Bugs as Drugs, Part Two: Worms, Leeches, Scorpions, Snails, Ticks, Centipedes, and Spiders

E. Paul Cherniack, MD

Abstract
In this second of a two-part series analyzing the evidence for the use of organisms as medicine, the use of a number of different "bugs" (worms, leeches, snails, ticks, centipedes, and spiders) is detailed. Several live organisms are used as treatments: leeches for plastic surgery and osteoarthritis and the helminths *Trichuris suis* and *Necator americanus* for inflammatory bowel disease. Leech saliva is the source of a number of anticoagulants, including the antithrombin agent hirudin and its synthetic analogues, which have been approved for human use. Predatory arthropods, such as certain species of snails, spiders, scorpions, centipedes, and ticks provide a trove of potential analgesic peptides in their venom. A synthetic analogue of a snail venom peptide, ziconotide, has been approved for human use and is used as an alternative to opioids in severe pain cases. Arthropods, such as ticks, have venom that contains anticoagulants and centipede venom has a protein that corrects abnormalities in lipid metabolism.
(*Altern Med Rev* 2011:16(1):50-58)

Introduction

Part one of this series delineated the use of insects as a source of medical therapy. However, many other organisms are present on earth in vast quantities and, like insects, have been used globally in traditional medicine for centuries (Figure 1). In some cases, whole live arthropods or extracts have been utilized; in others, individual substances have been refined. In addition, as with insects, other organisms might potentially be a source of new medicines or be more readily available in some parts of the world than conventional medicines.

Leeches

Leeches have been used to treat human illness for centuries. The tombs of Egyptian pharaohs contained pictures of leeches, and descriptions of leech medical treatments appear in ancient Greek and Roman texts.[1-3] The popularity of the use of leeches in the 18th and 19th century in Europe caused them to become scarce.[1,4]

Leeches are placed on the skin and guided to the desired site by applying a sucrose solution.[5] They can be prevented from migrating by cutting a hole in the center of a piece of gauze, where the head of the leech is inserted. Leeches are then allowed to feed for 10-20 minutes, after which they stop on their own and detach from the site. Although a leech only consumes about 5 mL of blood, once they are removed they can cause leakage of blood from the host for a number of hours.[5]

Wound Healing

Leeches have been used to aid wound healing after plastic surgery. In certain parts of the world, leeches are commonly applied to treat venous congestion at surgical wound sites. A questionnaire given to 62 plastic surgery units in Great Britain and Ireland found that 80 percent had used leeches 10 times per year on the average.[2] The majority of surgical units provided patient education prior to application, and in only 10 percent of cases did patients refuse leeches. Safety precautions included written protocols about the use of leeches, wound disinfection prior to use, nurse monitoring (including remaining during the whole course of treatment), and leech counting before and after use to make sure none remained in the wound.

Leech therapy is appropriate in plastic surgical situations in which there is more arterial repair than venous repair, such as fingers, auricles, and skin flaps.[1] Leeches have also been used after breast surgery to relieve the possible complication of venous congestion at the nipple.[6] An additional use of leeches in plastic surgery is in the treatment of ring avulsion injuries.[7]

E. Paul Cherniack, MD – The Geriatrics Institute, University of Miami Miller School of Medicine, Division of Geriatrics and Gerontology, and the Geriatrics and Extended Care Service and Geriatric Research Education, and Clinical Center (GRECC) of the Miami Veterans Affairs Medical Center
Correspondence address: Miami VA Medical Center, Room 1D200, 1201 NW 16th Street, Miami, FL 33125
Email: evan.cherniack@va.gov

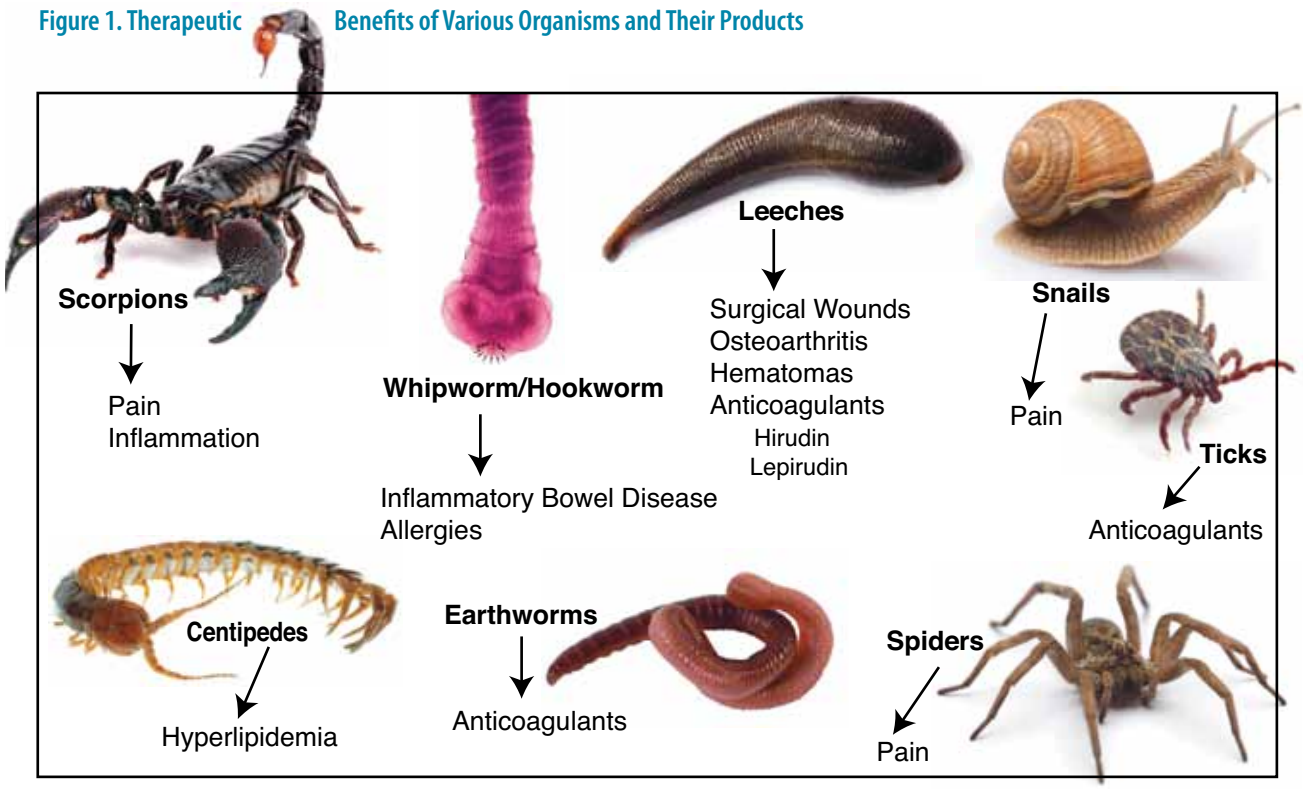

Figure 1. Therapeutic Benefits of Various Organisms and Their Products

While there are many descriptions of leech use, there are no published clinical trials of the efficacy of leeches in surgery. Leech therapy can also cause infections, which occur in 2.4-20 percent of plastic surgical repairs.[8] *Aeromonas hydrophila*, a symbiote found in the leech gut that aids in blood digestion, is the most common organism found in these infections.[8-11]

Post-phlebitis Syndrome

Leeches have also been used to alleviate post-phlebitis syndrome, in which venous valves are obliterated by a deep vein thrombosis.[1] In an uncontrolled case series, 40 patients had 7-12 leeches placed on their legs every 3-4 weeks. After leech treatment, 70 percent of subjects claimed they could walk further, 52 percent stated they had less pain, 40 percent had better leg skin color, and 12 percent had reduced leg swelling. There were no infections or significant blood loss.

Key words: arthropod, insect, leech, worm, snail, tick, scorpion, centipede, spider, helminth

Osteoarthritis

Leeches can treat osteoarthritis pain. In a small pilot trial, four leeches were applied for 60-90 minutes to the joints of 10 subjects (mean age 69) with osteoarthritis of the knee, while six control subjects did not receive leeches.[12] All subjects were able to have physical therapy and take pain medication as they desired. One month later, the subjects who received the leeches had a significantly lower pain score (1.3 on a 0-10 scale, $p<0.001$), while those not receiving leeches did not improve.

In a second study, 51 subjects with knee osteoarthritis were provided either leech therapy or topical diclofenac.[13] Subjects assigned to leech application were treated as in the pilot study; control subjects applied diclofenac gel to their knees twice a day for 28 days. Subjects rated their symptoms using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scale for pain, stiffness, and function. Those individuals who received leech therapy had a 12-point better score than those who applied diclofenac ($p=0.0317$). After one week, there was a significant difference between groups in pain score; but, by the end of three months, the differences in scores were no longer significant.

In an additional investigation, 113 individuals with knee osteoarthritis were divided into three groups: (1) subjects received a single application of leeches, (2) subjects received two leech treatments,

and (3) subjects received a simulated leech therapy consisting of a needle prick and a wet gauze wrapped up to simulate the size and shape of a leech.[14] Both leech therapy groups had significantly better pain scores on the WOMAC scale than the control group. Adverse effects of leech therapy were not observed in any of the three osteoarthritis studies.

Therapeutic Substances from Leeches

The therapeutic capacity of the leech is based on secreted anticoagulants that are released while feeding. Specific substances have been refined and utilized in situations where it is necessary to prevent thrombosis.[15] Unlike conventional anticoagulants, these anticoagulants have the advantage of being selective for specific clotting factors, without affecting others. At least 14 different anticoagulants have been obtained from leeches.[1]

Hirudin, which is a specific inhibitor for thrombin, was initially isolated from the leech salivary gland.[15] It has been applied in the treatment of coronary ischemic syndromes, heparin-induced thrombocytopenia, and disseminated intravascular coagulation (DIC). Probably the most widespread use in clinical investigation has been its use to treat coronary ischemia.[16] In the Organization to Assess Strategies for Ischemic Syndromes (OASIS) and Gusto IIB trials, hirudin was compared with a conventional agent, heparin, in the treatment of acute coronary syndromes. Subjects receiving hirudin in the OASIS trial had a small but statistically significantly lower number of cardiac events compared to heparin-treated subjects (6.5% versus 4.4%); however, a day after therapy, there was a large rise in events in hirudin-treated subjects.[17] In the Gusto trial, there were comparable reductions of coronary events using both drugs, but individuals receiving hirudin were more likely to achieve therapeutic serum anticoagulation targets.[18] Hirudin was compared to heparin in a series of trials – Thrombolysis and Thrombin Inhibition in Myocardial infarction (TIMI).[16] Both drugs resulted in a similar reduction in coronary events and a comparable number of adverse events.

Hirudin has been produced synthetically and used in the treatment of deep venous thrombosis and for coronary angioplasty preparation. There have been small clinical trials with modified subcutaneous versions of the molecule, lepirudin, for these conditions.[19-21] In two uncontrolled trials of lepirudin for the resolution of heparin-induced thrombocytopenia, 88.7- and 92.6 percent of the individuals tested had restoration of normal platelet levels. However, there was no lower risk of bleeding in these subjects than in historical controls.[21] In a multi-center investigation of 155 individuals with deep vein thrombosis, lepirudin-treated subjects had fewer new pulmonary emboli than heparin-treated subjects (3% for 1.25/mg/kg/12h lepirudin versus 27% for heparin).[22] Lepirudin and another modified hirudin, desirudin, have been produced by yeast and used as anticoagulants in subjects with renal insufficiency.[23]

A topical cream manufactured from hirudin was used to shrink hematomas caused by musculoskeletal injuries.[24] Sixty individuals (ages 18-65), who had a bruise created by musculoskeletal injury, and pain, warmth, or discoloration rated greater than 50 on a 0-100 visual analogue scale, were randomized to the hirudin or a placebo cream for five days. Although both creams resulted in a significant reduction in hematoma symptoms, the hirudin cream resulted in a larger absolute reduction in visual analogue scale score.

Worms

Earthworms provide another source of medicinally-useful products. Baked earthworms with bread have been used to treat kidney stones. Burnt earthworm ashes mixed with rose oil have been used to alleviate alopecia and dried earthworms were historically ingested to relieve jaundice in Iran.[25] North American Indians applied dead earthworms topically to relieve arthralgia and treat injuries.[25] Earthworm powder also reduced alcohol-induced elevations in liver enzymes in rats with alcoholic hepatotoxicity.[26]

Fibrinolytic Effects

Earthworm powder mixed with coconut milk has been used as a medicine in the Far East to treat infections and as a fibrinolytic agent.[25] Earthworm powder fed to rats increased partial thromboplastin (aPTT) and thrombin times (TT).[27] Dried powder from *Lumbricus rubellus* fed to rats with an arterio-venous shunt decreased the weight of the thrombus.[28]

Earthworms provide a trove of anticoagulants.[25] One is lumbrokinase, an array of enzymes that degrade protein, such as plasminogen activator and plasmin. Lumbrokinase degrades experimental clots in rodents and rabbits. A factor Xa inhibitor, eisenstasin-derived small peptide (ESP), has been isolated from the midgut of the *Eisenia andrei* worm.[29] Six other fibrinolytic enzymes and a

glycolipoprotein complex that includes serine peptidases have also been identified.

These anticoagulant products may play a role in the treatment of ischemic events. In a pilot trial, 10 patients with stable angina were given 600,000 units of oral lumbrokinase three times daily for one month. Participants had pharmacologic stress tests before and after treatment and 39 percent showed improvement in summed stress scores based on technetium scans; the statistical significance of the result was not provided in the report.[30]

Antimicrobial/Antineoplastic

Earthworms have a rudimentary immune system and contain antimicrobial and antineoplastic substances.[25] The bacteriolytic substances include fetidins (which have serine proteases and promote clots), lysenins, lumbricin, eiseniapore, coelomic cytolytic factor (CCF-1), and erythrocytolytic proteins. Some of these agents have been tested for antineoplastic potential. One substance, eisenin, destroyed human neoplastic cells from several human cancer cell lines.[25]

Inflammatory Bowel Disease

Other worms may also contain medicinally valuable molecules. In a mouse experimental model of colitis, motility disruption was improved with a homogenized protein extract from the worm *Schistosoma mansoni*.[31]

Populations in sub-Saharan Africa have a relatively low incidence of inflammatory bowel disease.[32] It has been theorized, based on epidemiological studies (some of which include African emigrant populations), that chronic intestinal helminth infection may be at least in part responsible. Helminth infection has been postulated to upregulate the CD4+ subset of T-regulatory cells that suppress inflammation. In a patient who developed ulcerative colitis after treatment with a helminth infection, increased populations of T-regulatory cells were found in infected colonic regions.[33] A helminth infection might also stimulate mast and goblet cells, causing excess mucus and water to enter the gut lumen and potentially suppressing inflammatory bowel disease.[34]

The helminth *Trichuris suis* (a whipworm parasite in pigs) was given to human subjects to deliberately induce infections in order to treat inflammatory bowel disease. Twenty-nine subjects (ages 18-72) swallowed 2,500 live *T. suis* ova every 21 days for six months.[35] The progression of Crohn's disease (CD) was measured with the Crohn's Disease Activity Index (CDAI). A CDAI score of 220-450 was an inclusion criterion for participation (>450=severe disease; <150=remission). After three weeks, 75 percent had a response (reduction in score by at least 100 points). By week 12 the respondents' mean score was reduced by two-thirds ($p<0.0001$).

In a preliminary study, three patients with CD and four people with ulcerative colitis ingested one dose of *T. suis* ova. These individuals were monitored every other week for three months and were able to tolerate helminth treatment safely.[36] In an additional investigation, 54 persons with ulcerative colitis ingested 2,500 *T. suis* ova or a placebo every two weeks for three months. Intention-to-treat analysis indicated a 43.3-percent successful response (decrease in the Ulcerative Colitis Disease Activity Index by at least four points) in *T. suis* treated subjects and a 16.7-percent response in the placebo group ($p=0.04$); the treatment caused no adverse effects.[37] Although theoretical concern has been raised that helminthic treatment might lead to invasion beyond the gut into the lymphatics and vasculature, this has not been observed in clinical trials.[38] One possible solution might be to treat patients with antihelminthic therapy at the end of the course of worm treatment.[39]

Allergies/Asthma

Therapeutic helminth infections might be used to treat allergies. Epidemiological evidence suggests that populations in which helminth infections are prevalent have lower incidences of allergies, and studies show benefit in animal models.[40] Helminth therapy may augment production of interleukin-10 (IL-10) and transforming growth factor-beta (TGF-β), increase the number of T-regulatory cells, enhance the production of polyclonal IgE that occupies binding sites on mast cells, and prevent granulocyte signaling mechanisms.[40] In a preliminary dose-ranging trial for asthma treatment, 10 healthy subjects drank water with different doses of the hookworm *Necator americanus* larvae for three months.[41] Serum from the individuals was measured for IgE and IgG levels, and stool was examined for egg content. All doses (from 10-100 larvae per person) resulted in quantifiable fecal egg counts, transient increases in serum IgE and eosinophil counts, and a gradual rise in IgG. Subjects experienced adverse reactions: nine had abdominal pain and diarrhea, four reported malaise, and two developed a cough or wheezing. At the end of the trial, all were

successfully rid of worms using an antiparasitic drug. One hundred persons with an allergy to grass pollen took eight doses of 2,500 *T. suis* ova or a control substance for three weeks.[42] Allergic symptoms were not improved in the treatment group and one-third of those individuals developed diarrhea.

Scorpions

There are 13 families and approximately 1,400 species and subspecies of scorpions. Most of the medical research to date has been on just two of these, the Chinese scorpion (*Buthus martensii* Karsch (BmK), which has subsequently been renamed *Mesobuthus martensii*) and the Indian black scorpion (*Heterometrus bengalensis* C.L. Koch).

Venom, extracts, scorpion tails, or even whole scorpions from the Chinese scorpion have been used in traditional Chinese medicine to relieve pain and treat meningitis, epilepsy, stroke, and rheumatic diseases.[43,44] Extracts of Chinese scorpion venom contain numerous small neuroactive peptides, protease inhibitors, phospholipase, hyaluronidase, and mucopolysaccharides that have anti-inflammatory properties, inhibiting nitrous oxide and interleukin-1β in human chondrocytes.[45,46]

At least five peptides have been identified from Chinese scorpion venom that have anti-inflammatory and antinociceptive properties.[43] One peptide, J123, blocks potassium channels that activate memory T-cells.[47] The venom also contains a 61-amino acid peptide that has demonstrated antiseizure properties in an animal model[44] as well as other constituents that act as analgesics in mice, rats, and rabbits.[48-54] The polypeptide BmK IT2 stops rats from reacting to experimentally-induced pain.[55] A protein called BmBKTx1 created from Chinese scorpion venom prevents the breakdown of the tumor suppressor protein p53,[56] and another called "AGAP" retards the growth of intraperitoneal tumors in mice.[52] Chinese scorpion venom contains a hyaluronidase, which was used to suppress hyaluronan, a cell adhesion factor that promoted metastasis in an *in vitro* human breast cancer cell line.[57]

A protein from the Indian black scorpion, bengalin, caused human leukemic cells to undergo apoptosis *in vitro*.[58,59] Bengalin also improved biochemical markers of osteoporosis in female rats.[60,61] The peptide chlorotoxin, found in the venom of the scorpion *Leiurus quinquestriatus*, retarded the activity of human glioma cells *in vitro*.[62]

Venom from the Venezuelan scorpion, *Tityus discrepans*, has antimicrobial and antiparasitic activity. The venom inhibited the growth of Leishmania species *in vitro*.[63] Alpha-toxin found in the venom retards inactivation of sodium channels at the neuromuscular junction that might enhance neuromuscular reflexes and airway contraction, theoretically making it a suitable treatment for sleep apnea.[64]

Snails

Snails, first described as medication during the Roman Empire, have had multiple medical uses, including treatment for mental illness, syncope, vertigo, and infectious diseases.[65] Predatory sea snails produce peptides known as conotoxins that target specific cellular ion channels, such as N-type Ca^{++} channels. They may be useful as analgesics and cardioprotective agents.[66,67] Ziconotide is a synthetic equivalent to a 25-amino acid peptide derived from snail venom that is administered intrathecally as an alternative to opioids in cases of severe pain. When 220 subjects with chronic pain were randomized to receive either ziconotide or a placebo drug for three weeks, those receiving ziconotide had a significantly greater reduction in visual analog scale score (a mean percentage reduction of 14.7% versus 7.2% for those given a placebo, p=0.036). In a long-term, open-label pain trial, the most frequent side effects were memory impairment (11.3% of 78 patients studied for up to three years), and dizziness, nystagmus, and slurred speech (8.5% for each).[68] Other snail peptides, such as conantokin, leconotide, and conotoxin MVIIA, have been successfully used in rat pain models.[69,70]

Snail toxin peptides have also been used as agents to treat ischemia. CGX-1051 given to rats, rabbits and dogs reduced the size of infarcts in experimentally-induced ischemia.[71,72]

Centipedes

The Chinese red-headed centipede, *Scolopendra subspinipes mutilans* L. Koch, is a predatory arthropod that has been used in traditional Far Eastern medicine. The potential benefits of Chinese red-headed centipede products on lipids was tested when a substance, centipede acidic protein (CAP), was isolated from an alcohol extract comprised of powdered, dried Chinese red-headed centipedes and injected into rats.[73] Rodents receiving CAP had lower levels of cholesterol, triglycerides, and LDL than saline-injected control animals. Chinese red-headed centipede venom is also the source of two peptides, scolopin 1 and 2, that are antibacterial *in vitro*.[74]

Table 1. Summary of Research on Medical Benefits of Arthropods and Annelids

Treatment	Potential Uses	Human Studies	Randomized Controlled Trials
Leeches	Osteoarthritis, deep venous thrombosis, wound healing after plastic surgery	√	√
Earthworms	Antimicrobial		
Helminths	Inflammatory bowel disease	√	√
Snail venom	Analgesia		
Centipede venom	Hyperlipidemia		
Spider venom	Analgesia		
Tick venom	Vascular and thrombotic disease	√	

Spiders

Spider venom contains an array of peptides and other compounds that include substances that alter neuronal sodium, calcium, and potassium channels and glutamate and acetylcholine receptors.[43] A peptide from the Chinese rose tarantula spider (*Grammostola spatulata*), GsMTx4, blocked pain reactions in rat experimental pain models.[75] Psalmotoxin 1, another peptide obtained from the South American tarantula (*Psalmopoeus cambridgei*), acts on encephalin and opioid receptors in mice to mediate analgesic effects.[76]

Ticks

Ticks are a source of anticoagulants. Contact phase inhibitor is a protein from the tick *Ixodes ricinus* saliva that *in vitro* inhibits human kallikrein and factors XIa and XIIa, and increases aPTT.[77] Contact phase inhibitor reduces venous and arterial thrombi and protects against embolic events in rodents. Another protein, ixolaris, found in salivary glands of the tick *Ixodes scapularis*, blocks the action of factors X and Xa in human *in vitro* models and decreases the formation of venous thrombi in rodents *in vivo*.[78] A protein that acts as a factor Xa inhibitor from the tick *Ornithodoros moubata* has been used in experimental femoral artery balloon angioplasties in rabbits.[79]

Future Directions and Conclusions

Several live organisms, such as leeches and helminthes, have shown a remarkable versatility to treat surgical infections, inflammatory bowel disease, and allergies. Newer applications of such organisms and their products have been proposed. *N. americanus*, for example, might be used to treat inflammatory bowel disease, which could be more easily utilized than *T. suis* because as few as 10 ingested larvae may have a systemic effect.[39] Worm extracts and antigens, such as cystatin, ES-62, *Dirofilaria immitis* derived antigen, thioredoxin peroxides, *Ascaris suum* suppressive protein, *Nippostrongylus brasiliensis* excretory-secretory products, and *A. suum* extract might also potentially be used as allergy treatments.[40]

There is a wealth of possible medicinal substances derived from a variety of arthropods or annelids. At least 10 have been identified from leeches, 30 from earthworms, 40 from scorpions, and thousands from snails.[1,25,46,67] Two substances,

the anticoagulant hirudin and the analgesic analogue of the snail venom peptide ziconotide, have been approved by the U.S. Food and Drug Administration for human use. Other anticoagulants in leech saliva that might ultimately prove to have clinical use include destabilase, which breaks down fragmin, and seven other substances that prevent Factor Xa action, including antistasin.[1] Leech saliva also contains several antiplatelet agents, such as apyrase, which breaks down ATP, collagenase, hyaluronidase, and eglin.[1]

Currently, efforts are underway to isolate newer and smaller molecules from these organisms or create synthetic versions that have similar or enhanced medicinal properties. Several new peptides are being designed that have hirudin's antithrombin activity, and a new analgesic spider venom peptide that acts on a specific pain receptor (PX23) has recently been identified.[80,81] A summary of medicinal benefits can be found in Table 1.

References

1. Eldor A, Orevi M, Rigbi M. The role of the leech in medical therapeutics. *Blood Rev* 1996;10:201-209.
2. Whitaker IS, Izadi D, Oliver DW, et al. *Hirudo medicinalis* and the plastic surgeon. *Br J Plast Surg* 2004;57:348-353.
3. Papavramidou N, Christopoulou-Aletra H. Medicinal use of leeches in the texts of ancient Greek, Roman and early Byzantine writers. *Intern Med J* 2009;39:624-627.
4. Massart D, Sohawon S, Noordally O. Medicinal leeches. *Rev Med Brux* 2009;30:533-536. [Article in French]
5. Abdelgabar AM, Bhowmick BK. The return of the leech. *Int J Clin Pract* 2003;57:103-105.
6. Gross MP, Apesos J. The use of leeches for treatment of venous congestion of the nipple following breast surgery. *Aesthetic Plast Surg* 1992;16:343-348.
7. Tuncali D, Terzioglu A, Cigsar B, Aslan G. The value of medical leeches in the treatment of class IIC ring avulsion injuries: report of 2 cases. *J Hand Surg Am* 2004;29:943-946.
8. de Chalain TM. Exploring the use of the medicinal leech: a clinical risk-benefit analysis. *J Reconstr Microsurg* 1996;12:165-172.
9. Mory RN, Mindell D, Bloom DA. The leech and the physician: biology, etymology, and medical practice with *Hirudinea medicinalis*. *World J Surg* 2000;24:878-883.
10. Ardehali B, Hand K, Nduka C, et al. Delayed leech-borne infection with *Aeromonas hydrophilia* in escharotic flap wound. *J Plast Reconstr Aesthet Surg* 2006;59:94-95.
11. Lineaweaver WC. *Aeromonas hydrophila* infections following clinical use of medicinal leeches: a review of published cases. *Blood Coagul Fibrinolysis* 1991;2:201-203.
12. Michalsen A, Moebus S, Spahn G, et al. Leech therapy for symptomatic treatment of knee osteoarthritis: results and implications of a pilot study. *Altern Ther Health Med* 2002;8:84-88.
13. Michalsen A, Klotz S, Ludtke R, et al. Effectiveness of leech therapy in osteoarthritis of the knee: a randomized, controlled trial. *Ann Intern Med* 2003;139:724-730.
14. Andereya S, Stanzel S, Maus U, et al. Assessment of leech therapy for knee osteoarthritis: a randomized study. *Acta Orthop* 2008;79:235-243.
15. Markwardt F. Hirudin as alternative anticoagulant – a historical review. *Semin Thromb Hemost* 2002;28:405-414.
16. van de Loo A, Bode C. Hirudin in acute coronary syndromes. *Semin Thromb Hemost* 2002;28:459-466.
17. No authors listed. Comparison of the effects of two doses of recombinant hirudin compared with heparin in patients with acute myocardial ischemia without ST elevation: a pilot study. Organization to Assess Strategies for Ischemic Syndromes (OASIS) Investigators. *Circulation* 1997;96:769-777.
18. Cannon CP, Braunwald E. Hirudin: initial results in acute myocardial infarction, unstable angina and angioplasty. *J Am Coll Cardiol* 1995;25:30S-37S.
19. Deitcher SR. Clinical utility of subcutaneous hirudins. *Am J Health Syst Pharm* 2003;60:S27-S31.
20. Sohn JH, Kang HA, Rao KJ, et al. Current status of the anticoagulant hirudin: its biotechnological production and clinical practice. *Appl Microbiol Biotechnol* 2001;57:606-613.
21. Lubenow N, Greinacher A. Hirudin in heparin-induced thrombocytopenia. *Semin Thromb Hemost* 2002;28:431-438.
22. Schiele F, Lindgaerde F, Eriksson H, et al. Subcutaneous recombinant hirudin (HBW 023) versus intravenous sodium heparin in treatment of established acute deep vein thrombosis of the legs: a multicentre prospective dose-ranging randomized trial. International Multicentre Hirudin Study Group. *Thromb Haemost* 1997;77:834-838.
23. Fischer KG. Hirudin in renal insufficiency. *Semin Thromb Hemost* 2002;28:467-482.
24. Stamenova PK, Marchetti T, Simeonov I. Efficacy and safety of topical hirudin (Hirudex): a double-blind, placebo-controlled study. *Eur Rev Med Pharmacol Sci* 2001;5:37-42.
25. Cooper EL, Ru B, Weng N. Earthworms: sources of antimicrobial and anticancer molecules. *Adv Exp Med Biol* 2004;546:359-389.

26. Prakash M, Gunasekaran G, Elumalai K. Effect of earthworm powder on antioxidant enzymes in alcohol induced hepatotoxic rats. *Eur Rev Med Pharmacol Sci* 2008;12:237-243.
27. Hahn BS, Jo YY, Yang KY, et al. Evaluation of the *in vivo* antithrombotic, anticoagulant and fibrinolytic activities of *Lumbricus rubellus* earthworm powder. *Arch Pharm Res* 1997;20:17-23.
28. Kim YS, Pyo MK, Park KM, et al. Dose dependency of earthworm powder on antithrombotic and fibrinolytic effects. *Arch Pharm Res* 1998;21:374-377.
29. Joo SS, Won TJ, Kim JS, et al. Inhibition of coagulation activation and inflammation by a novel Factor Xa inhibitor synthesized from the earthworm *Eisenia andrei*. *Biol Pharm Bull* 2009;32:253-258.
30. Kasim M, Kiat AA, Rohman MS, et al. Improved myocardial perfusion in stable angina pectoris by oral lumbrokinase: a pilot study. *J Altern Complement Med* 2009;15:539-544.
31. Ruyssers NE, De Winter BY, De Man JG, et al. *Schistosoma mansoni* proteins attenuate gastrointestinal motility disturbances during experimental colitis in mice. *World J Gastroenterol* 2010;16:703-712.
32. Fiasse R, Latinne D. Intestinal helminths: a clue explaining the low incidence of inflammatory bowel diseases in Sub-Saharan Africa? Potential benefits and hazards of helminth therapy. *Acta Gastroenterol Belg* 2006;69:418-422.
33. Buning J, Homann N, von Smolinski D, et al. Helminths as governors of inflammatory bowel disease. *Gut* 2008;57:1182-1183.
34. Radford-Smith GL. Will worms really cure Crohn's disease? *Gut* 2005;54:6-8.
35. Summers RW, Elliott DE, Urban JF Jr, et al. *Trichuris suis* therapy in Crohn's disease. *Gut* 2005;54:87-90.
36. Summers RW, Elliott DE, Qadir K, et al. *Trichuris suis* seems to be safe and possibly effective in the treatment of inflammatory bowel disease. *Am J Gastroenterol* 2003;98:2034-2041.
37. Summers RW, Elliott DE, Urban JF Jr, et al. *Trichuris suis* therapy for active ulcerative colitis: a randomized controlled trial. *Gastroenterology* 2005;128:825-832.
38. van Kruningen H. Potential danger in the medical use of *Trichuris suis* for the treatment of inflammatory bowel disease. *Inflamm Bowel Dis* 2005;11:515.
39. Reddy A, Fried B. An update on the use of helminths to treat Crohn's and other autoimmunune diseases. *Parasitol Res* 2009;104:217-221.
40. Erb KJ. Can helminths or helminth-derived products be used in humans to prevent or treat allergic diseases? *Trends Immunol* 2009;30:75-82.
41. Mortimer K, Brown A, Feary J, et al. Dose-ranging study for trials of therapeutic infection with *Necator americanus* in humans. *Am J Trop Med Hyg* 2006;75:914-920.
42. Bager P, Arnved J, Ronborg S, et al. *Trichuris suis* ova therapy for allergic rhinitis: a randomized, double-blind, placebo-controlled clinical trial. *J Allergy Clin Immunol* 2010;125:123-130. e1-3.
43. Rajendra W, Armugam A, Jeyaseelan K. Toxins in anti-nociception and anti-inflammation. *Toxicon* 2004;44:1-17
44. Wang Z, Wang W, Shao Z, et al. Eukaryotic expression and purification of anti-epilepsy peptide of *Buthus martensii* Karsch and its protein interactions. *Mol Cell Biochem* 2009;330:97-104..
45. Kim KS, Cho HS, Lee SD, et al. Inhibitory effect of *Buthus martensi* Karsch extracts on interleukin-1beta-induced expression of nitric oxide (NO) synthase and production of NO in human chondrocytes and LPS-induced NO and prostaglandin E2 production in mouse peritoneal macrophages. *Toxicol In Vitro* 2005;19:757-769.
46. Goudet C, Chi CW, Tytgat J. An overview of toxins and genes from the venom of the Asian scorpion *Buthus martensi* Karsch. *Toxicon* 2002;40:1239-1258.
47. Shijin Y, Hong Y, Yibao M, et al. Characterization of a new Kv1.3 channel-specific blocker, J123, from the scorpion *Buthus martensii* Karsch. *Peptides* 2008;29:1514-1520.
48. Shao J, Kang N, Liu Y, et al. Purification and characterization of an analgesic peptide from *Buthus martensii* Karsch. *Biomed Chromatogr* 2007;21:1266-1271.
49. Chen B, Ji Y. Antihyperalgesia effect of BmK AS, a scorpion toxin, in rat by intraplantar injection. *Brain Res* 2002;952:322-326.
50. Guan RJ, Wang M, Wang D, Wang DC. A new insect neurotoxin AngP1 with analgesic effect from the scorpion *Buthus martensii* Karsch: purification and characterization. *J Pept Res* 2001;58:27-35.
51. Tan ZY, Xiao H, Mao X, et al. The inhibitory effects of BmK IT2, a scorpion neurotoxin on rat nociceptive flexion reflex and a possible mechanism for modulating voltage-gated Na(+) channels. *Neuropharmacology* 2001;40:352-357.
52. Liu YF, Ma RL, Wang SL, et al. Expression of an antitumor-analgesic peptide from the venom of Chinese scorpion *Buthus martensii* karsch in *Escherichia coli*. *Protein Expr Purif* 2003;27:253-258.
53. Xiong YM, Lan ZD, Wang M, et al. Molecular characterization of a new excitatory insect neurotoxin with an analgesic effect on mice from the scorpion *Buthus martensi* Karsch. *Toxicon* 1999;37:1165-1180.
54. Shao JH, Wang YQ, Wu XY, et al. Cloning, expression, and pharmacological activity of BmK AS, an active peptide from scorpion *Buthus martensii* Karsch. *Biotechnol Lett* 2008;30:23-29.
55. Bai ZT, Liu T, Pang XY, et al. Suppression by intrathecal BmK IT2 on rat spontaneous pain behaviors and spinal c-Fos expression induced by formalin. *Brain Res Bull* 2007;73:248-253.
56. Li C, Liu M, Monbo J, et al. Turning a scorpion toxin into an antitumor miniprotein. *J Am Chem Soc* 2008;130:13546-13548.

57. Feng L, Gao R, Gopalakrishnakone P. Isolation and characterization of a hyaluronidase from the venom of Chinese red scorpion *Buthus martensi*. *Comp Biochem Physiol C Toxicol Pharmacol* 2008;148:250-257.
58. Gupta SD, Gomes A, Debnath A, Saha A. Apoptosis induction in human leukemic cells by a novel protein Bengalin, isolated from Indian black scorpion venom: through mitochondrial pathway and inhibition of heat shock proteins. *Chem Biol Interact* 2010;183:293-303.
59. Das Gupta S, Debnath A, Saha A, et al. Indian black scorpion (*Heterometrus bengalensis* Koch) venom induced antiproliferative and apoptogenic activity against human leukemic cell lines U937 and K562. *Leuk Res* 2007;31:817-825.
60. Haldar S, Das Gupta S, Gomes A, et al. A high molecular weight protein Bengalin from the Indian black scorpion (*Heterometrus bengalensis* C.L. Koch) venom having antiosteoporosis activity in female albino rats. *Toxicon* 2009;55:455-461.
61. Gomes A, Haldar S, Giri B, et al. Experimental osteoporosis induced in female albino rats and its antagonism by Indian black scorpion (*Heterometrus bengalensis* C.L.Koch) venom. *Toxicon* 2009;53:60-68.
62. Deshane J, Garner CC, Sontheimer H. Chlorotoxin inhibits glioma cell invasion via matrix metalloproteinase-2. *J Biol Chem* 2003;278:4135-4144.
63. Borges A, Silva S, Op den Camp HJ, et al. *In vitro* leishmanicidal activity of *Tityus discrepans* scorpion venom. *Parasitol Res* 2006;99:167-173.
64. Conduit R, Sasse A, Hodgson W, et al. A neurotoxinological approach to the treatment of obstructive sleep apnoea. *Sleep Med Rev* 2007;11:361-375.
65. Bonnemain B. Helix and drugs: snails for western health care from antiquity to the present. *Evid Based Complement Alternat Med* 2005;2:25-28.
66. Becker S, Terlau H. Toxins from cone snails: properties, applications and biotechnological production. *Appl Microbiol Biotechnol* 2008;79:1-9.
67. Bingham JP, Mitsunaga E, Bergeron ZL. Drugs from slugs – past, present and future perspectives of omega-conotoxin research. *Chem Biol Interact* 2010;183:1-18.
68. Webster LR, Fisher R, Charapata S, Wallace MS. Long-term intrathecal ziconotide for chronic pain: an open-label study. *J Pain Symptom Manage* 2009;37:363-372.
69. Hama A, Sagen J. Antinociceptive effects of the marine snail peptides conantokin-G and conotoxin MVIIA alone and in combination in rat models of pain. *Neuropharmacology* 2009;56:556-563.
70. Kolosov A, Goodchild CS, Cooke I. CNSB004 (Leconotide) causes antihyperalgesia without side effects when given intravenously: a comparison with ziconotide in a rat model of diabetic neuropathic pain. *Pain Med* 2010;11:262-273.
71. Lubbers NL, Campbell TJ, Polakowski JS, et al. Postischemic administration of CGX-1051, a peptide from cone snail venom, reduces infarct size in both rat and dog models of myocardial ischemia and reperfusion. *J Cardiovasc Pharmacol* 2005;46:141-146.
72. Zhang SJ, Yang XM, Liu GS, et al. CGX-1051, a peptide from Conus snail venom, attenuates infarction in rabbit hearts when administered at reperfusion. *J Cardiovasc Pharmacol* 2003;42:764-771.
73. Wu Y, Li J, Wang J, et al. Anti-atherogenic effects of centipede acidic protein in rats fed an atherogenic diet. *J Ethnopharmacol* 2009;122:509-516.
74. Peng K, Kong Y, Zhai L, et al. Two novel antimicrobial peptides from centipede venoms. *Toxicon* 2010;55:274-279.
75. Park SP, Kim BM, Koo JY, et al. A tarantula spider toxin, GsMTx4, reduces mechanical and neuropathic pain. *Pain* 2008;137:208-217.
76. Mazzuca M, Heurteaux C, Alloui A, et al. A tarantula peptide against pain via ASIC1a channels and opioid mechanisms. *Nat Neurosci* 2007;10:943-945.
77. Decrem Y, Rath G, Blasioli V, et al. Ir-CPI, a coagulation contact phase inhibitor from the tick *Ixodes ricinus*, inhibits thrombus formation without impairing hemostasis. *J Exp Med* 2009;206:2381-2395.
78. Nazareth RA, Tomaz LS, Ortiz-Costa S, et al. Antithrombotic properties of ixolaris, a potent inhibitor of the extrinsic pathway of the coagulation cascade. *Thromb Haemost* 2006;96:7-13.
79. Ragosta M, Gimple LW, Gertz SD, et al. Specific factor Xa inhibition reduces restenosis after balloon angioplasty of atherosclerotic femoral arteries in rabbits. *Circulation* 1994;89:1262-1271.
80. Corral-Rodriguez MA, Macedo-Ribeiro S, Pereira PJ, Fuentes-Prior P. Leech-derived thrombin inhibitors: from structures to mechanisms to clinical applications. *J Med Chem* 2010;53:3847-3861.
81. Grishin EV, Savchenko GA, Vassilevski AA, et al. Novel peptide from spider venom inhibits P2X3 receptors and inflammatory pain. *Ann Neurol* 2010;67:680-683.