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Earthworm as a biopharmaceutical: from traditional to precise

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Review

Abstract: As a traditional Chinese medicine (TCM), earthworm (Dilong) was applied in China for ages. Some active proteins and compounds in earthworm were characteristic composition as a pharmaceutical, including fibrinolytic enzymes, lumbricinase, collagenase, superoxide dismutase, cholinesterase, catalases, glycosidases, metallothionein, calmodulin-binding protein, proteins with proliferation improving activity, lysenin, eiseniapore, antitumor protein, glycoprotein extract, gut mobility regulation peptide, antibacterial peptide, carbamidine, lumbricin, lumbricofibrin, terrestrolumbrolysin, purin, vitamin B, tyrosine, succinic acid, lauric acid, unsaturated fatty acid and so on. These earthworm components had more or less pharmacological and clinical functions on effects on the nervous system, blood system, the cardiovascular system, the respiratory system, uterus smooth muscle, and its anticancer function and sperm-killing function. Lumbricin metalchelatin (LMT) among these active components has detoxification function. LMT is one of the most common heavy metal detoxification, which are small cysteine-rich and non-ribosomal peptides that chelate soft metal and metalloid ions, such as cadmium, zinc and arsenic. LMT function of scavenging free radicals plays an important role in the process of the anti-aging, anti-oxidative stress and cell apoptosis, etc. No matter whole dry body or abstracted components, earthworm has been used to treat at least 19 human diseases in clinical, including treatments of tracheas, bronchial asthma, epilepsy, high blood pressure, schizophrenia, leg ulcers, mumps, eczema, urticaria and anaphylaxis diseases, chronic prostatitis, burns and scald, fractures, erysipelas, chronic lumbago, blood-deficiency apoplexy, acute injury of soft tissues, vertigo, hematemesis and hematuria, digestive ulcer, vesical calculus and cancer.

Key words: Functional component; Pharmacological effect; Clinical application; Lumbricin metalchelatin; Earthworm.

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Introduction

Earthworms have been used as a traditional Chinese medicine (TCM) for at least 2,300 years in China. However, only a few species of earthworm can be used as medicine. As mentioned in Chinese pharmacopoeia in 2015 (1), TCM Dilong (*Pheretima*) refers in particular to whole body of four kinds of wild earthworm, *Pheretima aspergillum* Perrier, *Pheretima guillelmi* Michaelsen, *Pheretima vulgaris* Chen, and *Pheretima pectinifera* Michaelsen. The first one, distributed in Guangdong, Guangxi, Fujian and Hainan Provinces, is called as “Guang Dilong”, and the other three species, are called as “Hu Dilong”, they distributed in Shanghai, Henan, Shandong, Jiangsu and Anhui Provinces. In ancient Chinese medicine books, such as “Qianjinfang”, “Danxifang”, “Jixiaofang” and “Bencangmu”, it is recorded that “Dilong” (a Chinese medicinal name for earthworm) was used as an antipyretic and anaesthetic, for detoxification, treatment of hypertension and hastening parturition, as well as many common ailments, such as arthritis, itching, burns, carbuncles, erysipelas, and inflammation.

Decoction preparing method was common with whole body, roots, seeds, or leaves of medicine herbs in (TCM). Since last century, researchers focused on chemical composition and physiological effects of earthworm. Some nitrogenous substances extracted from the earthworm can be used as an anti-histamine to treat asthma (2-7). In recent years, as development of fine chemistry and analysis technology, some active compounds from earthworm, such as

linmbritin and terrestrolumbrolysim, have been isolated. More pharmaceutical and physiological components were found and isolated from earthworms, including fibrinolytic enzymes, lumbricinase, collagenase, superoxide dismutase, cholinesterase, catalases, glycosidases, metallothionein, calmodulin-binding protein, proteins with proliferation improving activity, lysenin, eiseniapore, antitumor protein, glycoprotein extract, gut mobility regulation peptide, antibacterial peptide, carbamidine, lumbricin, lumbricofibrin, terrestrolumbrolysin, purin, vitamin B, tyrosine, succinic acid, lauric acid, unsaturated fatty acid (8-10). The pharmaceuticals from earthworm, as green biomedicine, have developed fast in China and Asia. Lumbricin preparations have been used as a common drug to help treat or prevent cardiovascular and cerebrovascular diseases. Earthworm is becoming an international medicine, even though the original utilization of earthworm was known as a traditional Chinese medicine.

Collection and process of Dilong in TCM

Collection of *Pheretima*

Wild earthworms of *Pheretima* were normally collected from soil in spring, summer and autumn. In China, a water solution of plants *Polygonum dydropiper* and *Camellia sinensis* was used. The solution was sprayed on the field to stimulate earthworms to emerge out of the soil (similar to formalin method). Earthworms were collected, washed in warm water, and then put them into plant ash to be choked and bored. The dead earthworms were cut, and

the gut was washed out. Then the processed earthworm were dried under sunshine or with heat. It could be stored in a cool, dark and dry place to minimize mildew and pest insects attack (11).

Processing of *Pheretima*

First, earthworms were cut into 1-2 cm sections, soaked in water for half a minutes, and rinsed 1-2 minutes, and made into meal. The dried earthworm tissues were kept in wooden boxes stored in a dry and ventilated place; second, earthworms were put in bamboo baskets and sprayed with a little water, and then immediately cut into 3-5 mm sections with the medicine-cutter. The soil and sand was rinsed from the earthworms in a water bowl; then dried under sunshine or by heat. This method was to cut up earthworms quickly and completely into distinct sections, rinse soil and sand easily and quickly, and retain most medical ingredients in the earthworm sections; third, earthworms were moistened by sprinkling them with Shaoxing wines for one hour. Wheat bran was heated by frying and the earthworms were mixed with hot wheat bran, and then the mixture fried until the earthworms became a deep yellow color. The mixture was taken out and the wheat bran was sieved, and fried earthworms were spread out, cooled and stored; at the end, earthworms were moistened with old rice vinegar for one hour. When the vinegar was absorbed into the earthworm bodies, the earthworms were spread on enamelware to a depth of 3 cm and placed in an oven at 100 °C for 2 hours. When the toasted earthworms became a deep yellow color, they were cooled and stored in bottles (11).

As fast development of molecular biology and relative instrumental analysis, fine ingredient from earthworm, both qualitative and quantitative, was procedurally conducted (12).

Characteristic composition as a pharmaceutical in earthworm

Earthworms usually contain some characteristic compositions of lumbriferrine, terrestrolumbrin, lumbricin, hypoxanthine and other purines, pyrimidines, choline and guanidine. The fat of earthworm is composed of octade acids, palmitic acids, high-chain unsaturated fatty acids, linear and odd carbon fatty acids, branched fatty acids, phosphatide, cholesterol etc (13,14). The yellow chloragogenous cells and organs of *Lumbricus terrestris* contain rich amounts of carbohydrates, lipids, protein, pigments and some alkaline amino acids. The yellow pigment perhaps consists of riboflavin or its analogues (15). The tissues of *Pheretima spp.* Contains large amounts of microelements, Zn 59.1 ug/g, Cu 25.4ug/g, Fe 1735.5 ug/g, Cr 10.93 ug/g, Mo 0.25 ug/g, Ca 1019.2 ug/g, and Mn 1143 ug/g (16). These of *Allolobophora caliginosa* contain crude protein 57.96%; crude fat 6.53%, crude ash 21.09%, crude fiber 0.36%, and N extract 14.06%. These of *Eisenia fetida* contain crude protein 64.61%; crude fat 12.29%, crude ash 10.16%, crude fiber 0.27%, and N extract 12.67%. These of *E. Rosea* contain crude protein 63.71%, crude fat 12.29%, crude ash 10.66%, crude fiber 0.21%, N extract 12.67% (17). The blood and body fluids of *Lumbricus terrestris* contains small concentrations of glucose (0.01-0.05 ug/ml) (18), considerably lipids, including 35.14% neutral

fat, 41.74% glucolipid, and 23.12 % phosphatide. The C-chain of the fatty acid is between 10 to 22 C. The neutral fat consists mainly of lauric acid, oleic acid, myristic acid and decanoic acid. The fatty acids of glucolipids are decanoic acid and some short chain fatty acids. The fatty acids of phosphatide are oleic acid, decanoic acid, linoleic acid and behenic acids. The proportion of unsaturated fatty acids is higher than that of neutral fatty acids and saccharides (19). A P-peptide substance exists in the gut wall of *Lumbricus terrestris* (20). Some active enzymes occur in the yellow chloragogenous cells and organs of *Lumbricus terrestris* in high concentrations, including catalase, peroxidase, dismutase, β -D-glucosyl enzyme, alkaline phosphatase, esterase, S-amino- γ -ketoglutaric dehydrogenase and porphyrin synthetase. The body fluids of *Eisenia spp.* contained at least 18 proteins with molecular weights between 1,000 and 95,000 Da (21). The dormant species of *Allolobophora caliginosa* contain a protein, which can hydrolyze collagen (20). Scientists from Japan, China and Korea found and separated enzymes from the earthworm gut and body fluids, which can dissolve fibrin (22). These enzymes have been developed as innovative medicines to treat cerebral thromboses and myocardial infarction (21). Sun (1998) found and separated a kind of acid antibacterial peptide, tetradecapeptide, which has produced a disease-resistant, nutrient earthworm preparation (23). Then, 6 other kinds of antibacterial peptide, including a peptide family, were found (24, 25).

Pharmacological and clinical functions of earthworm

Effects of the nervous system

Zhang first reported in 1950's that earthworms could reduce blood pressure (26). Xu et al. observed the phenomenon of significantly reducing blood pressure of anesthetized dogs that were injected with macerated earthworm extracts in hot water and ethanol solution. To understand the depressive mechanisms of earthworms, Xu conducted a medicine-pouring experiment with a rabbit heart separated from the body. The results showed that 0.001% earthworm macerated extracts dissolved in hot water and ethanol solution can increase the heart beats, but when the dose was increased to 0.05-1%, heart beats decreased. Up to a dose of 0.1g/kg body weight, the rhythm of the rabbit heart decreased progressively. At a dose 15 to 17 times higher than a clinical enema to the dog, there were no changes in rates of heart rhythm and electrocardiograms actively. Xu pointed out that the depressive mechanisms of earthworms couldn't be explained only by restraints on the heart (27). An effective depressive component B1 from earthworm tissues was separated with an ion-exchange column. It can reduce significantly a type of high blood pressure, which was caused by exciting the sciatic nerves from the central area forwards and confirmed that earthworms can cause anti-excitation similar to that produced by caffeine. The main function of depressives was located in the central nerve of the upper spinal cord and can influence the central nervous system by directly refluxing some inner receptors and causing the expansion of some internal blood vessels (28). Kuang reported that a 100% extract of earthworm tissues could improve metabolism of dopamine (DA) and 5-hydroxy tryptamine (5-HT), monoamine nerve medium of the cen-

tral nervous system, so it can have a protective function against blood-deficiency brain death (29).

Effects of earthworms on blood system

Rao (1986) reported that the enzymes in earthworm body fluids could dissolve fibrin thrombosis (30). Li (1988) studied extracts from earthworms to restrain the formation of thrombi, by comparing six indices of thrombosis including viscosity angle, development time of pro-thrombosis, formation time of a characteristic thrombus, dissolving time of the fibrin thrombus, length of the thrombus and dry weight (31). Cheng studied the effects of different extracts of earthworms on the rates of decomposition of experimental thrombus of rabbits, with whole blood coagulum, blood plasma with platelets and with pure fibrin coagulum of humans. The results showed that crude extracts of earthworms and protein extracts had significant effects in dissolving various kinds of thrombi of rabbit and humans. The thrombus-dissolving function was discovered by directly hydrolyzing fibrin (32).

Effects of earthworms on the cardiovascular system

Shen (1982) reported that earthworm injections (0.5g/ml) could act against arrhythmia, earthworms restraining arrhythmia of various experimental models made with chloroform-adrenaline, ectisine, barium chloride, respectively. It can also cause short conduction stops between the atrium and ventricle, which is not due to K^+ in the agent (33).

Effects of earthworms on the respiratory system

An effective asthma-calming component from earthworms was separated early in the 1930's. This component was used in experiments with rabbit lungs and reported that the component made bronchiectasis. Hence it could be used to resist asthma caused by histamine and pilocarpine. This component was injected venous to the body cavity of experimental animals, 50% of the animals could withstand the lethal dose of histamine (34, 35,36).

Effects on uterus smooth muscle

A kind of substance, which can make the uterus smooth muscle contract, (1964) was separated in 1964. Experimental results showed that this substance significantly increased the tension of the pregnant or non-pregnant uterus (37). In 1982, Xu reported earthworm injections increased contractions of the mouse uterus more than the standard solution of pituitrin (0.01mg/ml) (38).

Anticancer function

Earthworm extracts were used successfully to cure transplanted cancer, in S-180 cell of rats (39) and it made the cancer cells significantly smaller after the treatment for 88 days' enema with a dose of 5mg/ml, without any adverse side-effects (40). Han et al. separated some components by a dialysis method and observed their effects on MGC803 gastric cancer cells in participation of 3H-TdR. The results showed that some earthworm components could inhibit 3H-TdR participation of MGC803 gastric carcinoma ($p < 0.01$), and still had an inhibitory function, even when the component was heated up to 56 °C for half an hour. ($p < 0.05$). This means that the dialysis components of earthworm have a strong heat-resistance on a

limited scale (41-Han, 1991). Further results showed that some other components could directly inhibit the growth of cancerous cells (42,43). Sun et al. (1989) compared the cancer-killing ability of four treatments, including cancer cell suspension, earthworm extract-blood porphyrin derivative- laser, blood porphyrin derivative-laser, and earthworm extracts. The toxicity rate on cancer cells was highest in the treatment with an earthworm extract-blood porphyrin derivative-laser. With the chemical-luminous method, Sun (1991) concluded the mechanism by which the earthworm extract increased the cancer-killing capability of blood porphyrin; derivative-laser at least is by increasing active oxygen (44). A kind of new anticancer medicine, "FU NAI KANG" has been developed in China (45).

Sperm-killing function of earthworms

Succinic acids and hyaluronic acids in earthworm tissue can agglutinate and kill sperms (46-47). Results showed that the earthworms contain 200 ppm of arsenic. This arsenic toxicity can be decreased by washing and is comparatively low in experiments with rabbit, rats and dogs that took enema or were intravenously injected with earthworm extracts. The toxic and side effects are below the level harmful to human health. Sperm-killing function of earthworm can be used for family planning in China (48).

Antidotal action of earthworm and more

The environment where earthworms lived in is full of toxic substances, both chemical and biological sources. It is generally acknowledged that earthworm itself exhibited all sorts of detoxification mechanism. Expression of detoxifying protein is one of the important mechanisms to detoxification. Heavy metal is one of the most common toxic substances in soil, and consider the strong accumulation of heavy metals in the earthworm, it raised high attention on how earthworms resist poison of heavy metal, and detoxification of heavy metals (49). Lumbricus metalchelates (LMT) is one of the most common heavy metal detoxification, which are small cysteine-rich and non-ribosomal peptides that chelate soft metal and metalloid ions, such as cadmium, zinc and arsenic. Among them, it is low molecular weight, rich in cysteine, special non-enzyme protein, which can be induced and combined by a large number of metals. It has characteristics, such as stable space structure, high metal content and hard to be broken down by enzymes. To date, researchers have made some achievements on earthworm LMT in heavy metal detoxification, scavenging free-radicals, tumor pathology. Due to LMT stable space structure, it has strong heat-resistance and not easy to be broken down by enzymes. LMT's metal content is high, it contains 6 to 12 metal atoms per molecule. LMT participates in the storage, transportation and metabolism of trace elements. It functions in antagonizing ionizing radiation, scavenging free-radicals and enhancing the body's response to various stresses. LMT could also be used as a biological antidote, environment monitoring agent of heavy metals, and treatment of metal metabolic disease. With hydro sulphonyl, LMT chelates toxic metals and transfers them out of the body (50).

MT-3 is one of the brain specific members of the MT family. Combined with zinc and copper, it has important

function in neuromodulation. Research suggests that in many organisms, MT plays an important role in the regulation of the basic metal elements, and inhibition and detoxification of some metal elements. MT by binding with heavy metals, is currently the most ideal clinical biological chelating antidote.

In nerve cells, metal elements (such as zinc, Cu, Fe) imbalance can lead to proteins interaction. It may cause the disorder of protein aggregation or eventually metal metabolic disorder disease. For example, Wilson's disease is a congenital Cu savings disorder. The disease is caused by Cu metabolic defect. In treatment of the disease, the effective method was to use zinc compounds for inducing synthesis of MT in the liver and bowel, so as to extend the Wilson's patient life. Due to its scavenging body free-radicals, MT is also added in the cosmetics and anti-aging products. In general, excessive free-radicals products in body often lead to many diseases. Many experiments have proved that MT has protective effect on the nervous system. In the transgenic mice, the expression of MT-1 can change encephalitis, promote restoration of the brain. Therefore, MT-1 is a protective factor for nerve cells. Through ischemia and wound model of rat, it was found that a MT-3 participates in restoration of the central nervous system damage. Parkinson's disease is due to a 6-hydroxy dopamine induced by free-radicals in certain brain by MT isomer inducers, such as oxidative stress, cytokines and inflammatory process. MT can prevent the nerve poison, which has to do with MT scavenging free-radicals (51).

LMT has strong ability of scavenging free-radicals in the body, of a protein, its abilities was much higher than that of SOD in removing hydroxyl free-radicals, and glutathione (CSH) in clearing oxygen free-radical ability. And its strong antioxidant activity can be used as a complement of antioxidants in the body. Ionizing radiation can produce a large number of free radicals. It could directly or indirectly damage organisms. According to a study, it showed that oral MT can prolong survival time of mice with one large dose of ionizing radiation, reduce the immune system damage with one large dose or multiple small doses of ionizing radiation. A large number of Cys, raw material as disulfide bond, can be absorbed when oral MT into the body, for repairing the rupture of radiation effects in the body.

LMT function of scavenging free radicals plays an important role in the process of the anti-aging, anti-oxidative stress and cell apoptosis, etc. LMT is a kind of potential negative regulatory factors in cell apoptosis, and has certain protective effect on the normal body cells in chemotherapy.

Combining with the characteristics of LMT and metal, we have produce LMT by breeding earthworm and plan to use genetic engineering means to establish high expression of LMT system. In order to control heavy metal pollution of soil, it was reported that MT gene of tobacco can significantly improve the absorption of Cu₂ in contaminated soil, in order to restore pollution land of heavy metal (52).

Clinical application of earthworm

Treatment of tracheas and bronchial asthma

Fried earthworm powder was taken orally 3-4 times per day, with 3-4g each time, for treatment of bronchial

asthma (53). Mixtures of earthworm, inner bone of *Sepiella maindroni* and coagulum of *Bambusa textilis* were ground to a fine powder for treatment of bronchial asthma (3, 54). An earthworm preparation, "Chuan-shu-ning pill", was used for treatment of 44 cases of asthma patients, and 84.09% of the patients responded favorably to the treatment. This method is characteristic of lasting and moderate antiasthma (55). A single earthworm injection was used to treat 275 cases of bronchial asthma and asthma bronchitis and 78% of patients recovered fully, especially children, the therapeutic effects were better than for adults (56). According to a report by Huang Wenda, a 30% earthworm injection was used to treat children sudden asthma and adult stubborn asthma with a single dose of 0.1-2ml for children and 2ml for adults, once a day when the asthma broke out. After 10 to 30 minutes' treatment, the breathing became smoother. The special sounds of asthma eased, phlegm was produced easily. With two to four treatments, the symptoms of asthma disappeared entirely (2, 57). Usually a 1ml earthworm extract preparation (equal to 1g earthworm) for adults was used as intramuscular injections on the first day and 2ml per day for a second dose if no side effects appeared on the first day. Ten-day period was regarded as a course of treatment (58). Some reports said earthworm mixtures from several species were better than single species earthworm preparations, in curing of 101 hot asthma cases. A dose of 2ml intramuscular injection per day, once every other day, resulted in 88.1% of the patients responding to the treatment in one to two weeks (59). A mixture of earthworm and toad powder was used to treat 107 cases of chronic tracheitis, and the rate of efficacious treatment reached 92.5%. The results of germ culture and bacterial checks showed that the earthworm tissue components were effective in controlling inflammation and repairing mucosa membranes. Earthworm powder was used in treatment of 100 cases of children with asthma, and the therapeutic effects were good, especially for hot asthma (60).

Treatment of epilepsy

An earthworm pill, a secret recipe handed down from generation to generation, which was composed mainly of earthworms (*A. caliginosa*) and vermilion. The pill looked like green germ and was wrapped up in goldleaf. Tests over 50 years confirmed its therapeutic effects against epilepsy. An earthworm mixture soup was suitable to treat epilepsy. This consisted of earthworms 9g, whole scorpions 5g, and dry fruit of *Forsythia suspensa* 9g, flowers of *Loniceraesquirolia* 12g, stems of *Uncaria hynchophylla* 15g and plaster stone 30g. A 50% earthworm injection was used intramuscularly to control epilepsy attacks, 3 to 5 times, and 85.7% cases was controlled effectively (61). Zhu concocted a material medica of earthworms 3 to 6g, used once a day, to treat 20 cases of partial epilepsy, and whom was coordinating with chemical treatments, 16 cases recovered fully, 3 cases were improved and in one case there was no effect (62). Zhang used another earthworm soup to treat 12 cases of epilepsy, dosed once a day for 10 to 20 days. In four cases there were no epilepsy attacks for one year, in five cases there were no epilepsy attacks for half a year, and there were obvious decreases in attack times in the other patents (17).

Treatment of high blood pressure

An earthworm tincture, applied twice a day at 20ml per dose, was used to cure 34 cases of hypertension in patients whose treatments with other medicines had low effects. High blood pressure was usually reduced within 4 to 10 days by this earthworm treatment (63). An earthworm extract, named "Earth dragon B1" was used 3 times per day and 2 ml once, to treat 11 cases of hypertension; the results showed an effective ratio of suppression of 90.9% without any obvious side effects. An earthworm mixture soup was also used to treat 17 cases of hypertension with very good therapeutic effects (64, 65). A main ingredient extracted from earthworm was injected intramuscularly to cure high blood pressure, and an 86.6% improvement rate occurred in 30 cases, depressor function showing in various phases of hypertension and were better than chemical treatments to control high blood pressure (66).

Treatment of schizophrenia

It was reported that earthworms were used to treat 110 cases of schizophrenia, which were divided into two groups, 60 cases in the first group with treatment of earthworm powder and 50 cases in the second group with treatment with an earthworm injection. During a 60-day treatment course, 18 patents showed improved condition in the first group and 11 in the second group. Fresh earthworms were more effective than dried ones. A batch of 300 fresh earthworms was stewed with eggs and eaten as a dish to cure schizophrenia (67).

Treatment of leg ulcers

An earthworm ointment for external use, «Xin-fu-shuang» in Chinese, was used to treat 50 cases of leg ulcers, 17 cases recovered fully and 37 cases improving. It functioned to stop pain, to depose of rotten tissues, remove pus and improve the growth of muscle buds (68). A syrup, made up of earthworms and powdered sugar, was put on the ulcers with good treatment results (4). Earthworms were also used orally to cure leg ulcers. The method was to rinse earthworms with cold water and wrap up in a skin of soya-bean milk. This was taken before dinner. A patient took 300 earthworms and recovered fully (69).

Treatment of mumps

Earthworms were rinsed and put into a container, and the same amount of powder sugar added to the container, and the earthworms submerged. The earthworms gradually secreted yellow-white mucus and this mucus was put on to affected parts covering them with gauze. The mucus was changed once every 2 to 3 hours. Observation of 20 cases showed that was a better method of treating mumps because of its fast detumescence and antifever effects. In another report, 170 cases of mumps were cured fully within 1 to 3 days using this method (70).

Treatment of eczema, urticaria and anaphylaxis diseases

Earthworm tissue extracts were used to treat 35 cases of eczema with injections at acupoints and 14 patients recovered fully, 13 improved, 5 responded to treatment and in 3 patients there were no effects. A sample of 60g earthworms was mixed with 30g sugar. And the mix was put on the affected part, 4 to 5 times daily. This method was used to treat skin clonic itches and repeated eczema attacks

(71). Earthworm injections were used to treat 100 cases of urticaria. Patients were treated once per day with 2ml per treatment, resulting in an 84% of cure rate (72). Another 50 cases of treatment with this method showed that 15 patents were recovered fully, 24 improved, 9 responded to the treatment and in 2 there were no effect (73). A liniment of earthworms and glycerin extract was applied externally to treat strip bleed. In all 9 cases, 3 patients were cured fully in three days; another recovered in four to ten days. Fresh earthworms were pounded with chive roots until it became pulped. The earthworm pulp was stirred evenly with sesame oil. It was reported that 26 cases of strip bleed recovered fully using this method. Earthworms can also be used to treat regular lupus erythematosus. The method used was to mix fresh earthworms with powder sugar and make an infusion. The infusion was applied to affected places and 15 cases were cured with 2 to 4 treatments. A formula made up from the whole bodies of *Pheretma aspergillum* or *Allolobophora caliginosa trapezoides*, roots of *Rehmannia glutinosa* and root of *Paeonia suffruticosa* were used satisfactorily to treat some anaphylaxis diseases (74, 75).

Treatment of chronic prostatitis

232 chronic prostatitis patients were treated with earthworm mixtures, and the therapeutic result was that 128 cases healed fully, 62 cases were improved and 42 cases responded to treatment but relapsed when the treatments stopped. This prescription was made up of dry earthworms of *Pheretma aspergillum* or *Allolobophora caliginosa trapezoides* 20g, roots of *Paeonia suffruticosa* 20g, stem of *Aristolochia manshuriensis* 15g, seeds of *Plantago aristata* 15g, roots of *Astragalus membranaceus* 30g and 10g roots of *Glycyrrhiza uralensis* (11).

Treatment of burns and scald

A sample of 15 earthworms were put into a sugar solution and soaked for 10 hours to produce an infusion. Using this earthworm infusion on the wounded surface, 50 cases of burns and scalds (I to II°) recovered fully in one week (76). According to another record, mixtures of dried earthworms 120g, sugar 60g and a little borneol were stirred into an earthworm-sugar solution. The solution was put on the burned or scalded limb, 4 to 6 times for one day, and the wound usually recovered in 5 to 7 days, without any scars and other harmful effects (77). Burn ointment 101, a Chinese traditional medicine, contains tissues of *Allolobophora caliginosa* and other medicinal herbs. Application to 5011 cases of burns and scalds (first and second degree) showed that 98.7% of wounds recovered fully. In 32 cases of serious burns and scalds, 23 were completely cured (78).

Treatment of fractures

Using earthworms to treat 63 cases of femur fracture, the pain stopped within one hour of treatment, tumescence disappeared within 24 hours and the bone grew well over an average of 38.7 days. Li observed 264 cases of new fractures of femur stem (within 7 days) and found that the healing time of fractures was 3.6 days earlier after earthworm treatments compared to that after other treatments ($p < 0.05$). Li considered that this effect might relate to lipid and nucleic acids in the earthworm bodies (79, 80).

Treatment of erysipelas

A mixture of fresh earthworms tissues and red sugar was applied to affected parts with erysipelas, to cure 11 patients in 3 to 5 days (81). Treatment of sequelae of encephalitis B. Fresh earthworms were stewed into a soup. The soup was taken orally to treat 10 cases of sequelae of encephalitis B, 30 days, as a treatment, and satisfactory therapeutic results obtained (82).

Treatment of chronic lumbago

An earthworm powder, made up of earthworms and *Caesalpinia sappan*, was used to treat 50 cases of chronic lumbago. In 50 patients, 48 cases recovered fully. In treatment of skin crevices, another skin-nursing ointment, made up of earthworm powder and pearl powder, was used to treat skin crevices, twice per day. In records of 42 cases of treatment, 41 cases recovered fully (83).

Treatment of blood-deficiency apoplexy

Guo reported that extracts from fresh earthworms were used to treat 381 cases of blood-deficiency apoplexy, with an average effective cure rate being 79% (84).

Treatment of acute injury of soft tissues

The prescription for treatment of acute injuries of soft tissues consists of 3g dried bodies of *Pheritma aspergillum* or *Allolobophora caliginosa trapezoides*, 3g central stem of *Casalpinia sappan*, 3g dry fruit of *Daemonorops draco* spp, 3g plant of *Oynanchum chinense*, 3g dry fruit of *Forsythia suspensa*, 3g root-tuber of *Aconitum carmichaeli*, 3g dry fruit of *Zanthoxylum bungeanum*, 9g dry flower of *Carthamus tinctorius* and 9g stem of *Cinnamomum camphora*. This medicinal earthworm/herb mixture was soaked in 500ml of 75% Ftorl solution for 48 hours. The infusion was applied directly to the affected part(s), 3 to 4 times daily. Of 122 cases of acute injury of soft tissues in this way, 104 cases recovered fully and 18 cases improved. The cure took from 5 to 70 days (85).

Treatment of vertigo

An earthworm tissue mixture soup was used to treat vertigo. Of 32 treatments, 20 cases were cured fully, 7 proved, 2 responded to the treatment and 3 had no effects (86).

Treatment of hematemesis and hematuria

A batch of 50 fresh earthworms was mixed with 250g red sugar, and a yellow secretion emerged from the body pores of earthworms. Patients took this secretion orally 20ml per treatment and stopped spitting blood within 2 hours. The disease healed fully when a dose of 100ml of the secretion was taken (87,88).

Treatment of digestive ulcer

A dried earthworm powder was taken orally to treat 40 cases of digestive ulcers, 2g per dose and 3 to 4 times per day, with 34 cases that recovered fully and 6 cases improved (89).

Treatment of vesical calculus

Chen reported that earthworms were applied to treat 5 cases of cystolith with significant therapeutic effects (90).

Treatment of cancer

Earthworm mixture soups termed numbers I, II and III were taken orally for 2 to 4 months to treat 44 cases of cancer patients. The average cure rate was 89.9%, which suggested that this prescription was workable in both theory and clinical practice (91).

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