Medical Leech Therapy – An Overall Perspective

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Abstract

Complementary medical methods have a old history, but modern medicine just recently focuses on their possible modes of actions. Medical Leech Therapy (MLT) or Hirudotherapy, an old technique, has been studied by many researchers for possible effects on various diseases such as inflammatory diseases, osteoarthritis and after different surgeries.

_Hirudo medicinalis_ has widest therapeutic usage among leeches, but worldwide, many different species were tested and studied. Leeches secrete more than 20 identified bioactive substances such as antistasin, eglins, guamerin, hirudin, saratin, bdellins, complement and carboxypeptidase inhibitors, etc. They have analgesic, anti-inflammatory, inhibitory to platelet functions, anticoagulant, regulatory to thrombin functions, destructive to extracellular matrix and antimicrobial effects, but with further studies, effect spectrum may widen. The technique is relatively cheap, effective, easy-to-apply and its modes of actions are significantly enlightened for certain diseases.

In conclusion, for treatments of certain diseases, MLT is not an alternative, but is a complementary and/or integrative choice. MLT is a part of multidiciplinary treatments, affects with various bioactive substances. These substances have serious variability on species level and different species should be evaluated for both treatment capability and particularly secretive molecules. There is a huge potential for novel substances and these can be future therapeutics.

Keywords: _Hirudo medicinalis_; Hirudotherapy; Leech

Introduction
Medical Leech Therapy (MLT) or Hirudoherapy is a kind of complementary and integrative treatment method applied with blood-sucking leeches. One or more leeches are attached to the skin of problematic area and the purpose is to gain potential utilities of leech saliva that is secreted while leech feeding. MLT is actively on usage for centuries and even the term “leech” was provided from the word “laece (physician)”. First recorded applications were observed in ancient Egypt [1,2]. In addition, Chinese, Arabic, Anglo-Saxon, Ancient Greek and Roman medical records have many parts of MLT. In 17th-Century Europe, MLT reached to its widest application area [1,3]. Since 1900s, attention of medical professionals decreased, but in the last 30 years, MLT has become an important part of many scientific researches [1,4].

Leeches live in fresh waters and are segmented, hermaphrodite, carnivorous worms. They are very sensitive to vibrations on the water, touch-feeling, light, warm, sound and various chemicals. They are multi-segmented including “brain parts” and each segment has different organs such as ganglions and testicles. Two sucker parts work for creeping and adherence; the anterior one has three jaws including many teeth. They generally bite from warm parts of the host and suck its blood with rhythmic contractions [3,5]. Feeding usually takes almost 40 minutes and a leech digests 10-15 ml of blood per feeding. Digestion is done by many enzymes and mutual microorganisms such as *Aeromonas hydrophila* and *Pseudomonas hirudinia* [6,7].

Medical Leech Therapy was previously tested and is widely used after plastic, reconstructive and microsurgical applications, in cardiovascular diseases, deep vein thrombosis, postphlebitic syndrome, complications of diabetes mellitus, tinnitus, acute and chronic otitis and in decreasing pain of osteoarthritis [4,8]. There are more than 600 leech species, but *Hirudo medicinalis, Hirudo troctina, Hirudo nipponia,*
*Hirudo quinquestriata, Poecilobdella granulosa, Hirudinaria javanica, Hirudinaria manillensis, Haementeria officinalis* and *Macrobdella decora* are the most applied ones worldwide [3].

In many studies, it is found that leeches have various bioactive molecules in their secretions. More than 20 molecules and their modes of action were identified, but there is still a huge dark zone awaiting for exploration. In overall, they have analgesic, anti-inflammatory, inhibitory to platelet functions, anticoagulant, regulatory to thrombin functions, destructive to extracellular matrix and antimicrobial effects [6,9-15]. It is believed that with further studies, more indications may emerge due to recently-enlightened effect mechanisms. In this article, it is aimed to put together the information about MLT, provide an overall vision and to take a broad look to modes of actions.
Leeches Work With Secreted Proteins

To date, many scientific studies published and some part of effect mechanisms were enlightened. Although over than 100 particular proteins with different molecular mass were observed in leech secretions, only a few of them were identified which have a major role in action [16]. The effect mechanisms were divided into six titles to make them more understandable, but these mechanisms are actually tightly bounded to each other and should be evaluated as a whole (Table 1). Following a leech bite, it has to open a "sucking" pathway (extracellular matrix destruction), inhibit adhesion, aggregation and coagulation (inhibition of platelet functions, anticoagulant effect), increase the blood flow, protect itself (antimicrobial activity) and try not to be noticed (analgesic and anti-inflammatory effects).

*Extracellular Matrix Destruction:* Following the bite, leeches immediately release hyaluronidase (27.5 kDa) and collagenase (100 kDa) enzymes to easily penetrate the tissue and spread its bioactive molecules. These enzymes also have supportive effect on antimicrobial activity [9,10,14].

*Analgesic and Anti-inflammatory Effects:* It is believed that leeches somehow show analgesic and anti-inflammatory effect for not to be noticed by the host while feeding [15]. Despite of this, any analgesic molecule directly acting on this way could not be isolated from leech secretions until now. So, studies focused on indirect mechanisms to achieve this goal. For example, some studies indicate that some kinds of kininases and “antistasin” molecule may inhibit the kinin-kallikrein mechanism which is a major pain-composer route [17]. On the other hand, more informations were showed for anti-inflammatory effects.
Antistasin actually identified from *Haementeria officinalis* (Mexican medical leech) and it serves as a potent Factor Xa inhibitor and has inhibition activity on kinin-kallikrein system [17]. Factor Xa is the prothrombin activator, acts with a critical role in the common pathway of coagulation cascade [18]. Kinin-kallikrein system is also connected to coagulation cascade and has a major role in inflammatory response [19]. Researchers claim that antistasin has both anticoagulant and anti-inflammatory effect, but current studies often focus on the anticoagulant act which seems to be main predominant mechanism of action [20]. “The Ghilantens” were also found in secretions of *Haementeria ghilianii* (Amazonian Leech) and they show high structural homology with antistasin. There is only a few data about their anticoagulant effects and other possible functions are controversial due to lack of additional studies [21,22].

Leech Derived Tryptase Inhibitor has three isoforms (a,b,c) and affects by inhibiting proteolytic enzymes of mast cells. LDTI, a Kazal-type like serine protease inhibitor, especially inhibits mast cell tryptase, but also trypsin and chymotrypsin [23]. Mast cell tryptases are serine proteases in cell granules and their release causes inflammatory reactions. These effects are strongly related to kinin-kallikrein system, chemotaxis, leukocyte activation, vasoactive actions and accordingly pain-generator interactions. Their levels are correlated with allergic and inflammatory diseases such as anaphylaxis, asthma and arthritis [24,25]. LDTI found to be an inhibitor of mast cell tryptase, trypsin, chymotrypsin, thrombin and plasmin, on the other hand inhibitory effects on Factor Xa, plasma kallikrein and neutrophil elastase are controversial [26]. Even with the inhibition act on mast cell tryptase, potential benefits of anti-inflammatory effects can be foreseen. However, recombinant LDTI showed
inconstant actions in different studies, so it is hard to comment on actual clinical effects of LDTI [26,27].

Eglin C is an inhibitor of human neutrophil elastase and cathepsin G [14]. These two enzymes are immune serine proteases in chymotrypsin family which are stocked in azurophil granules of polymorphonuclear neutrophils and released as a part of inflammatory response [28,29]. Inhibition by Eglin C causes decreasing level of free oxygen radicals in neutrophils and prevents tissue inflammation and destruction. In test models, Eglin C was shown as a potential therapeutic agent for shock and emphysema [14]. Further studies are needed to show other potential effects, but the molecule itself is very promising. Other isolated “Eglins” affect on similar ways resulting anti-inflammatory effect. Another leukocyte elastase inhibitor is sistein-rich Guamerin which was isolated from *Hirudo nipponia* (Korean medical leech). From the same leech, Piguamerin was also isolated and has an inhibitory effect on kallikrein and trypsin. As previously stated, Hirustasin (*Hirudo antistasin*) is a serine protease inhibitor and affects as an inhibitor of kallikrein, trypsin, chymotrypsin and cathepsin G. It was isolated from *Hirudo medicinalis* (European medical leech) ve *Haementeria officinalis* (Mexican medical leech) [10,14]. Separately, Bdellins and bdellastasin were detected as trypsin, plasmin and sperm acrozine inhibitor [14,21]. Besides, human neutrophil elastase and cathepsin G have activating effect on Factor X (prothrombin activator) and enhancing activity on FXII and tissue factor, so, as a result, their inhibition by these substances may cause additional anticoagulant outcomes, but this area is still in the darkness and needs further studies [29,30].

Complement C1 has a critical role in classic pathway of complement system [31]. In leech secretions, Complement C1 inhibitor is a 60-70 kDa protein, but the effect mechanism is partially shown [32]. This protein might be just one part of protein-pool
that inhibit complement system by many ways. In addition, “The original” C1 inhibitor in humans suppresses FXIIa, FXIa, plasma kallikrein and thrombin. As understood, this substance inhibits both coagulation cascade and kinin-kallikrein system [33]. Currently, there is no data of similar effect of leech C1 inhibitor, but it is possible and needs further studies.

There is a contentious mechanism causing inhibitory effect is on carboxypeptidases (kininase 1). The enzymes carboxypeptidase N and M work in kinin degradation resulting agonism to B receptors that causes bradykinin-related inflammatory response [19]. Inhibition of carboxypeptidases by leech secretions should not affect on bradykinin action via B2 (constitutive) receptors, but may prevent B1 (inducible) receptors. Although these two receptors basically work with similar mechanism, researchers stated that B1 receptors seems to be related with chronic inflammation whereas B2 receptors with acute. Strong relations were found between B1 and inflammatory diseases such as multiple sclerosis, asthma and rheumatoid arthritis. But, studies indicated that bradykinin action is not limited only with these receptors, so possible anti-inflammatory effects of carboxypeptidase inhibition is controversial and should be tested separately [34].

*Increasing The Blood Flow:* It is necessary to increase the blood flow for both feeding of the leech and therapeutical results. The main components causing this result are “Histamine-Like” molecules which cause vasodilation and arised local vascular permeability [7,10,14]. Acetylcholine is also a component in leech secretions causing endothelial muscle relaxation and vasodilatation [10,14,35].

*Inhibition of Platelet Functions:* Destruction of blood vessel wall for sucking blood causes activation of platelets and coagulation cascade which are mortal for the leech.
For this reason, leech secretions contain many bioactive molecules to locally inhibit these actions.

In a normal host, wall destruction causes spread and debouch of collagen particles and they are targets of free vonWillebrand factors (vWF). This complex strongly binds to Glycoprotein Ib (GPIb) on platelets as vWF works like a bridge. With this binding, up-regulatory mechanisms occur, especially with the critical role of Adenosine diphosphate (ADP), and via Glycoprotein IIb-IIIa (GpIIb-IIIa) and fibrinogen, platelets bind to each other to make a plug and stop bleeding. This reaction also starts another chain of releasing substances such as thromboxane A$_2$, platelet activation and coagulation cascade [31]. In leech secretions, various molecules (Saratin, Calin, Decorsin and Apyrase) reacts against different parts of this chain [9,10,14].

Saratin, a 12 kDa protein, affects just in the beginning part of platelet adhesion, inhibits collagen-vWF reaction competitively. Some studies indicated promising results in animal experiments with recombinant saratin molecule as a potential local therapeutic agent for anti-thrombosis therapies and atherosclerosis [36]. Other leech-secreted proteins, Calin and leech antiplatelet protein (LAPP), show the same action on platelet adhesion [38]. Differently, Decorsin molecule, isolated from *Macrobdella decora* (American Medical Leech), is structurally similar to anticoagulant leech proteins hirudin and antistasin, but functionally it is an efficient GpIIb-IIIa inhibitor and acts potentially against platelet aggregation [38,39].

As previously stated, ADP has a critical role on platelet aggregation by especially activating GpIIb-IIIa receptors and arising affinity of platelets to vWF [31]. The enzyme Apyrase converts ADP to adenosine mono-phosphate (AMP) and blocks aggregation by indirectly inhibiting these receptor mechanisms. Because ADP also
has strong relations with arachidonic acid, platelet activating factor (PAF) and epinephrine reactions, so additionally Apyrase indirectly acts opposite way of these substances [14]. An additional molecule was also described which acts as inhibitor of PAF and thrombin-induced platelet aggregation by suppressing thromboxane produce in platelets [40-42].

Of note, the enzyme collagenase also destructs collagen particles, which starts all these adhesion and aggregation reactions, that causes additional supportive action to inhibitory effects [40].

**Anticoagulant Effect:** Coagulation during feeding is deadly for leech, so anticoagulant effect is necessary [15]. Coagulation cascade is a chain reaction and bioactive molecules in leech secretions have effects on various points. Hirudin and Gelin mainly work as thrombin inhibitor, Factor Xa inhibitor breaks the chain reaction and Destabilase has fibrinolysis effect [9,14]. Of note, thrombin has strong effect on platelet activation and ADP release and so these inhibitors may indirectly have negative impact on platelet functions [31].

Hirudin is a 7.1 kDa protein and irreversibly binds to thrombin which causes consuming of active thrombin and results as anti-thrombin action [7,15]. This substance is the most interested one and was the topic of research of many studies. In overall, there is a strong consensus about it to be a great alternative to heparin as a therapeutic agent, since it has higher anticoagulant ability and lower rates of adverse effects [15]. Gelin is an Eglin analogue and a potent thrombin inhibitor. Gelin also shows inhibitory effect on chymotrypsin, cathepsin G and neutrophil elastase [14].
Factor Xa inhibitor breaks the coagulation cascade chain and directly shows anticoagulant effect. Studies indicated that it has critical role in treatment of osteoarthritis and rheumatoid arthritis by MLT [9,10]. In addition, as previously stated, antistasin directly inhibits Factor Xa [17] and ghilantens, LDTI, C1 inhibitor and eglins have “possible” anticoagulant effects, potentially via direct and/or indirect inhibition of coagulation factors [21-25,29,30,33].

Destabilase is an enzyme with glycosidase activity and shows both antibacterial and fibrinolytic action [43,44]. This enzyme has various isoforms with different capacity of action, sourcing from different leech species [45]. Studies showed a major destructive action on stabilized fibrin and this enzyme should also be evaluated as an anticoagulant agent [43].

Recently, novel anticoagulant peptides from different leech species were identified (New Leech Protein-1, whitide and whitmanin). Additionally, many other peptides were also isolated, but their function is a mystery for now [46].

**Antimicrobial Effect:** To date, only a few research studies were accomplished and they indicated that mainly two molecules; Destabilase and Chloromycetyn, work for this intention [9,10,14].

As previously stated, Destabilase has beta glycosidase activity which directly disrupts beta 1-4 binds that are key of the peptidoglycan layer in cell walls of bacteria [14,47]. It is clear that this act is similar with Lysozyme (muramidase) that is commonly found in human saliva and lachrymal fluid [48]. Furthermore, additional studies stated that antimicrobial activity does not only depend on enzymatic glycosidase activity, but it has also non-enzymatic ways to show this action [44]. Of note, it is shown that dose dependently, even the denaturated form shows bacteriostatic effect on
*Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* [49].

Chloromycetyn is a potent antibiotic found in leech secretions, but unfortunately the data is limited about this issue [9]. Additionally, theromacin, theromyzin and peptide B were isolated as antimicrobial peptides [50,51].

**Other Possible Actions:** Interestingly, many *in vitro* studies were published indicating anti-cancer effects of leech saliva extracts. Since coagulation is related to metastasis and tumor progression, blocking the cascade can be an anti-tumor act [52]. Hirudin has been studied upon this topic and very promising results about metastasis, especially with mesothelioma were reported. In addition, other anticoagulant derivates were claimed to have similar effect, but also reducing cell growth and tumor angiogenesis [15,46]. The extracts were found to be provoking apoptosis and cell differentiation and causing cell cycle arrest. The main action mechanisms seem to be depending on suppressing ongogenic gene expression and upregulating apoptotic chains [46]. On the other hand, effects against cell degeneration were also reported. Eglin C, bdellastasin, destabilase, bdellins and hirudin were found to be very protective and/or stimulative actions positively, especially on neurons, but these studies are on just preliminary phase [15].

Leech saliva extracts were also studied for possible effects on cerebral ischemia-reperfusion injury. Although, as previously stated, leech saliva extracts cause provocation on apoptotic mechanisms, these studies indicated that saliva extracts cause opposed action by protecting cerebral cells from ischemia-reperfusion injury. Significant changes on superoxide dismutase, nitric oxide, malondialdehyde levels and expression of adhesion molecules were detected on cerebral cells with leech saliva extracts. Pteridines are isolated as potential antianoxic substances, but it is clear that this actions cannot be related to only one substance type [46].
Conclusion

Medical leech therapy has a very old history and but only recently, the exact effect mechanisms started to be clarified. In overall, when a leech bites, hyaluronidase and collagenase open the doors to the tissues and blood vessels, vasodilatation occurs by histamin-like molecules, platelet functions, kinin activity and coagulation cascade are inhibited and inflammatory reactions are suppressed. In addition, analgesic and antimicrobial effects are observed. Interestingly, experiments on mice showed positive act on wound/tissue repair [6,9-14].

There is no consensus on the application duration and number of simultaneously-applied leeches. Professionals usually suggest a maximum application of 4-5 leeches at the same time and a maximum duration of 6-8 hours, but the clinical evaluation has the critical point. The total duration of MLT is another unclarified issue. Physicians should consider “the bleeding period” after the application which may cause blood loss over than wanted. Clinical patient monitoring and laboratory tests (blood count) are strongly recommended [7]. Necessity of blood transfusion is related to duration, number of applied leeches, patient conditions and comorbidities [7,53].

Junction diseases such as osteoarthritis and epicondylitis, extremity vein diseases and flap surgeries are the major indications. MLT is also useful for soft-tissue and periorbital hematoma, purpura fulminans, macroglossia, penile replantation, post-phylebitic syndrome and echimosis. In addition, anticoagulants obtained from leeches are used in peripheral arterial occlusions and infectious myocarditis [9-13,54,55]. Their use in dentistry is additionally tested [56]. MLT is not recommended when there is hemorrhagic diathesis, during anticoagulant therapies, leukemia, bone narrow suppression, dialysis, cirrhosis, patients on chemo- or radiotherapy and cachexis [7].
There are some potential complications about MLT. Allergies to leeches and its secretions should be considered [57]. Infection is a serious condition which shows wide variability from local infections to bacteremia. Prophylactic antibiotherapy significantly reduces the risk of leech-borne infections. Infectious agent varies depending on etiology, leech species, application area and patient conditions, but by far, Aeromonas spp. is the most common one [7,53,58-63]. Furthermore, leeches were shown to be vectors of some viruses, fungi and parasites in animals [64-67], but it seems leech applications to the humans have also infection potential with these agents [68,69]. On the other hand, these complications are rare and the most common side effects are itching and bleeding on the application area. These adverse effects can be eliminated by little interventions. Orthostatic hypotension and vasovagal symptoms may occur especially in elderly patients. Regional lymphadenopathies were additionally reported. MLT usually leaves scar and so, patient should be informed especially before applications to particular body parts [6,7,54,70]. Since this therapy has a potential risk of blood-transmitted diseases, reuse of leeches is strictly forbidden [71].

In conclusion, MLT is a valuable traditional medicinal technique with strong biochemical actions. Although modes of action and bioactive substances still await for further exploration, its utilities in certain medical conditions are obvious. Indications and potential complications should be evaluated including prophylactic antibiotherapy and application frequency, dosage and delivery timing depend on the patient and physician’s opinion. It must be strongly noted that MLT is not a treatment method by itself, but it can be an important part of multidisciplinary approach.
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Table 1: Potential Bioactive Substances in Leech Secretions

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<tr>
<th>Modes of Action</th>
<th>Substance</th>
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<tr>
<td>Analgesic and Anti-inflammatory Effect</td>
<td>Antistasin$^{10,14,17}$, Hirustasin$^{10,14}$, Ghilantens$^{21,22}$, Eglin C$^{14}$, LDTI$^{23}$, Complement C1 inhibitor$^{32}$, Guamerin and Piguamerin$^{10,14}$, Carboxypeptidase inhibitor$^{14}$, Bdellins and Bdellastasin$^{14,21}$</td>
</tr>
<tr>
<td>Extracellular Matrix Destruction</td>
<td>Hyaluronidase and Collagenase$^{9,10,14}$</td>
</tr>
<tr>
<td>Increasing Blood Flow</td>
<td>Acetylcholine$^{10,14}$, Histamine Like Molecules$^{7,10,14}$</td>
</tr>
<tr>
<td>Inhibition of Platelet Function</td>
<td>Saratin$^{9,10,14,36}$, Calin$^{9,10,14,37}$, Apyrase$^{9,10,14}$, Decorsin$^{9,10,14,38,39}$</td>
</tr>
<tr>
<td>Anticoagulant Effect</td>
<td>Hirudin$^{7,9,14,15}$, Gelin$^{9,14}$, Factor Xa inhibitor$^{9,10,14}$, Destabilase$^{9,14,43-45}$, New Leech Protein-1, Whitide and Whitmanin$^{46}$</td>
</tr>
<tr>
<td>Antimicrobial Effect</td>
<td>Destabilase$^{9,14,43-45}$, Chloromycetyn$^{9,10,14}$, Theromacin, Theromyzin and Peptide B$^{50,51}$</td>
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