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Review

Earthworm as a Peripheral Nerve Regeneration Biomaterial: A Comprehensive Review

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Abstract

Many Earthworm (EW) related effects have been introduced so far including, wound healing effects as well as anti-microbial, anti-inflammatory and anti-thrombosis effects. EW biomaterials could also be a therapeutic agent for various neuroinflammatory conditions. Besides, indigenous people traditionally have practiced the extraction of medicinal compounds from EW. Therefore, the present study aimed to investigate the EW capacities in nerve regeneration. An exhaustive literature search was performed using databases including PubMed, Scopus, Web of Science and Google. A total of 13 studies were included. All of them included an animal model or were cell culture studies between 2009 and 2018. The description of these findings is given in table 1. Despite the limited number of publications and their controversial results, EW can be regarded as a new promising option for nerve repair, given the several pieces of evidence confirming the effects of EW biomaterials on nerve injury and regeneration.

Keywords: Earthworm; Nerve regeneration; Lumbricusin; Lumbrokinase; Traditional medicine

Introduction

Peripheral nervous system (PNS) injuries are common in clinical settings and are one of the primary sources of disabilities. PNS injuries affect muscle function, feel natural sensations and may lead to painful neuropathy. Peripheral nerves have a significant capacity to recover after an injury [1]. Regeneration of these nerves is a complex physiological reaction. A set of agents such as cell delivery (Schwann cell, macrophage and fibroblast), growth factors (NGF and BDNF), scaffold/substrate, polarized blood vessel development, and cytokine delivery are required to remodel the nerve regeneration environment [1,2].

Several earthworm (EW) products such as Lumbrokinase (LK), G90, coelomic fluid, and EW extraction material are used for scientific research. Many earthworm-related effects have been introduced so far. These include healing effects on diabetic [3] and non-diabetic [4] wounds, as well as antimicrobial [5] and anti-inflammatory [6] effects that may aid in the healing process of wounds. LK has antithrombotic effects through its fibrinolytic activity and suppressing platelet aggregation. It also has an antifibrosis function performed by decreasing fibronectin, laminin and collagen, showing a broad substrate specificity [7]. LK has also increased the blood flow rate [8]. Earthworms could be considered a therapeutic agent for treating various neuro-inflammatory conditions

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[9]. Notably, it can have a preventive and therapeutic effect on some neurological disorders such as Parkinson and cerebral infarction [10].

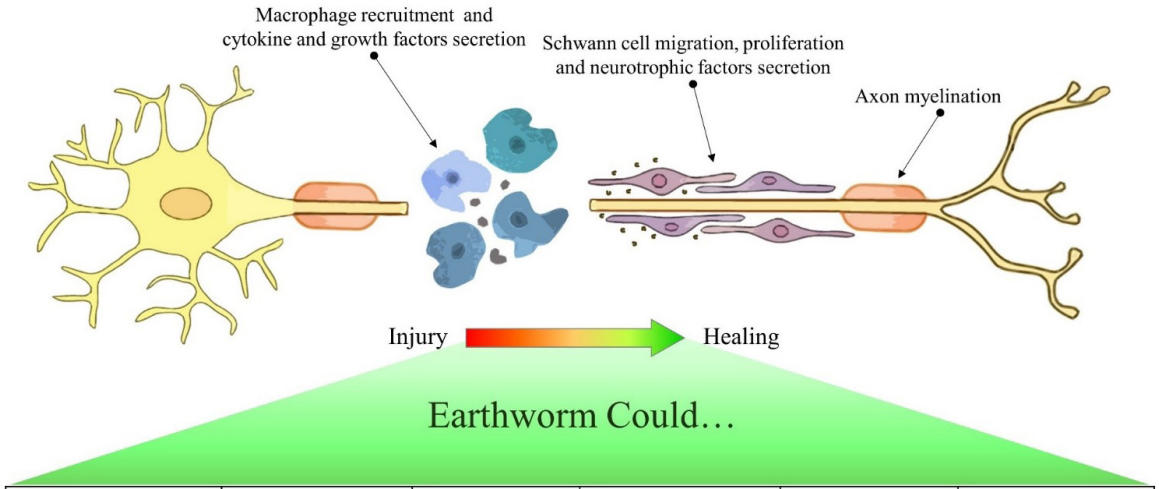
Indigenous people worldwide, particularly in Asia, have traditionally practiced the extraction of medicinal substances from earthworms [11]. The possible effects of earthworm therapy may come from its rich soil-based nutritional content [12]. In Persian Medicine (PM), mollusks such as snails [13] and earthworms [14] have been used as a therapeutic agent for wound healing and nerve injuries for about 1000 years ago [15-17]. Therefore, since no comprehensive review has been done on this topic, the present study aimed to investigate the evidence for earthworm capacities in nerve regeneration.

Materials and Methods

Inspired by PM an exhaustive literature search was conducted using databases including PubMed, Scopus, Web of Science and Google. The keywords used in this study were 'earthworm', 'nerve regeneration', and 'nerve repair' as well as MeSH terms or free-text words. Articles published in English were included. All *in vitro* and *in vivo* experiments, such as cell culture and animal models directly or indirectly related to EW effects on nerve regeneration were included. Studies with a mixture of materials in addition to earthworm biomaterials as a treatment were excluded. Primary search results were screened, and all studies assessing the efficacy of earthworms were covered due to the lack of clinical trials. Finally, references of the included study were reviewed for more relevant articles.

Results

No systematic reviews or clinical trials were found to have aimed at evaluating the nerve regenerative profile of earthworms. A total of 13 studies were investigated, including six animal models, five cell culture studies, and three studies with both types. All studies were published between 2009 and 2018. Most animal models used for nerve regeneration analysis were rats, especially Sprague Dawley rats. Fifty percent of nerve repair analyses were performed by bridge technique in sciatic nerves. Still, some other approaches such as cerebral infarction or side-stream-smoke injury have also been made. As far as the type of treatment was concerned, 55% of the studies chose oral treatment, followed by topical and intraperitoneal treatments, respectively. RSC96 and PC12 were the most commonly selected cell lines to evaluate a nerve regeneration effects of earthworms. In 61% of the studies, LK and Lumbricusin (LC) as bioactive materials of earthworm were used, followed by ethanol extract and water extract, respectively. A description of these findings is given in table 1 which shows earthworm extraction type, effective dose, cell lines and animal models properties and final effects. An overview of the earthworm neuronal regeneration impacts is shown in figure 1.



↓ Motor impairment	↑ Blood flow rate	↓Inflammation	↓ Autophagy	↓ Apoptosis	↑ NGF expression
↑ Neurite outgrowth	↑ Myelination	↑ NCV & NFI	↑ MQ. recruitment	↑ SCs. migration	↑ Proliferation

* : ↓, Decrease; ↑, Increase; SCs, Schwan cells; MQ, Macrophage

Figure 1. An overview of the earthworm neuronal regeneration effects

Table 1. Classified summary of included studies

Ref.	Earthworm specious	Extract / component	Effective dose	Cell type	Animal model	Effect	Conclusion
[25]	*Pheretima aspergillum*	EEE	125 µg/mL	RSC96	-	↑ Schwan cells migration ↑ MAPK pathway by pERK & P38 phosphorylation ↑ PA, MMP-9 & MMP-2	EEE might serve as a migration inducing and/or therapeutic drug for nerve regeneration.
[24]	*Pheretima aspergillum*	EEE	125 µg/mL	RSC96	-	↑ proliferation ↑ PI3K/AKT pathway by IGF-1 activation ↑ pBad, PCNA, cyclin D1, E, A	EW promotes the proliferation and survival of RSC96 cells via IGF-I signaling and PI3K protein.
[29]	*Pheretima aspergillum*	WEE	500 µg/mL	PC12		↑ Neurite outgrowth ↑ GAP43 & Synapsin 1	WEE can be a nerve growth promoting factor which can be applied for peripheral nerve regeneration.
			31.25 µg/mL (Topical treatment)		SD Rat (nerve bridge technique)	↑ Peripheral nerve regeneration ↑ Myelinated axon	
[8]	*Lumbricus rubellus* and *Eisenia fetida*	LK	600-1200 µg/kg (oral treatment)	-	Diabetic SD rat (silicone rubber conduit technique)	↑ Cutaneous blood flow rate ↑ NCV & amplitude and ↓ latency ↑ macrophages recruitment ↑ CGRP, IL-1β & PDGF	LK has remarkable effects on promoting peripheral nerve regeneration and functional recovery.
[30]	*Pheretima aspergillum*	LC	1 mg/kg (oral treatment)	-	SD Rat (left sciatic nerve clamping)	↑ NCV & NFI ↑ Myelinated axon	LC may enhance sciatic nerve regeneration and function recovery following injury.
[23]	*Lumbricus rubellus*	Protein fraction DLBS1033	50 µg/mL	RSC96	-	↑ Growth & Survival in Min. O_2 Condition ↑ NGF expressions ↑ PI3K by AKT, BCL-2 & BCL-xL activation	It can promote the growth and survival of Schwann cells by inducing NGF expressions. These mechanisms are probably achieved via PI3K pathway.
				PC12		↑ Neurite outgrowth	
[33]	*Pheretima aspergillum*	LC	1 g/mL (2 mL daily by oral treatment)	-	SD Rat (artificial conduit bridging surgery)	↑ NCV & TFI ↑ Amplification ratio for nerve regeneration	It can promote proximal axons to grow more lateral buds, promote nerve amplification effect and nerve function.
[31]	*Pheretima aspergillum*	EEE	100 µg/ml & 100 mg/kg (Topical and oral treatment respectively)	-	Diabetic wistar Rat (Diabetic ulcer stage II model)	↑ Nervous fiber density Topical treatment had better impact than oral treatment	EEE could promote regeneration of peripheral nerve in diabetics rats model.
[32]	*Pheretima aspergillum*	WEE	0.5 g/kg (Oral treatment)	-	SD Rat (middle cerebral artery occlusion)	↑ Nervous density or survival ↓ GFAP & S100B expression	WEE could improve neurological and functional recovery of rats after MCAo.

[36]	*Lumbricus rubellus* and *Eisenia fetida*	LK	1.2 mg/kg (i.p., twice a week)	-	Hamster	↓ Hippocampus apoptosis, autophagy and inflammation related injuries induced by SSS	LK exerts protective effects on hippocampus apoptosis and has therapeutic potentials against its abnormal function.
[27]	*Lumbricus terrestris*	LC	10 µg/mL	SH-SY5Y	-	↑ Proliferation by ↓ p27Kip1 ↓ Apoptosis induced by 6-OHDA,	LC induces neural cell proliferation and protects against cell-damaging agents.
[28]	*Lumbricus terrestris*	LC	10 µg/mL	MN-SCs	-	↑ Proliferation by ↓ p27Kip1 ↓ Apoptosis induced by 6-OHDA ↓ Motor impairment induced by 6-OHDA with TH content	LC could enhance the neural cell proliferation and recover behavioral impairment in a mouse model of PD.
[9]	*Lumbricus terrestris*	Lumbricusin analogue 5 (LumA5) isolation	20-80 µg/mL	BV-2 microglial	-	↓ iNOS, Cox-2, and proinflammatory mediators, including NO, TNF-α, IL-6, and IL-1β protein and RNA expression that stimulated by LPS ↓ phosphorylated protein of MAPKs and AKT that stimulated by LPS	LumA5 could be a candidate for development of a therapeutic agents for neuroinflammation-associated diseases. In addition, LumA5 has a low molecular weight (9-mer) resulting in low antigenicity.
			20-80 µg/mL	SH-SY5Y		↓ Neurotoxicity	
			1 and 5 mg/kg (i.p., once a day for three days)	-	Swiss Albino mice	↓ iNOS, Cox-2, and pro-inflammatory mediators, including TNF-α, IL-6, and IL-1β expression that stimulated by LPS	

Abbreviations:
↑, Increase; ↓, Decrease; 6-OHDA, 6-hydroxydopamine (Parkinson's disease mimicking agent); BV2, mouse microglia cell lines; CGRP, calcitonin gene-related peptide; EEE, ethanol extract of earthworm; EW, earthworm; GAP-43, growth associated Protein 43; GFAP, glial fibrillary acidic protein; IGF-1, Insulin-like growth factor 1; IL, Interleukin; iNOS, Inducible nitric oxide synthase; LC, lumbricusin; LK, lumbrokinase; MAPK, mitogen-activated protein kinase, MCAo, middle cerebral artery occlusion; MMP, matrix metalloproteinase; MNSCs, mouse neural stem cells; NCV, nerve conduction velocity; NFI, nerve function index; NGF, nerve growth factor; NO, nitric oxide; PA, plasminogen activator; PC12, is a cell line derived from a pheochromocytoma of the rat adrenal medulla; PCNA, proliferating cell nuclear antigen; PD, Parkinson's disease; PDGF, platelet-derived growth factor; PI3K/AKT, phosphatidylinositol 3-kinase/protein Kinase B; RSC96, neuronal Schwann cell; SD rat, Sprague Dawley rat; SH-SY5Y, human neuroblastoma cells; SSS, side-stream-smoke; TFI, tibial function index; TH, tyrosine hydroxylase (a marker for the respective numbers of dopaminergic terminals and cell bodies); TNF-α, tumor necrosis factor- α; WEE, water extract of earthworm.

Discussion

Peripheral nerve injury is a common disorder, with around 1 million patients demanding peripheral nerve surgery worldwide every year. There are numerous causes of nerve injury, including crushes, ischemia, piercing damage, and traction. While axon regeneration has been studied for more than a century, good functional outcomes for nerve repair are still presenting a challenge [18]. Earthworms have been a source of therapeutic agents in many countries since ancient times. Previous studies have shown their antibacterial, antioxidant, anti-inflammatory, wound healing and hepatoprotective impacts [19]. Like many other gastropods, the earthworm can regenerate its nerves. In addition to the peripheral nerves, in case of an injury, it can also repair its central nervous system, although its mechanism has not yet been completely understood [20,21]. Several studies on the neuronal regenerative

effects of earthworms have been carried out since around 2008. An overview of these features is shown in Figure 1 and are discussed as follow.

Earthworm effects on Neuronal proliferation, survival and growth

Most of the studies in this field have evaluated the proliferation effects of EW compounds using different methods. EW effects contribute to PI3K and MAPK signal transduction pathways, which are the most common pathways considered. It has been found that trophic factors such as insulin-like growth factor could promote nerve regeneration [22], and it seems EW induced PI3K pathway via IGF-1R [23,24]. Different results have been obtained regarding the MAPK pathway. Chang et al., for example, reported that MAPK pathway proteins, ERK and p38 significantly increased in the Schwann cell line (RSCs96) [25], and we already know that Schwann cell migration and proliferation are critical for the regeneration of injured nerves [26]. Karsono et al., on the other hand, showed that MAPK-related genes such as cFos, MYC, and CREB decreased in treated RSCs96 cells [23]. Therefore, the MAPK pathway did not show any activation. In the meantime, Seo et al. expressed a neuroinflammation regulatory role of LC. They showed that an isolated 9-meric peptide of LC reduced phosphorylated protein of MAPKs stimulated by LPS [9]. Thus, the impact of EW compounds on MAPKs signaling is still controversial.

Some other studies examined downstream mechanism of these pathways. Cell cycle proteins such as cyclin E, A, D1, pBAD and, PCNA increased in the RSCs96 cells after treatment [24]. By contrast, the rate of P27kip1 as the cyclin-dependent kinase inhibitor decreased. It has been found that overexpression of P27kip1 blocks the protective and proliferating effect of LC in human neuroblastoma cells (SH-SY5Y). Also, microscopic assays demonstrate that proliferation increased in neuronal cell lines [27,28]. On the contrary, LC treatment did not have any impact on the non-neuronal cell lines like human colonic epithelial (HT29) cells or adrenal carcinoma (SW13) cells [27]. Hence, EW biomaterial could stimulate the cell cycle for neuronal proliferation. Although earthworm compound and neuronal cell lines used in these studies were different, the results were consistent.

In *in vivo* studies, the results mentioned above were confirmed as well. Histological and microscopic examinations showed an increase in myelinated nerves [29,30] and nervous fiber density [31-33] in topically and orally treated rat models. Immunohistochemistry assays in the hippocampal dentate gyrus of mice revealed that chronic treatment with high-dose earthworm ethanol extract (EEE) reduced cell proliferation and neuroblast differentiation [34]. Therefore, it is essential to arrive at a precise dose.

Earthworm effects on nerve function

To evaluate the development of nerve regeneration, it is helpful to be able to test nerve injury models in the same study at different intervals. With regard to motor nerves, an electrophysiological examination could indicate whether or not nerve recovery occurs while the animal still remains alive. Walking track analysis is the most commonly used method for evaluating sciatic nerve motor function [18]. For PNS function studies, the most widely nerve model studies are the sciatic nerve and its branches, along with the tibial and peroneal nerves [1]. Sciatic function index (SFI) and tibial function index (TFI) are two types of walking track analysis. Nerve conduit velocity (NCV) is a common method for electrophysiological assay. There were four studies used SD rats as a nerve injury model and used the nerve bridge technique for evaluating nerve function after treatment. Oral treatment of LK and LC significantly increased NCV, SFI, and TFI [8, 30, 33]. However, Chen et al. found different results indicating that the electrophysiological tests did not increase significantly. They used water earthworm extract (WEE) as a topical treatment [29]. In another research, a mixture of icariin and the liquid extract of earthworm demonstrated that NCV, SFI, TFI, and Peroneal function index (PFI) increased significantly [35].

Earthworm effects on apoptosis

EW has also shown anti-apoptotic effects in *in vitro* and *in vivo* studies. Different apoptotic pathways have been so far evaluated in the literature. Huang et al. and Ji et al., for example, have conducted *in vivo* studies on LK and its anti-apoptotic effects. IP treatment of LK in hamster hippocampus tissue induced by sidestream smoke (SSS) was examined, and the results showed a decrease in the number of apoptotic cells by TUNEL staining method. It was also demonstrated that activated caspase 9, 8 and, 3 as well as Fas and FasL decreased; therefore, the apoptosis process was attenuated [36]. LK also demonstrated an anti-apoptotic effect on cerebral tissue and HUVEC cells through the JAK1/STAT1 pathway in an MCAO rat model [37]. Kim et al., conducted two studies on LC and apoptosis in SH-SY5Y cells and mouse neural stem cells (MNSCs). They induced apoptosis by 6-OHDA, and found that LC could decrease activated caspase 3, hence decreasing the apoptotic effect of 6-OHDA [27,28]. Therefore, EW compounds could play a protective role against apoptosis.

Earthworm effects on neurite outgrowth

One of the widely studied topics in neurodevelopment has been the *in vitro* analysis of neurite outgrowth.

There are a variety of well-characterized *in vitro* models that have been used to analyze chemical effects on neurite outgrowth. Pheochromocytoma of the rat adrenal medulla cell (PC12) is a cell line that has been extensively used in neurobiological research to evaluate chemical effects on neurite outgrowth [38].
Two studies were found that examined EW biomaterial effect on neurite outgrowth in *in vitro* models. Chen et al. used WEE on PC12 cell. Their morphological test showed increased neurite outgrowth levels along with an increase in GAP-43 and Synapsin1. The GAP-43 is a marker for growth cone, and synapsins-I is an essential phosphoprotein for regulating neurotransmitter secretion [29]. In their study, Karsano et al. used LC, and found that LC could increase neurite outgrowth on PC12 cells even more than the sample treated with nerve growth factor (NGF) [23]. Another interesting study worked on Caltubin (a snail tubulin-interacting protein). They transfected the Caltubin gene in PC12 cells and mouse cortical neurons and found that local synthesis of Caltubin increased neurite outgrowth. The N-terminal region of Caltubin interacts with beta-tubulin and stabilizes microtubules against depolymerization [39]. Like snail protein the Caltubin, EW biomaterials could be identified and evaluated as well.

Earthworm effects on neurotrophic factors and cytokines

In response to injury, neurotrophic factors are generated in target organs by Schwann cells. A nerve injury usually disrupts the interaction between the target organs and the neuronal cell body and leads to Wallerian degeneration (a breakdown of myelin sheath and axons). The cytokines released during Wallerian degeneration stimulate Schwann cells to release neurotrophins such as brain-derived neurotrophic factor (BDNF) and NGF. According to previous studies, the synthesis of NGF in sensory and sympathetic nerve target organs in the PNS supports the survival of sensory ganglia and nerves, including spinal sensory nerves and sciatic nerves [40,41]. Also, it has been found that BDNF expression is enhanced in injured peripheral nerves, which stimulate the outgrowth and survival of sensory and sympathetic nerves as well as motor nerves [42].
Slowed Wallerian degeneration due to a deficiency in macrophage infiltration has been considered the key reason for the lack of diabetic nerve regeneration [43]. It has been found that macrophages could release neurotrophic factors and cytokines during nerve regeneration [44,45].
Comparatively, LK-treated nerves could enhance the number of macrophages. They could stimulate the segments of the diabetic nerve, producing more cytokines and neurotrophic factors that are beneficial for regenerating nerve fibers. These results indicate that the increased macrophages observed in LK-treated rats could accelerate Wallerian degeneration and secrete more nerve growth-promoting substances following nerve injury, leading to an improved diabetic regenerative response [8].
Of all studies examining the impacts of EW biomaterial on BDNF, none has demonstrated an increasing influence on BDNF. Results in both cell culture and animal model experiments suggest that BDNF is not increased by EW treatment, and some outcomes have even shown a reduction of BDNF. LC treatment on RSC96 showed a decreasing impact on BDNF gene expression along with a decrease in Fibroblast growth factor (FGF) and Glial cell-derived neurotrophic factor (GDNF) [23]. Liu et al. and Yan et al. reported that not only did WEE and EEE not change the BDNF protein level in neuronal tissue of the animal model, but also its level was decreased to some extent [32,34]. On the other hand, mixed results have been obtained regarding the effect of EW on NGF. LC treatment on the RSC96 cell line increased NGF significantly, with 50% more gene expression and protein level than the control group [23]. Besides, in an *in vivo* study, oral LK treatment on SD rats increased NGF gene expression slightly but not significantly; while CGRP and PDGF were significantly increased in the same study [8]. CGRP is recognized as a neurotrophic peptide that could promote neuromuscular development and regeneration in the transected hypoglossal nerve [46]. IL-1β increased in this study as well, which theoretically suggests it could recruit macrophages and induce Schwann cell neuropeptide secretion [8].
Neurotrophic factors are expected to be an essential part of future clinical therapies for peripheral nerve injuries and diseases. The application of neurotrophic factors could be highly effective in facilitating nerve regeneration [1]. Taken together, EW effects on macrophage recruitment and neurotrophic factor secretion are promising and require more experimental research.

Earthworm effects on neuroinflammation

The anti-inflammatory activity along with antioxidant properties of EW may be due to the high polyphenolic content in EW tissue [47]. Neuroinflammation, an innate immune response of the nervous system, is usually involved in multiple neurodegenerative diseases, including ischemic injury, Alzheimer's disease, and Parkinson's disease [9,48]. COX-2 and iNOS are pro-inflammatory enzymes, and their reaction products are responsible for cytotoxicity in several neurological disorders. The activity and expression of iNOS and COX-2 are mediated by NF-κB. The transcription factor NF-κB is a crucial regulator of genes involved in cell survival/apoptosis and inflammation. One study indicated that LK decreased NF-κB phosphorylation and enhancement of NF-κB transcription-

al target proteins, iNOS/ COX-2, in the hippocampus of hamsters. So this suggests that LK neuroprotective effects appear to be partially dependent on the NF-κB/iNOS/COX-2 signaling pathway inhibition. LK administration resulted in a significant decrease in iNOS, thus weakening the formation and detrimental effects of abnormal amounts of peroxynitrite. It was presumed that LK protected hippocampus injury by reducing oxidative stress and ROS-induced inflammatory response induced by inducible NO [36].
Moreover, another study that used LC as an EW biomaterial also confirmed these results. They reported that LC could markedly reduce the expression of enzymes (COX-2, iNOS), cytokines (IL-6, IL-1β, TNF-α), and signal transduction factors (AKT, MAPKs, NF-κB) involved in inflammation triggered by LPS *in vitro* and *in vivo* [9]. These results indicate that LC and LK may be potential therapeutic agents for the treatment of various neuroinflammatory conditions.

Earthworm effects on blood flow rate

Nerve growth-promoting effects of EW biomaterial are believed to be possibly through the pathways associated with enhanced circulatory blood flow, improved macrophage infiltration, etc. in nerve regeneration. According to an examination of LK therapeutic effects on peripheral-nerve regeneration using well-defined sciatic nerve lesion paradigms in diabetic rats induced by the injection of streptozotocin, LK therapy could boost the rats' circulatory blood flow [8]. In addition, LK administration resulted in a remarkable decline in iNOS and an increase in eNOS protein levels in the brain, suggesting that the brain tissue could cope better with some toxic insults. eNOS activation with the production of NO decreases resting vascular tone and increases cerebral blood flow in response to diverse stimuli, alters neuronal signaling and facilitates angiogenesis and neurogenesis [36].
Moreover, crude EW extract has a thrombolytic effect that could significantly promote blood flow and remove stasis [49].

Other earthworm effects on the nerve regeneration process

Here we discuss some EW effects, such as Schwann cell migration stimulation and anti-autophagy on nerve regeneration.
As we know, Schwann cell migration and proliferation are critical for regenerating injured nerves [26]. It has been demonstrated that EW can stimulate Schwann cell migration and upregulate matrix-degrading proteolytic enzyme expressions such as plasminogen activators (PAs) and matrix metalloproteinases (MMP2/9) mediated by the MAPK pathways, ERK1/2 and p38. Therefore, data suggest that MAPKs (ERK1/2, p38), PAs (urokinase PA, tissue PA) and MMP (MMP2, MMP9) signaling pathways of Schwann cells regulated by EW could play a significant role in Schwann cell migration and nerve regeneration [25].
Autophagy plays a critical role in the maintenance of neuronal homeostasis because neurons are postmitotic cells. While autophagy is beneficial, excessive autophagy can also play a detrimental role in neurological diseases, leading to autophagic neuronal death. [50, 51]. Huang et al. designed an examination of the effects of SSS exposure on the hippocampus of hamsters. They stated that autophagy is enhanced in the hippocampus of SSS-exposed young hamsters, as indicated by the increased expression of autophagy-related proteins (i.e., Beclin-1, ATG7, LC3-I, and LC3-II). However, LK therapy significantly reduced the SSS-induced autophagy effect [36].
Compared to other results, since the effects of earthworm on anti-autophagy and Schwann cell migration stimulation are limited and unreliable, more investigations are needed.

Conclusion

While there are controversial outcomes regarding the impact of EW on nerve regeneration in a few cases, such as neurotrophic factor, nerve function, and neuronal proliferation pathways, and a limited number of publications on this topic are completely clear, we cannot easily ignore the relevant and confirming evidence in modern studies, as well as traditional medicine on the relationship between EW compounds and nerve injury regeneration. According to several pieces of evidence for the effects of EW biomaterials on nerve injury and nerve regeneration, a new tool of nerve repair in the field of these biomaterials can be promising. Therefore, extensive and further studies in the EW biomaterials area are required.

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Data availability

The datasets used and analyzed during the current study are available from the corresponding author.

Conflict of Interests

None.

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