Research progress on the pharmacological effects and clinical applications of leeches in the last three years

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Abstract. The present study shows that leeches have precise pharmacological effects and a wide range of clinical effects. This paper mainly reviews the pharmacological and clinical effects of leeches in the last three years regarding renal diseases, cardiovascular diseases, pulmonary diseases, liver diseases and other diseases, with a view to provide a reference for further resource utilization of leeches.

Keywords: leech; pharmacological effects; clinical applications

Preface: Leech is the dried whole of leech Whitmania pigra Whitman, leech Hirudo nipponica Whitman or willow-leech Whitmania acranulata Whitman in the leech family. Salty, bitter, flat; with little poison. Belongs to the liver meridian. Breaking blood and opening menstruation, expelling blood stasis and eliminating symptoms. Used for blood stasis and menstrual blockage, obstruction and masses, stroke and paraplegia, and fall and flutter injuries.[1]The pharmacological effects of leech are precise and widely used clinically, and it is often used in combination with Astragalus, Centipede and so on, and the efficacy of proprietary Chinese medicines containing leech is precise. This paper mainly collates the pharmacological effects and clinical applications of leech in renal diseases, cardiovascular diseases, pulmonary diseases, liver diseases and other diseases and its combination of drug pairs and proprietary Chinese medicines.

1. Pharmacological Effects

1.1 Pharmacological Effects of Leeches

1.1.1Pharmacological effects of leeches on kidney diseases

Leeches can alleviate the progression of focal segmental glomerulosclerosis . Yilun Zhang et al. found that leech could reduce extracellular matrix accumulation and slow down the progression of focal segmental glomerulosclerosis by decreasing TGF-β1 and type I collagen expression in renal tissues.[2]Wei Jingjing et al. found that leech could improve immune complex deposition caused by blood stasis in idiopathic membranous nephropathy, regulate Nephrin expression in podocytes, reduce glomerular tethered cells proliferation and glomerulosclerosis, and improve renal function. [3]Yang Xin et al. found that leech could interfere with the process of extracellular matrix accumulation to a certain extent by increasing the expression of MMP-9 protein and decreasing the of protein, thus delaying the process expression of TIMP-1 focal segmental glomerulosclerosis.[4]Liu Na et al. found that leech could not only reduce 24h urine protein quantification, increase plasma albumin and improve general status in FSGS rats but also maintain normal foot cell structure and function by regulating the expression of podocalyxin and VEGF proteins in kidney tissues of FSGS model rats, and delayed the the development of FSGS.[5]Leeches can treat IgA nephropathy. Fang Lan et al. used leeches in rats with IgA nephropathy hemoptysis and observed that leeches improved whole blood viscosity and plasma viscosity, downregulated IL-6, reduced urinary protein, and protected renal function in rats with IgA nephropathy hemoptysis.[6]Further research relied on application of isobaric tags for relative and absolute quantitation found that leech treatment of rats with IgA nephropathy hemoptysis resulted in significant differences in serum protein expression, which may be related to complement and coagulation cascade pathways, metabolic signaling pathways, purine metabolism and pyrimidine metabolism pathways.[7]Based on the PI3K/Akt/mTOR pathway, Cao H, Xie L, Zhao J

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et al. concluded that leeches may inhibit glomerular thylakoid cell proliferation and promote glomerular thylakoid cell apoptosis by decreasing the expression of PI3K/Akt/mTOR mRNA, thereby reducing the effect of renal fibrosis.[8]

Leech can retard interstitial fibrosis. Ren Tong et al. found that leechin may interfere with the expression of MCP-1, ICAM-1 mRNA and protein in the interstitial kidney of UUO rats, thereby improving the degree of interstitial injury and delaying the progression of interstitial fibrosis in UUO rats to different degrees. [9]It was also found that hirudin may inhibit the phosphorylation of p38 protein, improve the renal impairment, inhibit the expression of FN, a pro-fibrotic index, and upregulate the expression of E-Cadherin in UUO rats.[10]

In addition, Yang Fan et al. found that leech lyophilized powder may inhibit the activation of JAK2/STAT1/STAT3 signaling pathway in renal tissues by suppressing oxidative stress and inflammatory factor production in rats with diabetic nephropathy, thereby reducing mALB excretion and improving renal function, and thus exerting renal protective effects.[11]

1.1.2Pharmacological effects of leeches on cardiovascular diseases

Hirudin can regulate the expression of AMPK/Integrin β 1/FAK signaling pathway to optimize the ability of NRCFs to deal with Ang II-induced pro-fibrotic factors, and finally achieve the purpose of inhibiting Ang II-induced changes in cell biological behaviors such as proliferation, phenotypic differentiation, migration and secretion of NRCFs.[12]Li-Xu Xu isolated and purified a peptide HE4-1 with sequence EAGSAKELEGDPVAG from the stem of the broad-bodied golden leech, which could inhibit the migration of macrophages RAW264.7 by suppressing the phosphorylation levels of JNK and p38 in the MAPK pathway. [13]Another study found that natural leechin and JAK2 inhibitor AG490 could block thrombin-induced apoptosis of human microvascular endothelial cells by antagonizing thrombin-activated JAK2 pathway.[14]

1.1.3Pharmacological effects of leeches on lung diseases

Leech extract may treat radiation pulmonary fibrosis, which is a common complication of clinical radiation therapy for thoracic malignancies, and ionizing radiation-induced epithelial mesenchymal transition of alveolar epithelial cells is a key step in the development of RPF. Jifan Yang found that leech extract inhibited ionizing radiation-induced EMT in L2 cells most likely by inhibiting TGF- β /Smad pathway activation.[15]

In addition, hirudin can also treat idiopathic interstitial lung fibrosis. Zhang Jingrong et al. found that hirudin could reduce the expression of AKT and p-AKT proteins and slow down the progression of IPF. [16]Li Hong et al. found that hirudin acted as a treatment for pulmonary interstitial fibrosis in rats by decreasing the expression levels of PAI-1 gene and protein. Similarly, the therapeutic effect of hirudin was found to be significantly dose-dependent, and the combination of hirudin with hormone was more effective in treating interstitial fibrosis in rats.[17]

1.1.4Pharmacological effects of leeches on liver diseases

Leech ultramicro powder can regulate rabbit liver lipid metabolism and improve liver and kidney function. Wang Liu et al. found that 0.15% leech superfine powder could significantly reduce serum ALT, AST, ALP and liver TG, TC, LDL-c in rabbits on high-fat diet, and significantly increase serum ALB in rabbits on high-fat diet, thus promoting the metabolism and biotransformation of lipids in the liver of rabbits on high-fat diet, reducing the deposition of lipids in the liver, reducing the damage effect of high-fat diet on the liver of rabbits, and improving the liver function. [18]

They also found that 0.10%~0.15% leech ultramicro powder could significantly reduce the content of Apo B and MDH and increase the content of Apo E, HL and LPL in the liver of rabbits fed high-fat diet, and increase the expression of LPL gene mRNA in the liver of rabbits fed high-fat diet.[19]It was further found that leech ultramicro powder regulated rabbit liver lipid metabolism-related enzyme activities, as evidenced by the fact that 0.15% leech ultramicro powder could significantly reduce ACC, FAS and HMGCR activities and significantly increase CYP7A1 activities in rabbits fed high-fat diets.[20]

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In addition, Liu Yuqing et al. found that leech could protect hepatocytes by upregulating FXR gene expression, promoting SHP, UGT2B4, and BSEP gene expression, and inhibiting the cholestasis pathway, thus serving to alleviate ANIT-induced intrahepatic cholestasis and reduce liver tissue damage.[21]

1.1.5Pharmacological effects of leeches on other diseases

Yuan-Yuan Li found that leech extract may, to some extent, affect WERI-RB-1 cell cycle distribution, apoptosis and invasion through VEGF/PI3K/AKT pathway and HIF-1a and MMP-9 factors, thus exerting an inhibitory effect on RB. It was also found that leech extract may inhibit umbilical vein endothelial cells by suppressing VEGF and VEGFR2 expression.[22]Based on the BATMAN-TCM analysis tool, Guo Xuefeng et al. predicted that leeches may prevent femoral head necrosis through the PPAR signaling pathway, TNF signaling pathway, AMPK signaling pathway, NF- κ B signaling pathway, and TLR signaling pathway. [23]In addition, based on OCTA, it was found that the herbal leech might be able to inhibit mouse embryonic fibroblast proliferation by inhibiting TGF- β /TAK1/p38MAPK pathway activity. [24]

1.2 Pharmacological Effects of Drug Pairs Related to Leeches

1.2.1Pharmacological effects of Astragalus-leech

Several studies have found that Astragalus membranaceus in combination with leech can alleviate thylakoid proliferative glomerulonephritis. Li Zongguo et al. found that different active components of Astragalus and leech could inhibit the release of inflammatory factors and degrade extracellular matrix by reducing the expression of MMP-9 and NF- κ B proteins, thereby protecting the renal basement membrane in order to inhibit thylakoid cell proliferation and treat kidney injury. [25]Gu Yeyun found that each effective component and component combination of Astragalus leech may affect the expression of P38MAPK, NF- κ :B and I κ B factors through regulating the activity of P38MAPK/NF- κ B pathway to reduce the proliferation of GMC, inhibit EMC deposition and delay the progression of MsPGN disease process.[26]

Atherosclerosis is the main pathological basis of coronary artery disease, and macrophage foam formation and apoptosis occur throughout the development of atherosclerosis. Pan Yang found that 40μ g/ml of leechin, 100μ g/ml of astragalus polysaccharide and the combination of both interventions reduced lipid accumulation in macrophages, and the efficacy of the combination intervention was better than the effect of the drugs alone. The combined intervention reduced the rate of 0X-LDL-induced macrophage regression, and its mechanism of action in the treatment of AS may be related to the regulation of mitochondrial membrane potential and the expression of related pro-regulatory proteins Caspase3, Bax and anti-regulatory protein Bc1-2. [27]

1.2.2Pharmacological effects of leech-centipede

The leech-centipede pair can treat erectile dysfunction. Zhou Yu's study found that the high dose of leech-centipede pair could increase the protein content by upregulating the mRNA of nNOS and NOS proteins, thus achieving similar effects as PDE5 inhibitors. [28]A network pharmacology-based study found that leech-centipede pairs may act on AR and ESR1 targets through cholesterol and linolenic acid to treat DED, thereby restoring erectile function. [29]Another study showed that leech and centipede boiling solution may reduce apoptosis of rat cavernous and vascular endothelial cells by modulating the inactivation of CaSR/PLC/PKC signaling pathway, thereby improving erectile dysfunction.[30]

1.3 Pharmacological Effects of Proprietary Chinese Medicines Containing Leeches

The main proprietary Chinese medicines containing leeches include leech colonization delayed-release micro-pills, Chaohealth leech-bearing gall bladder, eyelotong formula and leech-snake tongluo capsule. Lin Baoqin found that the anti-thrombotic mechanism of leech colonic delayed release micro-pills is related to the inhibition of inflammatory response at the site of

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thrombosis and the inhibition of tissue factor synthesis and release.[31]Liu Jiawen found that leech-bearing gentian may have a protective effect on brain tissue in rats with cerebral ischemia-reperfusion by enhancing the antioxidant properties and reducing the inflammatory response in rats. [32]Feng Yanbing et al. found that the Eye Lutong formula inhibited the expression of VEGF and PDGF in retinal tissues and reduced the release of pro-angiogenic factors, thereby inhibiting retinal vascularization and exerting a protective effect on the retina and treating RVO. [33]Through the cyclic adenosine monophosphate signaling pathway, leech and snake Tongluo capsule can not only regulate hormone levels and metabolize organic hydroxyl compounds, thus inhibiting inflammation, but also promote blood circulation and regulate secretion, thus diastole blood vessels and ultimately achieve the purpose of treating stroke.[34]

2. Clinical Applications

2.1 Single Medicine

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Studies have shown that leeches are clinically effective in cardiovascular and cerebrovascular diseases. A clinical study by Lai Hsi-Nien et al. found that leech powder was effective in improving the compensation of cerebrovascular collateral circulation in patients with cerebral infarction caused by severe stenosis or occlusion of intracranial arteries. [35]Zhang Jiafang et al. achieved safe and effective prevention of deep vein thrombosis after lower limb fracture surgery by low-frequency pulse combined with leech therapy. [36]Wang Jijuan et al. found that leechin could effectively improve coagulation and endothelial function in hypertensive patients by inhibiting thrombin activity and protecting vascular endothelium through clinical studies. [37]In the treatment of angina pectoris in coronary heart disease, the addition of leech can significantly improve the clinical symptoms of patients compared with conventional western medicine treatment alone.[38]Through clinical observation, Jueying Chen found that wet and hot application of leech tincture could improve blood flow, increase the internal diameter of the punctured segment and reduce post-puncture pain in patients with autologous arteriovenous fistula.[39]

Leeches also have clinical efficacy in renal diseases. Liu Jiyao et al. concluded from a Meta-analysis of the efficacy and safety of leeches in diabetic nephropathy that leeches combined with conventional therapy had a more significant effect in delaying renal damage and protecting the kidneys in diabetic nephropathy than conventional therapy alone. [40]

In addition, Wang Meiquan et al. successfully treated patients with active rheumatoid arthritis by Zhuang medicine leech therapy. [41]Pan Weixing et al. successfully treated 30 cases of myofasciitis with live leech therapy. [42]Zhang Rong treated patients with ear reimplantation after auricular dissection by combining negative pressure closed drainage with live leech therapy, thus effectively improving skin flap stasis and promoting wound healing.[43]

2.2 Drug Pairs

The results of Zeng Boxi's study proved that leech-centipede suppositories were clinically effective in the treatment of chronic prostatitis, which improved the blood circulation in the prostate, caused the edema of the prostate to subside, relieved clinical symptoms, and had both analgesic effects, and had multiple effects such as increasing and improving prostate function, increasing lecithin in EPS, and reducing WBC and pus globules. [44]Qiao Tianci et al. found that roasted astragalus-leech granules could to some extent assist rt-PA intravenous thrombolysis to improve early neurological function, TCM symptoms and prognosis in patients with acute cerebral infarction with qi deficiency and blood stasis, and may reduce the occurrence of adverse effects such as bleeding and reinfarction in them, and no safety differences were observed.[45]In addition, compared with simvastatin, the lipid-lowering effect of rhubarb leech formula was better, especially for the treatment of hyperlipidemia in diabetic patients.[46]

2.3 Proprietary Chinese Medicines

The clinical effects of leech-containing Chinese patent medicines are mainly in cardiovascular and cerebrovascular diseases. Vascular blood Kang capsule has good effect on transient cerebral ischemia.[47]Jiang Ming's use of Qingbao Yiyuan Tang is effective in the adjunctive treatment of ischemic stroke and can promote normalization of neurological function without increasing adverse effects.[48]In addition, the clinical effect of leech and tongluo powder in the treatment of acute phase ischemic stroke is also very significant.[49]And compared with the application of conventional western medicine alone, leech Tongluo capsule combined with conventional western medicine was more effective in treating unstable angina pectoris and gi deficiency and blood stasis type carotid atherosclerotic plaque after PCI for coronary heart disease.[50][51]Wang Lihui et al. found that treatment of hemorrhagic stroke with leeches in combination with adding and subtracting peach kernel Chengqi Tang not only accelerated hematoma resorption but also promoted neurological recovery.[52][53]In progressive stroke, treatment with butylphthalein injection combined with leech capsule can significantly improve the degree of neurological deficit and reduce the level of serum C-reactive protein, with significant efficacy.[54]Chen Huijun treated acute cerebral infarction with Tonic Yang Returning Five Tang Plus in combination with Danshen Chuanxiongzin, thus reducing blood viscosity and improving coagulation factor levels with significant efficacy, which is worth promoting.[55]In addition, Tonic Yang Returning Five Soup with leech, Tu Yuan Plus Reduction and Leech Pill are all effective in treating venous thrombosis of the lower extremities.[56][57]Bai Jundong et al. found that Danshen leech whole worm soup combined with drug-coated balloon was clinically effective in the treatment of diabetic infrapopliteal arteriopathy, and patients had improved symptoms and recovered well without adverse effects, which was safe and reliable.[58]

Yang Jifan et al. used leech Huangqi Tang combined with TP regimen in patients with locally advanced NSCLC treated with concurrent radiotherapy and found that it significantly increased DLCO values and significantly decreased serum TGF- β 1 levels. The combination of leech and astragalus soup with TP regimen was effective in the treatment of locally advanced NSCLC, which could improve lung function, reduce inflammation and adverse effects, and reduce recurrence to a certain extent.[59]In addition, Ni Yan-Xia et al. found that the Chinese herbal medicine leech Huangqi Tang used for locally advanced NSCLC patients receiving synchronized radiotherapy significantly reduced the incidence of radiation pneumonia, especially grade II or higher radiation pneumonia.[60]Xu Hongying et al. used laparoscopic severing of the uterosacral nerve and combined with oral leech-neijin soup after surgery to treat chronic pelvic pain caused by endometriosis, which could effectively relieve pain and improve the degree of activity with good clinical efficacy.[61]Patients with lymphedema after radiotherapy for head and neck malignant tumors receiving leech tongluo drink can effectively relieve head and neck swelling and improve the quality of life.[62]Adding and subtracting leech and peach kernel soup has certain effect in improving the postoperative neurogenic pain of TLIF in patients with lumbar instability, which can reduce the postoperative pain of patients, shorten the postoperative pain time, and reduce the use of postoperative analgesic drugs.[63]

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