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Research progress in the use of leeches for medical purposes

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Highlights

This paper comprehensively analyzes and evaluates current research and the latest progress with regard to the application of leeches, their medical value, and their application prospects from various perspectives, so as to provide a reference for new viewpoints and directions for research on leeches.

Traditionality

Leeches were first recorded in the ancient book of traditional Chinese medicine *Shen Nong Ben Cao Jing* (*Shen Nong's Herbal Classic*, time of publication unknown) in the Eastern Han Dynasty, stating that they had the effect of removing blood stasis and eliminating symptoms. The dried leeches (leeches sun-dried after scalding with boiling water) have often been used in combination with other medicinal materials to treat stroke, coronary heart disease, traumatic injury, etc. In the European history of medicine, leech therapy was once considered as effective for all diseases. During the 17th and 18th centuries, the use of leech blood-sucking therapy reached its peak, and leeches were used in the treatment of liver and kidney diseases, rheumatism, tuberculosis, etc. In the 1970s and 1980s, with the rapid development of modern medicine and biotechnology, a large number of active substances with anticoagulant, antithrombotic, anti-inflammatory, and hemorheological effects were found in leeches. In 2004, leeches were approved by the FDA as a medical device for adjuvant therapy in plastic surgery and microsurgery.

Abstract

Leeches are invertebrates that have a long history of application in the development of human medicine in both the East and the West. This paper comprehensively analyzes and evaluates current research and the latest progress with regard to the application of leeches, their medical value, and their application prospects from various perspectives, so as to provide a reference for new viewpoints and directions for research on leeches. Modern research has revealed that leeches contain various bioactive components, which have pharmacological effects such as anticoagulation, antithrombosis, blood viscosity reduction, and anti-atherosclerosis. Leech therapy is an important treatment approach for venous congestion after microsurgery and is also an effective adjuvant treatment for diabetic feet, chronic pain, and tumors. Therefore, leeches are of importance for the research and development of new drugs, the restoration of blood supply after surgery, and the adjuvant treatment of diseases accompanied by blood blocking. In addition, leeches can also be used as model organisms for research in evolutionary biology and invertebrate neurophysiology as well as in neurophysiological, behavioral, and functional studies.

Key words: Leech, Medical value, Species, Distribution, Clinical application

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Abbreviations:

TCM, traditional Chinese medicine; LPS, lipopolysaccharide; VEGF, vascular endothelial growth factor; MWCNTs, multi-walled carbon nanotubes.

Competing interests:

The authors declare that they have no conflict of interest.

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Background

Leeches are widely distributed in lakes, rivers, and other wetland environments worldwide. They have a history of medical application in China dating back thousands of years and are a classic medicine used for promoting blood circulation and removing blood stasis. Leeches were first recorded in *Shen Nong Ben Cao Jing* (*Shen Nong's Herbal Classic*, time of publication unknown) in the Eastern Han Dynasty, stating that they had the effect of removing blood stasis and eliminating symptoms. According to *Ben Cao Gang Mu* (*Compendium of Materia Medica*, 1578 C.E.) in the Ming Dynasty, leeches were mainly used to treat internal injuries caused by falls, toxic swelling, and postpartum extravasated blood [1]. With regard to clinical application in traditional Chinese medicine (TCM), dried leeches (leeches sun-dried after scalding with boiling water) are often used in combination with other medicinal materials to treat diseases, such as stroke, coronary heart disease, diabetes, and traumatic injury [2, 3]. In the European history of medicine, leech therapy was once considered as effective for all diseases [4–6]. Hippocrates (460–370 B.C.E.) hypothesis was about body fluids imbalance-related illnesses. Galen (130–201 C.E.) inherited Hippocrates' theory and supposed that patients could correct the imbalance of bodily fluids and restore health through bloodletting, thus, treating diseases. Galen would prescribe bloodletting for almost all diseases, such as inflammation, mental disorders, and hemorrhoids [7]; during the Middle Ages, because blood sucking by leeches only caused small wounds and slight pain, they were accepted by most people, and doctors usually carried a small glass or tin cup filled with more than a dozen leeches when going out for home visits. During the 17th and 18th centuries, the use of leech blood-sucking therapy reached its peak, and leeches were used in the treatment of liver and kidney diseases, rheumatism, tuberculosis, epilepsy, and infectious diseases, resulting in severe decrease in the leech population and a drastic increase in price [8]. At the end of the 19th century, with the gradual formation and development of modern medicine concepts, as leech blood-sucking therapy did not conform to modern medicine concepts, and also because of many complications caused by non-standard leech sources and usage, the practice was reduced accordingly. In the 1970s and 1980s, with the rapid development of modern medicine and biotechnology, a large number of active substances with anticoagulant, antithrombotic, anti-inflammatory, and hemorheological effects were found in leeches [9–11], which laid a foundation for the development of new leech-derived drugs. In 2004, leeches were approved by the FDA as a medical device for adjuvant therapy in plastic surgery and microsurgery [12–14]. Therefore, leeches have great development potential and application prospects in modern drug development, clinical treatment, and other aspects. In this paper, pharmacological research on and the clinical use of leeches is summarized, so as to provide reference for new viewpoints and directions in research into leeches.

Biological characteristics

Leeches have long, flat bodies with annular bands, with one sucker at the front and one at the rear. The front sucker is in the mouth and has teeth for eating and sucking blood. The rear sucker has an adsorption function and can assist the front sucker in moving the body. They are hermaphroditic, with both male and female reproductive organs on the abdomen. However, they cannot fertilize themselves; two leeches are needed to complete fertilization. Their skin is elastic. Microscopic observation shows that leeches' epidermal cells are cylindrical, with capillaries, nerve endings, pigment cells, and epidermal fibers inserted into the intercellular space. In addition, gland cells on the epidermis can also secrete a large amount of mucus, which not only acts as a protective barrier but also has the function of exchanging gas, ions, and water [15].

According to the physiological habits of leeches, they can be divided into two types, namely non-hematophagous leeches and hematophagous leeches. The jaws of non-hematophagous leeches are relatively fragile, with small dental plates, and they often feed on aquatic insects or snails (e.g., *Whitmania pigra* Whitman, *Whitmania acranulata* Whitman). Hematophagous leeches have sharp teeth and can rapidly cut skin or mucosa to suck blood (e.g., *Hirudo medicinalis* L., *Hirudo nipponica* Whitman); they can ingest 5–20 mL of blood within 15 min to 2 h, and can maintain this for a long time; their salivary gland holes are located between adjacent small teeth, so that saliva can be injected into the wound at the moment of piercing the skin to suck the blood [16]. As the saliva contains anticoagulant and vasodilator components, it will cause continuous bleeding for several hours after being bitten [4, 12].

Species and distribution

As for taxonomy, Medicinal leeches are classified as the family Hirudinisae, order Arhynchobdellida, subphylum Clitellata, phylum Annelida. About 680 species of leeches have been found worldwide today. With the progress of scientific research at its current stage, only a few species under the genera *Whitmania* Blanchard and *Hirudo* Linnaeus, have been officially approved for clinical use [11]. Influenced by the difference in environment and regional distribution, the leech species used for medical purposes are also different between the East and the West to some extent. The 2015 edition of the *Chinese Pharmacopoeia* stipulates the following: *Hirudo nipponica* Whitman, *Whitmania pigra* Whitman and *Whitmania acranulata* Whitman. In Europe, three species of leech， *Hirudo* *medicinalis* L., *Hirudo verbena* Carena, and *Hirudo orientalis* Utevsky & Trontelj, are present in natural environment, but only *Hirudo medicinalis* is a legally protected species in this region. Currently, *Hirudo verbena* is the species most commonly available from authorized commercial leech farms [11]. The morphological characteristics, physiological habits, and distribution of the major families and genera are described below.

Hirudo Linnaeus

The body is medium in size, 30–150 mm in length, with longitudinal stripes on the back, five eyes arranged in an arch shape, eight pairs of sensory papillae on the back of the body, and six rows on the abdomen; the male and female reproductive organs have constant positions; they have three well-developed jaws, each with 35–100 sharp teeth; they inhabit rice fields, riversides, and lakesides, and feed on the blood of people and livestock; there are many species under the genus *Hirudo* Linnaeus, which can generally be distinguished by their back pattern arrangement [17]. The common ones are *Hirudo nipponica* Whitman, *Hirudo medicinalis* L., etc. (Table 1, Figure 1).

Whitmania Blanchard

The body is medium or large in size, the rear sucker is medium in size, and the back is usually longitudinally striated. Most have 17 segments, and each segment has five equal-sized rings. They have five pairs of eyes forming an arch pattern. The reproductive organs are at an interval of the fifth ring. The jaws are small, and there are two rows of irregular dental plates. They inhabit rocks or mud at watersides, feed on aquatic insects and snails, and do not suck blood. *Whitmania pigra* Whitman is a common species (Table 1, Figure 1).

Active ingredients in leeches

The presence of several bioactive substances in leeches constitutes the material basis for their extensive historical use in medicine worldwide. Research has revealed that leeches contain at least 51 compounds, including bioactive peptides, pteridine, phosphatidylcholine, glycosphingolipids, sterols, etc. [13, 18, 19], specifically, hirudin, hyaluronidase, histamine, saratin, calin, hyaluronidase, destabilase, lysozyme, tryptase inhibitor, etc. [7, 20–22] (Table 2).

It can be seen that hematophagous leeches have complex regulatory functions for various active ingredients in their blood-sucking process. When a leech punctures the skin to suck blood, the active ingredients in its saliva are injected into the body; the hyaluronidase and histamine reduce the permeability of blood vessels and promote the rapid entry of hirudin, calin, destabilization enzymes, and so on into the blood supply; block thrombin; inhibit platelet aggregation; and dissolve fibrin, thus inhibiting blood coagulation and allowing the leech to suck more blood [21, 23, 24]. In contrast, leeches contain effective active components that can mediate various vascular regulatory signaling pathways, such as antithrombotic, anticoagulant, anti-inflammatory, and anti-platelet aggregation pathways, thereby playing an important role in the clinical treatment of cardiovascular and cerebrovascular diseases, venous obstruction, and other diseases as well as for skin flap transplantation [9].

Advances in pharmacological research on leeches

Anticoagulant and antithrombotic effects

Coagulation is a complicated process involving a series of factors that hydrolyze and activate proteins in the blood to form fibrin deposits, thus preventing continuous outflow of blood. It is an important bodily defense mechanism. The basis for thrombus formation is fibrin and platelet deposition in blood vessels, and a thrombus is formed owing to factors such as vascular endothelial injury and slow blood flow, which induce coagulation and trigger its underlying mechanisms. It is an important cause of cardiovascular and cerebrovascular occlusion. Hirudin, a natural polypeptide isolated from the saliva of *Hirudo medicinalis* L., is a potent thrombin inhibitor that can directly bind to thrombin to inactivate it, dissolve fibrin, and inhibit the platelet aggregation caused by thrombin, thus inhibiting thrombosis; it can be used to treat blood coagulation diseases and prevent postoperative thrombosis, coronary thrombosis, and strokes [25]. However, the isolation of natural hirudin does not meet clinical needs. The development of recombinant hirudin has solved this dilemma: Cornelia obtained DNA containing leech protease inhibitor using the chemical oligodeoxynucleotide synthesis method [26], and transferred the synthesized gene into *E. coli*; it was found that the product had similar biological characteristics to the protein isolated from leeches [26]. Fortkamp synthesized a hirudin DNA segment chemically [27]. The synthesis method included preparing seven pairs of long oligodeoxynucleotide pairs and assembling and cloning them using a rapid and simple method. Over half of the transformed *E. coli* cells expressed a biological synthetic peptide, with biological characteristics similar to those of natural hirudin. Guo reproduced venous congestion in a rat skin flap model [28]. Following local injection of natural hirudin and recombinant hirudin, it was determined that the two types of hirudin could improve the survival rate of the skin flap, increase the expression of vascular endothelial growth factor, and promote angiogenesis at the injury site. Therefore, recombinant hirudin has considerable clinical value.

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Table 1 Species and appearance characteristics of leeches

Species		Physiological habits and appearance characteristics	Distribution	Literature
Hirudo Linnaeus	*Hirudo medicinalis*	Hematophagous, with a narrow, slightly cylindrical body, flat back and abdomen, a body length of 100–150 mm, and five pairs of eyes. The back of the body is olive green or brown, with many small papillae, and the venter is pale yellow, with a large number of irregularly shaped black spots. Chromosome number = 14.	Northern, central, and mid-western Europe	[29–31]
	Hirudo verbana	Hematophagous, with a narrow, slightly cylindrical body, flat back and abdomen, a body length of 80–100 mm, a body width of 10–12 mm, and five pairs of eyes. The back of the body is green or yellow-green, with two longitudinal wide stripes of diffuse orange dots, and the abdomen is yellow, with two black stripes. Chromosome number =13.	Eastern Mediterranean, the Balkans, and the Ukraine	[17, 29, 30, 32, 33]
	Hirudo orientalis	Hematophagous, with a narrow, slightly cylindrical long body, flat back and abdomen, a body length of 90–110 mm, a body width of 8–10 mm, and five pairs of eyes. The back of the body is grass green, with two orange-yellow pinstripes, surrounded by quadrangular or round black spots between them, and with black or yellow irregularly shaped striped spots on the abdomen. Chromosome number = 12.	Georgia, Iran, and Uzbekistan	[29]
	Hirudo nipponica	Hematophagous, with long and narrow, slightly cylindrical body, and slightly flat back and abdomen. The body length is 30–60 mm, and the body width is 4.0–8.5 mm. It has 103 rings and five pairs of eyes. The back of the body is grayish-green, with five yellow-white longitudinal stripes. There is one grayish-green longitudinal stripe on each side of the abdomen.	China, Japan, Russian Far East, and Mongolia	[15]
Whitmania Blanchard	*Whitmania pigra*	Non-hematophagous, with a spindle-shaped body, widest in the middle, flat in the back and abdomen, and bulging in the back. The body length is 60–130 mm, the body width is 13–20 mm. It has 107 rings and five pairs of eyes. The back of the body is dark green, with five black stripes mixed with a light-yellow color.	China and Japan	[15]
	Whitmania acranulata	Non-hematophagous, with a lanceolate body, a very small head, a tapering front end, and a gradually widening back end, with a body length of 28–67 mm, a body width of 3.5–8 mm, 105 rings in total and five pairs of eyes. The back of the body is dark brown with five yellow-brown longitudinal stripes and 20 pairs of crescent-shaped brown spots on the middle stripes of the back.	South China and Japan	[15]

Figure 1 Appearance characteristics of leeches

Table 2 Main active components in leech

Component	Molecular weight	Function	Effect	Literature
Hirudin	7.1 kDa	Thrombin inhibitor	Directly binds to thrombin, inhibits platelet aggregation, and dissolves fibrin, thereby preventing thrombosis	[34–36]
Hyaluronidase	27.5 kDa	Reduces viscosity and increases permeability	Makes it easier for substances to penetrate tissues and increases the absorption rate of substances by the body	[21, 36, 37]
Histamine	111 Da	Increases vascular permeability	Accelerates the absorption of substances and resists inflammation	[21, 36]
Calin	65 kDa	Binds to platelet collagen, thereby inhibiting adhesion of von Willebrand factor	Inhibits platelet aggregation, reduces platelet adhesion, and inhibits blood coagulation	[22, 36, 37]
Destabilase	126 kDa	Dissolves fibrin	Dissolves thrombi and resists inflammation	[22, 38–40]

In addition, many studies have revealed that hirudin cannot be isolated from the saliva of non-hematophagous leeches (*Whitmania pigra* Whitman), but their saliva still has strong anticoagulant and antithrombotic activities, indicating the presence of other effective and powerful anticoagulant and antithrombotic active components. Zheng isolated and determined the amino acid sequence of an oligopeptide, whitide [41], with thermal stability from dried *Whitmania pigra* Whitman, with a molecular weight of 1997.1 Da; whitide is highly resistant to trypsin and could potentially be developed into oral anticoagulant drugs. Chu isolated a fibrin hydrolase from dried *Whitmania pigra* Whitman with strong fibrinolytic activity [42], and its thrombolytic activity was related to enzyme concentration; it could be used to develop antithrombotic drugs and thus had high economic value. Ren used software to sequence the cDNA of the salivary gland of *Whitmania pigra* Whitman and found the active protein pigrin [43], which was cloned and synthesized using *P. pastoris* GS115. Experiments to assess the antithrombotic activity and coagulation time of pigrin in animal models revealed that pigrin reduced thrombosis in animals in vivo, but did not prolong bleeding time under effective doses; therefore, it is a potential lead compound for developing antithrombotic drugs.

Anti-platelet aggregation

Platelets are important blood cells that play an essential role in coagulation and hemostasis during wound repair. However, excessive platelet aggregation will cause thrombosis, which can lead to ischemic cardiovascular and cerebrovascular diseases. Through summarizing related studies on leeches and platelet aggregation published in ancient books, pharmacopoeias, reports, and papers from 1980 to 2018, Jiang found that leeches had a very good anti-platelet aggregation effect [35], the mechanism of which was related to reducing thromboxane A2, increasing prostacyclin I2, reducing Ca^{2+}, regulating nitric oxide, and increasing nitric oxide synthetase activity. In addition, the pteridine compounds obtained and identified from leeches have chemical structure similar to those of anti-platelet aggregation agents (such as ticlopidine, clopidogrel, and ticagrelor). Therefore, leeches are a relatively good research subject for natural anti-platelet drugs based on traditional medical research.

Reducing blood viscosity

Blood viscosity is an important indicator of the nature of blood flow, and an important property for the regulation of blood circulation. Increased blood viscosity will decrease the rate of blood flow and increase the deposition of lipids and blood cells in blood vessels; this can narrow blood vessels, cause metabolic disorders, and lead to the formation of thrombosis in severe cases, making it the main cause of ischemic cardiovascular and cerebrovascular diseases. Wang used high-molecular-weight dextran to develop a high-viscosity rat blood model [44]; aspirin and water extract of *Whitmania pigra* Whitman dry powder were respectively administered and hemorheological indices and pathological changes were assessed in aortic specimens; metabolic changes were described based on the non-targeted metabonomic profiling of liquid chromatography–mass spectrometry. The results showed that both aspirin and leech water extracts could reduce the blood viscosity of rats and improve thoracic aortic tissue lesions; metabonomic results showed that leech extract could significantly improve metabolic disorders caused by high blood viscosity and restore metabolites to normal levels. Compared with aspirin, leech extract had a greater positive effect; the difference in the endogenous metabolites involved cysteine, methionine metabolism, tricarboxylic acid cycle, arachidonic acid, etc., suggesting that leech extract also plays a certain role in regulating metabolic disorders.

Anti-atherosclerosis

Atherosclerosis is a chronic vascular inflammatory disease that causes lipid plaque rupture and thrombosis, and is an important cause of acute cardiovascular and cerebrovascular diseases. Studies have found that inflammation plays an essential role in atherosclerosis and thrombosis. When inflammation occurs in the body, neutrophils rise and activate the coagulation function by directly binding to von Willebrand factor to promote platelet aggregation and activation; activated monocytes express tissue factor and release inflammatory cytokines (IL-1β, TNF-α), thereby regulating the anticoagulant system through the downregulation of thrombomodulin and endothelial protein C receptor [45]. Lu studied the anti-atherosclerosis mechanism of *Whitmania pigra* Whitman ethanol extract by using a cell model induced by lipopolysaccharide (LPS) or oxidized low-density lipoprotein [46]; it was found that leech extract pretreatment for 48 h could significantly inhibit the lipopolysaccharide-induced expression of intercellular adhesion molecule-1, vascular cell adhesion molecule-1, IL-6, and TNF-α in endothelial cells and weaken the reactive oxygen species accumulation and macrophage apoptosis induced by low-density lipoprotein [46]. Li used a wound-healing experiment and the Boyden chamber model to evaluate the migration inhibition effect of *Whitmania pigra* Whitman enzyme extract on LPS-induced vascular smooth muscle cells [47], and found that leech extract could inhibit the LPS-induced up-regulation of inflammatory factors and adhesion molecules in rat vascular smooth muscle cells; its mechanism was mainly associated with the regulation of the P38 MAPK/NF-κB signaling pathway, indicating that leeches can play a role in preventing atherosclerosis.

Anti-tumor

Cancer has a high mortality rate and is one of the major threats to human life [7]. Glioma is one of the most common intracranial cancers. Currently, its clinical treatment effect and prognosis are poor. Up-regulation of the ERK/MAPK signaling pathway has been proven to be involved in the amplification of mitotic stimulation and promote the proliferation of malignant glioma cells. Zhao used hirudin as a treatment intervention in glioma cells [48], using MTS and Annexin V staining methods to detect cell proliferation and apoptosis rate, and western blot and immunofluorescence staining methods to detect the expression of ERK/MAPK signals. Hirudin was found to reduce the proliferation rate of glioma cells, promote tumor cell apoptosis, and down-regulate the expression of ERK1/2, which indicates that hirudin is a potential clinical treatment for glioma. Li administered culture solution containing hirudin at different concentrations to hepatoma HepG2 cells and assessed the effect of hirudin on the proliferation rate, apoptosis, migration, and invasion of hepatoma HepG2 cells, as well as the expression of vascular endothelial growth factor (VEGF) [49]. The proliferation inhibition rate for HepG2 cells increased with the increase in hirudin concentration and action time in a dose-time dependent manner; VEGF expression significantly decreased with the increase in hirudin concentration, which indicated that hirudin could inhibit the proliferation, apoptosis, migration, and invasion of HepG2 cells by reducing the expression of VEGF. In addition, Shakouri used a combined treatment of active ingredients from leech saliva and liposome leech saliva extract and evaluated the targeted anti-tumor activity of the extract using the human breast cancer cell line MCF-7 [50]. The liposome active ingredient had a relatively good inhibitory effect on the MCF-7 cell line, indicating that leeches are of considerable value in anti-tumor mechanism research and drug development.

Clinical application of leeches

Cardiovascular and cerebrovascular diseases

The continuous progress in modern medical theories and pharmaceutical technology has promoted the innovation and development of TCM preparations. Many classic leech-containing TCM prescriptions that have achieved good therapeutic effects after many years of use have been improved upon and modified in modern TCM preparations, and have been approved for the treatment of cardiovascular and cerebrovascular diseases with good clinical therapeutic effects (Table 3).

Adjuvant treatment after microsurgery

Microsurgery is a surgical operation that uses microscopic instruments to anastomose small vessels, veins, and arteries during flap transplantation or resection [7]. Venous obstruction is a common complication after operation. As the inflow of an artery is greater than the outflow of a vein, the obstruction leads to increased venous pressure, which in turn leads to vascular rupture, blood stasis, pain, edema, and in severe cases, blood flow stagnation, thrombosis, and tissue necrosis [23]. Therefore, it is very important to relieve venous obstruction after surgery. Leech therapy is now recognized as an irreplaceable adjuvant therapy. The hematophagous characteristics of leeches can be utilized to suck out blood blockages at the congestion site, with the simultaneous injection of anticoagulant and thrombolytic active substances into the body to promote restoration of the blood supply to new tissues [7, 51, 52]. Herlin summarized 43 cases of leech-assisted skin flap transplantation from 1960 to 2015 [4], and found that the success rate of leech therapy was 65%–85%, the optimal application frequency was 2–8 h and average total duration 4–10 days; and 50% of patients needed a blood transfusion. Ciprofloxacin and trimethoprim-sulfamethoxazole are recommended in the treatment to prevent complications from leech infection [4].

Anti-inflammation and analgesia

Pain is a very common physiological stress reaction, with the degree ranging from mild to severe. Generally, pain dissipates by itself, but long-term severe pathological pain considerably decreases quality of life. Migraines are a common condition that can cause severe pain, and even disability and social burden. Traditional treatments can cause drug overuse, abuse, or addiction. Leech therapy has been successfully used for severe persistent headaches. Ansari analyzed seven migraine patients for whom traditional oral therapy had failed [53]; following the provision of consent, leech therapy was carried out behind the ear. Two months later, the frequency of headaches decreased and the quality of life improved. Wang conducted a comprehensive analysis of 264 cases of osteoarthritis treated with leech therapy [54], and found that medical leech therapy could significantly improve the pain and prognosis of patients. It can be supposed that leech therapy can improve long-term chronic pain, and its mechanism may be related to anti-inflammation and analgesia and the presence of active substances that promote blood circulation, suggesting considerable future application in pain relief therapy [54, 55].

Adjuvant therapy for tumors

Most advanced-stage tumors are accompanied by severe pain. Kalender recorded a 62-year-old male patient suffering from renal cell carcinoma and leiomyosarcoma [56]. After radiotherapy and systemic analgesia failed, leeches were used to relieve pain in the lumbar region. Two months later, the patient's physical condition improved without pain, suggesting that leech therapy has potential value for improving cancer-related pain. In addition, malignant tumor patients are often accompanied by a hypercoagulable state. Tang selected 20 patients with primary liver cancer accompanied by a hypercoagulable state who underwent transarterial chemoembolization for primary liver cancer and took leech capsules (see the "*Pharmacopoeia of the People's Republic of China*, Volume I, Edition 1995" for the preparation method with mung bean powder) after discharge [57]; blood coagulation, routine blood tests, and other indices were measured before each interventional treatment; the results showed that after the leech capsule treatment, the prothrombin time and activated partial thromboplastin time were slightly prolonged when compared with those before treatment, and fibrinogen and D-dimer were decreased, but there was no significant difference before and after treatment, and the toxicity and side effects of interventional chemotherapy and hemorrhaging were not increased; this indicates that the combined application of leech capsules, to a certain extent, does improve the coagulation function of hypercoagulable patients. Miao selected 56 patients with advanced malignant tumors aged 36–74 years from the same period (29 males and 27 females [58]; 11 cases of lung cancer, 11 cases of esophageal cancer, 8 cases of breast cancer, 6 cases of gastric cancer, 3 cases of liver cancer, 2 cases of pancreatic cancer, 7 cases of colorectal cancer, and 8 cases of gynecological malignant tumor); all patients were diagnosed pathologically, and the plasma platelet count was $> 300 \times 10^9$/L; they were not treated with any other blood-activating or anticoagulant drugs, and were prescribed with a Chinese herbal medicine decoction (10 g of Sanleng (*Sparganii Rhizoma*), 10 g of Ezhu (*Curcumae Rhizoma*), and 3 g of Shuizhi (*Hirudo nipponica*). The platelet count was reexamined after 5–10 days of administration (the plasma platelet count was $< 300 \times 10^9$/L, indicating effective treatment). After 5–10 days of treatment, the platelet count decreased in 49 cases, with an effective rate of 87.5%, indicating that the three Traditional Chinese medicinal materials Sanleng (*Sparganii Rhizoma*), Ezhu (*Rhizoma curcumae*) and Shuizhi (*Curcumae Rhizoma*) have a positive effect on thrombocytosis in patients with advanced malignant tumors.

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Table 3 Traditional Chinese medicine preparations containing leeches

Name	Composition	Indication	Mechanism of action	Literature
Traditional Chinese patent drug Naoxintong capsules	Shuizhi (*Whitmania pigra/Hirudo nipponica*), Dilong (*Pheretima*), Quanxie (*Scorpio*), Chishao (*Radix Paeoniae*), Moyao (*Commiphora myrrha*), Taoren (*Prunus persica* Batsch), Danggui (*Angelicae Sinensis*), Sangzhi (*Mulberry Twig*), Niuxi (*Achyranthis Bidentatae Radix*), Chuanxiong (*Rhizoma Ligustici Chuanxiong*), Danshen (*Salviae Miltiorrhizae Radix et Rhizoma*), Ruxiang (*Boswellia carteri*), Guizhi (*Cassia Twig*), Huangqi (*Astragali Radix*), Jixueteng (*Spatholobus Stem*)	Cerebral infarction, coronary heart disease, angina pectoris	Inhibits dendritic cell maturation and inducible nitric oxide synthase expression to reduce atherosclerosis; reduces oxidative damage of myocardial cells; promotes autophagy and inhibits myocardial hypertrophy of H9c2 myocardial cells; anti-platelet aggregation	[59, 60]
Traditional Chinese patent drug Tongxinluo capsules	Shuizhi (*Whitmania pigra/Hirudo nipponica*), Wugong (*Scolopendra subspinipes mutilans*), Chantui (*Cryptotympana pustulata Fabricius cicada*), Tubiechong (*Steleophaga plancyi*), Xiezi (*Scorpio*), Renshen (*Panax ginseng*), Ruxiang (*Boswellia carteri*), Bingpian (*Borneolum syntheticum*), Shaoyao (*Paeonia lactiflora* Pall), Dazao (*Ziziphus jujuba*), Tanxiang (*Santalum album*), Jiangxiang(*Dalbergia odorifera*)	Angina pectoris and coronary heart disease	Improving endothelial cell function, lowering lipids, reducing inflammation, preventing apoptosis, and enhancing angiogenesis.	[61]
Traditional Chinese medicine injection Shuxuetong injection	Shuizhi (*Whitmania pigra/Hirudo nipponica*), Dilong (*Pheretima aspergillum*)	Acute cerebral infarction	Promotes anticoagulation, promotes fibrinolysis, improves blood rheology, regulates blood lipids, relieves inflammatory reaction, and protects the blood brain barrier	[62, 63]
Traditional Chinese patent drug Zhixiong capsules	Shuizhi (*Whitmania pigra/Hirudo nipponica*), Chuanxiong (*Rhizoma Ligustici Chuanxiong*), Danshen (*Salviae Miltiorrhizae Radix et Rhizoma*), Gegen (*Puerariae Lobatae Radix*), Yimucao (*Leonurus japonicus*)	Cerebral arteriosclerosis, stroke recovery	Reduces fibrinogen content, inhibits thromboxane B_2 level, prevents migration of inflammatory cells, resists platelet activation, and reduces total cholesterol and low-density lipoprotein	[64, 65]
Traditional Chinese patent drug Shenyuandan capsules	Shuizhi (*Whitmania pigra/Hirudo nipponica*), Dilong (*Pheretima aspergillum*) Danshen (*Salviae Miltiorrhizae Radix et Rhizoma*), Yanhusuo (*Corydalis Rhizoma*), Huangqi (*Astragali Radix*), Baifuzi (*Typhonii Rhizoma*), Xuanshen (*Scrophulariae Radix*)	Angina pectoris, atherosclerosis	Inhibiting the inflammatory response NF-κB signaling pathway by regulating IRS-1/PI3K/Akt	[66]
Traditional Chinese patent drug Dahuang Zhechong pills	Shuizhi (*Whitmania pigra/Hirudo nipponica*), Qicao (*Holotrichia diomphalia* Bates), Dahuang (*Rheum officinale* Baill), Tubiechong (*Eupolyphaga sinensis*), Huangqin (*Scutellaria baicalensis Georgi*), Gancao (*Glycyrrhiza uralensis Fisch*), Taoren (*Prunus persica* Batsch), Xingren (*Prunus armeniaca L.*), Shaoyao (*Paeonia lactiflora Pall*), Dihuang (*Rehmannia glutinosa* Libosch), Ganqi (*Rehmannia glutinosa* Libosch), Mengchong (*Tabanus bivittatus Mats*)	Liver diseases, gynecological diseases, atherosclerosis	Inhibits endothelin -1 to stimulate vascular smooth muscle cells proliferation and platelet derived growth factor expression, and improves atherosclerosis	[67]
Traditional Chinese patent drug Maixuekang capsules	Shuizhi (*Whitmania pigra/Hirudo nipponica*)	Acute coronary syndrome	Inhibits platelet aggregation in coronary atherosclerosis, promotes anti-inflammation, and protects of vascular endothelial function	[68]

Diabetes

Diabetes mellitus is a metabolic disease characterized by hyperglycemia, and complications such as blindness, diabetic foot, renal failure, and cardiovascular diseases occur in the later stages of the disease that seriously threatens human health. According to statistics, the death rate of diabetic foot is second only to cancer, and traditional treatment methods have little effect. Shirbeigi reported a case of treating a 77-year-old diabetic foot patient with leech therapy combined with a honey and curcumin dressing: a leech was placed on the affected part; after treatment, the wound was covered with honey and curcumin, and ciprofloxacin was taken orally for 10 days [69]; the results showed that after leech treatment for two days, the pain completely disappeared; after three weeks, the toe wound recovered; after 12 weeks, the wound trace disappeared, indicating that leech therapy combined with honey and curcumin dressing can effectively prevent the progress of diabetic foot. Zaidi used leeches to treat a 60-year-old woman with diabetic foot who was about to face amputation, and used immature papaya as the wound dressing to help heal the wound; after 20 days of treatment, the pain stopped and she no longer needed painkillers; after three months, the necrotic area disappeared and the wound healed [70]; this suggests that leech therapy can be used as a treatment for severe diabetic foot.

Process of leech therapy

Since leech therapy involves direct contact between the leech and human blood circulation systems, the possibility of infection is very high (2.4%–26%), and once infection occurs, the flap repair rate is less than 30%. The degree of infection may be limited to the leech attachment site and surrounding soft tissues, or may develop into life-threatening septicemia and bacteremia [52, 71]. Complications, such as allergies and anemia, may also occur after treatment [72]. Therefore, treatment must be carried out in strict accordance with the operating procedures:

1. The leeches used for treatment must come from a special leech farm with formal medical certification [73]. The use of leeches collected from a natural environment (possibly contaminated by viruses, bacteria, fungi, and even parasites) is prohibited. Healthy, flexible, and touch-sensitive leeches should be selected for treatment, and their feeding temperature should be kept at 4–13°C [11].

2. Before starting treatment, patients should be questioned about their disease and allergy history. Leech therapy is not recommended for patients suffering from arteriosclerosis, hemophilia, hematological malignancies, anemia, or hypotension and septicemia; during pregnancy or lactation; with an unstable physical condition, history of leech allergy or severe allergic constitution, and scar formation tendency; or patients using anticoagulant and immunosuppressive agents. Written informed consent should be obtained from patients if the treatment conditions are met [14].

3. Clean the patient's skin thoroughly with warm heparin saline before treatment to enhance vasodilation. Dip a gauze into sterile water and cut a 1 mm hole in the middle to cover the fixed position to prevent the leech from moving. Then, puncture the treatment area with a syringe to encourage the leech to attach to the designated area for blood sucking, which can last for 30–90 min [74].

4. During the leech therapy, doctors and nurses must closely monitor the patient for allergic reactions, signs of infection, and congestion. In addition to monitoring the patient's vital signs, the blood volume should be monitored at least two to three times per day, and the wound site should be checked at any time to ensure that the leeches do not migrate or detach too early. Be careful not to forcibly remove the leech. After treatment, the leech should be removed quickly. Leeches should never be reused. They should be killed in 70% ethanol and put into bags specially used for biological waste disposal.

5. Antibiotic prevention is an effective method to reduce the infection rate. During leech therapy, quinolones (e.g., ciprofloxacin), aminoglycoside, and other antibiotics can be injected to prevent infection [52, 75]. Patients with open wounds should continue oral antibiotic treatment until the wound is closed. Leech therapy usually lasts 2–6 days. Hematological examination should be carried out daily, and when hemoglobin is lower than 8 g/dl, timely blood transfusion is required [76, 77].

Basic research (model animal)

Leeches are a typical model animal for studying animal system neuroscience worldwide [78]. In a leech's central nervous system, there are six fusions that form the head ganglia, 21 highly similar body ganglia, and seven fused tail ganglia; each ganglion contains about 200 pairs of neurons, which are connected with adjacent neurons through thousands of axons and have important research value in neuron development, regeneration, and repair [79, 80]. Françoise proposed that leeches were a relatively good model for studying the activation mechanism of microglia because they could repair themselves after injury to the central nervous system [81]. Rodet proved that leech neurons could respond to lipopolysaccharide through MyD88-dependent signaling pathways [82], thus promoting cell regeneration in the central nervous system. Mumcuoglu found that activated leech macrophages (HmAIF-1+) and granulocytes (CD11b+) could express TLR4 and its receptor CD14 [83], which is similar to the research results in vertebrates, indicating that leeches are a valuable model for studying molecular biological mechanisms. In addition, leeches can also be used as a good substitute model to study harmful materials. Girardello used leeches as a model to evaluate the effect of multi-walled carbon nanotubes (MWCNTs) on the immune system [84]; the results showed that MWCNTs could lead to a decrease in the cell proliferation rate and an increase in the apoptosis rate, which suggested the potential of MWCNTs to induce a strong inflammatory reaction, and proposed the use of leeches as a substitute model to study the harmful effects of nanomaterials [84].

Prospectives

At present, pharmacological research on the separation and purification of leech active ingredients, recombinant hirudin and its derivatives, and TCM preparations containing leeches and leech extract has made great progress. Leech therapy has achieved good results in the auxiliary treatment for blood supply recovery, diabetic foot, and long-term pain after clinical microsurgery. Leeches, as a model animal, have also made certain research achievements in neuronal development, regeneration, and repair possible. However, there are still many problems in the research on leeches at this stage, including the following:

1. There is a history of leech application in many countries and regions worldwide, so a summary of leech species and treatment methods used in different regions is needed;
2. The separation and content comparison of active components from different species and genera of leeches in different regions are needed;
3. Hematophagous leeches and non-hematophagous leeches both have good biological activities, so the differences among the main active components that play a role should be compared, and whether there are respective emphases in clinical application should be investigated;
4. The development of new clinical drugs with good anticoagulant, antithrombotic, and anti-tumor effects is necessary;
5. Quality control and production standards of TCM preparations containing leeches is necessary;

Study on the modern pharmacology and pharmacokinetics of TCM preparations is necessary;

6. The infection rate of leech therapy is relatively high, so standardized quality control should be implemented in leech cultivation;
7. The possibility of using leech nerve cells for genetic mental disease research and as drug screening models, etc., should be investigated. These problems are worthy of our deep exploration and consideration to prove the effectiveness and scientific merit of leeches in clinical treatment, and to promote the development of new drugs containing leeches in treating cardiovascular and cerebrovascular diseases, as well as tumors.

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