saliva extract such as ghilanten[46], lefaxin[2] and therostatin[47] 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)[48]. Moreover, hementin and hementerin were characterized from *H. ghilianii*[49] and *H. depressa*[10] 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[10].

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[18]. 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 tissues[18,50]. 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[18,51]. 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[50]. 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 complications[17]. 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[18,52]. 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[18].

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[53]. 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 complications[54]. Leeches were also used to decongest completely amputated ears[55]. 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[56]. 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[57]. Others outlined that bloodletting by leeches in combination with vascular endothelial growth factor may improve flap survival[58]. Furthermore, leech application was prescribed as a postoperative care in patients who underwent a surgical operation for replantation of the fingertip[59]. 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[60].

Penile replantation is commonly associated with venous insufficiency. By the year 1996, some researchers reported for the first time the efficacious usage of leeching to relieve the postoperative venous congestion in a 37-year-old man who had a completely amputated penis[61]. Penile replantation by nonmicrosurgical operations achieved a great success when accompanied with hirudotherapy. Leeching-treated patients revealed no edema and normal functions, such as emptying, sensation, and erection[62].

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[17]. 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[63]. 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[52].

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[64]. 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[65]. 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[66]. 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[66]. Later, an antimetastatic and anticoagulant protein named ghilanten was purified from the salivary gland secretion of the proboscis leech, *H. ghilianii*[67]. It was reported that ghilanten could suppress metastasis of melanoma, breast cancer, lung cancer, and prostate cancer[68]. 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[65].

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[69,70].

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[71]. 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[72].

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[73]. 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[74]. 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[75].

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[73]. 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[32]. Calin, isolated from *H. medicinalis*, has been proven to obstruct the formation of thrombi as described above[43]. 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[2,6].

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[73]. 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[76]. 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[77].

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 amputation[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).

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[78].

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[21].

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 components[79,80]. 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[81]. 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*[82]. 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[83]. 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[84].

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[78]. 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[85].

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[86]. 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[88].

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[89]. 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[90]. Other reports indicated leech therapy as an analgesic for iliosacral joints pain and cervicobrachialgia syndrome[17].

OTHER APPLICATIONS OF LEECHING

Dentistry:

Although, the benefits of leeching in dentistry have not been established yet, many reports mentioned leech application in dental abnormalities[21]. Traumatic and postoperative macroglossia (tongue swelling) have been associated with life-threatening complications, especially, airways occlusion[91]. 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[92]. Other case reports described the use of the medicinal leech in the treatment of sublingual hematoma and massive lingual hematoma[93,94]. 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[17].

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[95]. 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[17].

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[17].

SAFETY AND COMPLICATION OF LEECHING

Infection is the most common complication of leeching and occurs in 2-36% of the patients[51]. Several bacterial strains have been encountered in these infections involving *Aeromonas* spp., *Pseudomonas* spp. and *Vibrio* spp. The main infection-causing 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[16,21]. On the other hand, there is no reports on the leech therapy-transmitted diseases, even though, physicians who practice leeching are advised to use a leech once[17].

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[21]. Blood loss because of the prolonged hemorrhage and skin marks (scars) left by impaired healing of leech bites are also reported as postleeching complications[51].

CONCLUSION

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.

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