British Journal of Applied Science & Technology
4(4): 578-593, 2014

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Animals and Animal Toxins in Traditional and Modern Medicine

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Authors' contributions

Author AK wrote an assay in German on this topic as part of her study program. Author TE wrote the paper. Author HJG contributed clinical pictures and corrected the manuscript. All authors read and approved the final manuscript.

Review Article

Received 29th June 2013
Accepted 5th October 2013
Published 16th November 2013

ABSTRACT

Background: Animals and animal toxins have a long-lasting tradition in diverse human cultures all over the world. In addition to medicinal plants, animals are important components of many traditional medicines.
Methods: By using selected examples, we give an overview of animals, animal toxins, and derivatives to illustrate their pharmacological and therapeutic potential and use.
Results: Animals have been used in traditional and modern medicines for treatment purposes. Medicinal leeches (*Hirudo medicinalis*) are applied for microsurgical operations and for prevention of tissue necrosis due to congestion and insufficient supply of blood capillaries. Patches of blister beetles (*Cantharis vesicatoria*) have been topically applied in China since centuries against warts and poxvirus-caused molluscum contagiosum.
Snake venoms are a rich source for drug development. Some examples are batroxobin (reptilase) from *Bothrops moujeni* and *B. atrox*, and others. Bioactive peptides have also been identified from spider and scorpion venoms such as GsMTx-4 from the Chilean rose Tarantula (*Grammostola spatulata*) or chlorotoxin from the Yellow Israeli scorpion (*Leiurus quinquestriatus quinquestriatus*). Amphibian venoms have frequently been used as arrow poisons by indigenous tribes. The marine biodiversity is also a rich resource for new drugs. A large array of toxins can be found in sea snails. Important examples are ω-conotoxin from *Conus purpurascens*, contukalin-G from *C. geographus*, and ACV1 from

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C. victoriae. Examples among many others are aplidine from *Aplidium albicans*, pseudopterosin from the *Pseudopterogorgia* genus, and dolastatin from *Dolabella auricularia*.
Conclusion: Animals or compounds isolated from snake, spider or scorpion venoms, frog toxins, and toxins from marine organisms reveal astonishing pharmacological activities. Cross-disciplinary approaches between organic and medicinal chemistry, pharmacology, toxicology, molecular biology, and medicine are required to explore the full potential of animal toxins and their derivatives as important candidates in the drug development pipeline.

Keywords: Drug development; natural products; toxicology; traditional medicine; venom.

1. INTRODUCTION

Traditional medicines are largely based on therapies consisting of medicinal herbs, but also of animals (or parts of them) and minerals [1-4]. Although this is true in many cases, it should not be overseen that some of the most toxic compounds can be found in plants (*e.g*. aristolochic acid) [5,6]. Hence, medicinal plants may occasionally also reveal toxicities [7-10].

Animals and animal toxins have been used since ages in many human cultures worldwide. The toxic secretions of frogs have been used since ages by indigenous tribes for poisoning of arrows for hunting or warfare. In addition to medicinal plants, animals and even minerals are important components of many traditional medicines, including Chinese medicine, Ayurveda in India and folk medicines of the American and African continent as well as complementary and alternative medicine in industrialized countries [11,12]. Whereas traditional medicines frequently apply whole animals or animal preparations for treatment purposes, modern academic medicine has recognized the pharmacological value of isolated compounds from animals as lead compounds for chemical derivatization and further drug development [13-15].

Therefore, the purpose of the present review is to give an overview of the therapeutic potential of animals and animal toxins for therapy and as resource for drug development. Selected examples are chosen to illustrate the value of animal toxins for current and future strategies to combat diseases. The reader is also referred to reviews dealing with specific aspects of the therapeutic potential of animal toxins [13-15,16-21].

2. CLINICALLY APPLIED ANIMALS

2.1 Medicinal Leeches (*Hirudo medicinalis*)

When fully stretched, the adult medicinal leech (*Hirudo medicinalis*) can measure 15 to 20 cm with a width of one to two cm. In clean water habitats and optimal nutritional conditions, leeches are getting up to 5 years old [22]. Leeches have been traditionally used in medicine since ages. In the 1960s medicinal leeches were used by two Slovenian surgeons (Derganc and Zdravic). They reported that they used leeches to support a procedure in which a flap of tissue was used to close a wound [23]. Later, the option to use leeches for therapeutic purposes was rediscovered in the 1980s especially in reconstructive surgery. They can be used for microsurgical operations [24,25]. Furthermore, tissue necrosis due to congestion

and insufficient supply of blood capillaries can be avoided by the use of leeches, which release hirudin into the human blood. This compound inhibits coagulation, enhances lymph flow, has an antithrombotic action and reveals vascular anticonvulsant properties [26]. The bite of a leech with its three-star-formed stoma is not very painful. It may suck up to about 10 mL of blood. A blood meal saturates a leech for up to two years. However, medicinally used leeches are killed after single use [27,28].

2.2 Maggots of the Common Housefly (*Lucilia sericata*)

The treatment of wounds with maggots was internationally widely distributed until 1940, but was forgotten with the dawn of the era of antibiotics. It was only with the emergence of antibiotic-resistant bacterial strains that maggot therapy has experienced a renaissance in the USA since the 1980s. In the 1990s, biosurgery was reintroduced in Germany [29, 30].

Maggots keep the open skin areas and wounds clean and bacteria-free [31,32]. This is why maggots are primarily used for chronic wounds and ulcers, especially if conventional therapy was not successful [33]. Usually, 10 maggots per cm^2 wound surface are applied for a few days. Maggots can be maintained on pork liver and used for medicinal purposes, if sufficient numbers of maggots have grown in the culture [34]. They feed on dead skin parts that are resolved by their secretions. Like vacuum cleaners, maggots clean wounds from dead tissue. Necrotic tissues lack microcirculation, so antibiotics may significantly lose their local clinical availability. In addition to dead tissue, also bacteria are eaten by maggots [35]. This observation is particularly important in relation to the growing number of multi-resistant strains of bacteria, which are unresponsive to other treatments. Because maggots of some species do not differentiate between dead and healthy tissue, their use may even worsen the clinical picture. Therefore, only certain maggots may be considered for medicinal purposes, *e.g.* those of the common house fly (*Lucilia sericata*). They are maintained under sterile and standardized conditions on fresh pork liver [29].

2.3 Blister Beetle (*Cantharis vesiculata*)

A well-known example for the therapeutic application of insects is the blister beetle (*Cantharis vesicatoria*) (Fig. 1A). They have been traditionally used in China and Europe since centuries [36]. Their topical applications have been described [37]. Patches of ground*Cantharis* are active against warts and molluscum contagiosum, a dermatological disorder caused by MC poxvirus [11]. The active principle is cantharidin which is a protein phosphatase inhibitor (Fig. 2). Cantharidin exerts antiviral and anti-cancerogenic activity [38-42] and was also shown to induce DNA damage and apoptosis [40]. As cantharidin is highly toxic if used as systemic treatment, less toxic derivatives, *e.g.* norcantharidin, are under investigation. For topical application, *Cantharis* patches are used, which are safe and non-toxic. They produce intra-epidermal blisters, which usually heal without scarring [43] (Fig. 1B).

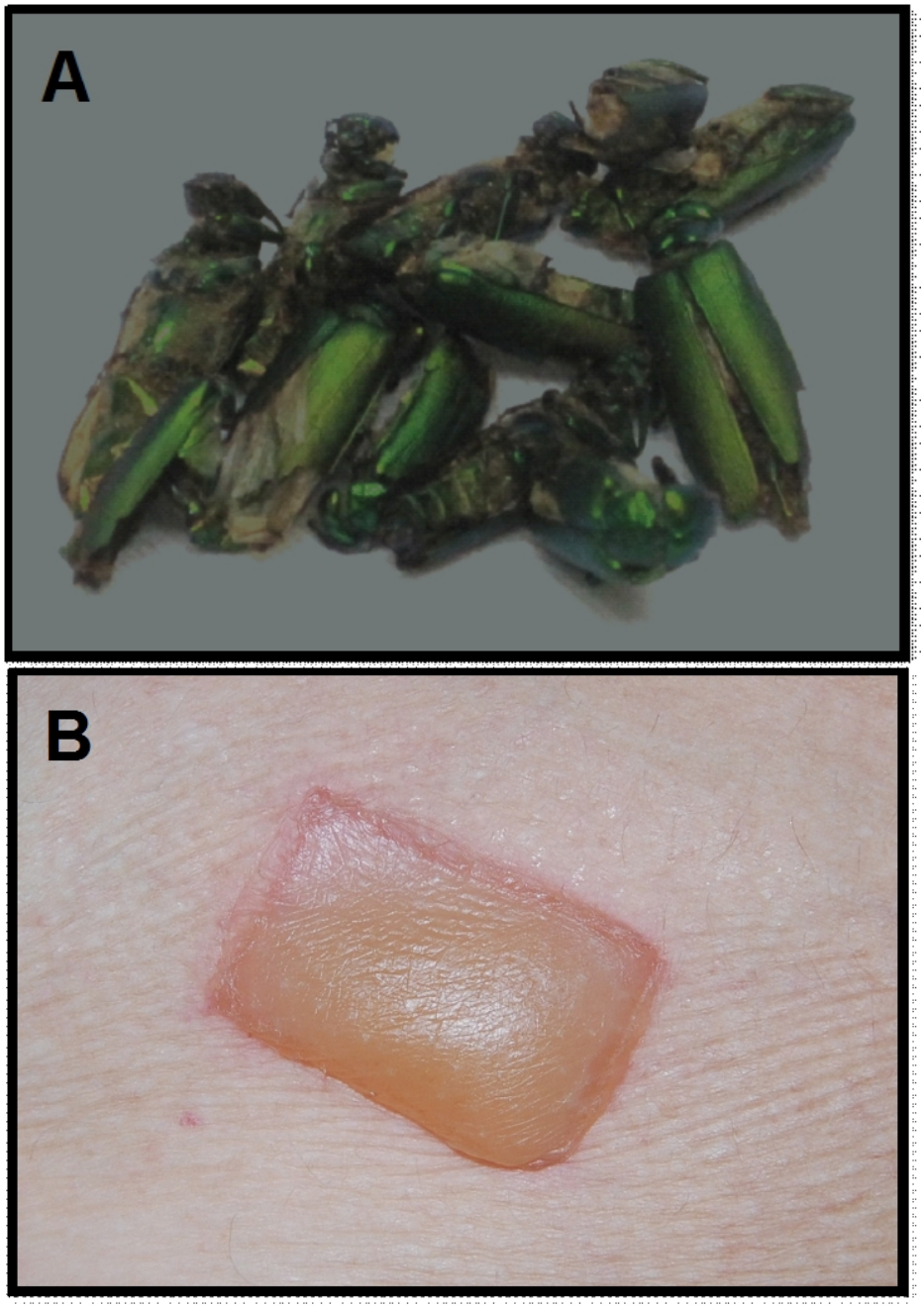

Fig. 1. Insect-based therapy. (A) Blister beetles (*Cantharis vesicatoria*). (B) Blister on skin induced by therapeutic *Cantharis* patch.

3. Animal Venoms and Toxins

Scientists have always been fascinated by the extraordinary precision of venoms. For instance, a bite of a king cobra (*Ophiophagus hannah*) or a sting of a thick-tailed scorpion

(Androctonus) specifically targets the nervous system of the victim [44]. Neurotoxins very specifically act on the conduction of nerve fibers or transmission at synapses. Furthermore, neurotoxins may eventually exert toxicity towards organs such as heart, liver, and kidneys [45]. A classic strategy in neurobiology to discover novel compounds is to harvest the venom of a toxic species and to isolate the active principle from a mixture of compounds. This can then be systematically analyzed by pharmacological and molecular biological methods.

Attention has to be paid on the difference between poisons and venoms. The latter are produced in specialized tissues called venom glands and the corresponding animals are called venomous animals such as scorpions, snakes, sea snails, spiders and others. The venom is injected into the victim by bite or sting. Poisons are produced in non-specialized tissues and the corresponding animals are called poisonous animals such as some fishes, frogs and others. Poison must be inhaled, ingested or delivered via touch.

3.1 Snake Venoms

Most of venoms derived from snake bites or scorpion stings contain neurotoxins acting directly on ion channels of nervous and muscular tissues. Snake venoms are complex mixtures of enzymes and toxins. They are classified as neurotoxins or hematotoxins, which either promote or prevent blood coagulation. Specific proteins of snake venoms can provoke different symptoms such as lowering of blood coagulability, thrombosis, and damage to blood vessels resulting in bleeding and secondary bleeding effects, *e.g*. hypovolaemic shock or organ damage [46].

The enzyme batroxobin (also known as reptilase) of the Brazilian Pit Viper species, *Bothrops moujeni* and *Bothrops atrox*, can medically be used to improve the flow properties of blood. On the other hand, it is also used as "fibrin glue" in operations [47]. Surgeons may stop diffuse bleeding from bleeding sources like liver or lung tissue by spraying it on the bleeding site [48].

Some hematological coagulation tests are based on reptilase from snake venoms. It is produced in snake farms by "milking" the venom glands. The teeth are placed on a membrane-covered container and the venom glands are massaged. The effluent toxin is freeze-dried and ground into granules. Reptilase promotes the conversion of fibrinogen to fibrin clotting *in vitro* and is used in laboratory medicine for the detection of coagulation disorders [49]. The so-called “reptilase time” is a measure for the duration to clot after addition of reptilase to a blood sample. The reference range is between 15 and 23 seconds. Values above the reference range indicate a delayed clotting, whereas values below the lower limit indicate sufficient coagulation and therefore in general have no diagnostic relevance [49]. In contrast to other tests such as the thrombin test, reptilase-induced clotting is not hindered by heparin or hirudin so that reptilase can also be used for heparinized blood samples [50].

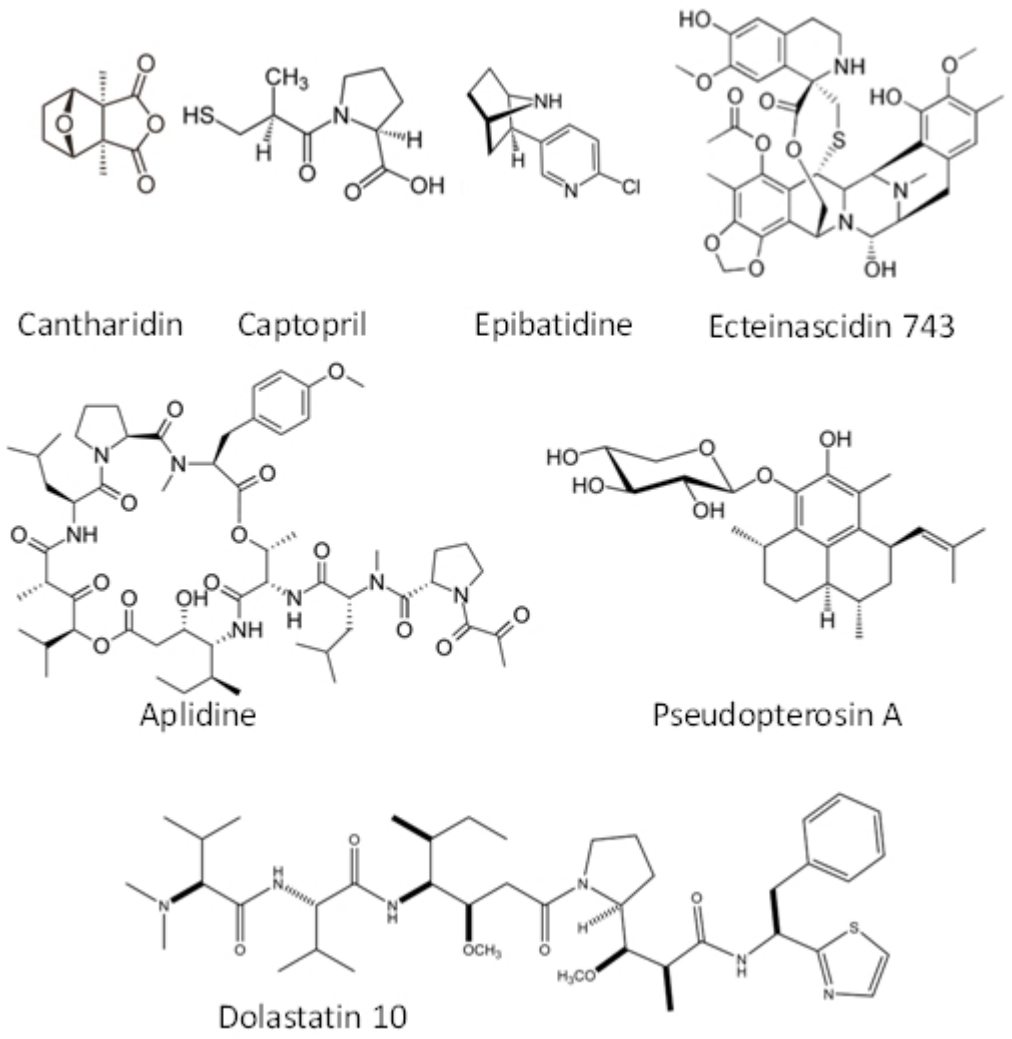

Fig. 2. Structures of different animal toxins

Reptilase reveals a moderate anti-coagulant effect *in vivo.* In contrast to thrombin, reptilase is not activated by coagulation factor XIII leading to fibrin monomers to form a fibrin thrombus instead of fibrinogen cross-links [51]. Therefore, the fibrin formed by reptilase is more readily degradable than the thrombus formed by thrombin. Thus, reptilase leads to fibrinogen deficiency in the blood circuit by conversion of fibrinogen to fibrin and its subsequent rapid enzymatic degradation [52]. This process is termed defibrination and leads to reduced blood coagulation. Reptilase is therefore clinically used for the dissolution of blood clots as well as for prevention of thrombosis and embolism [50].

The discovery of substances in the venom of the Yarará pit viper (*Bothrops jararaca*) raised considerable attention, because they can block angiotensin converting enzyme (ACE), which is important for the regulation of blood pressure [53]. A pentapeptide isolated from the snake was used for derivatization of pharmacologically improved molecules and was the starting point for the development of a large group of drugs, the ACE inhibitors which are

indispensible nowadays in the treatment of hypertension and congestive heart failure. The lead drugs of this class are captopril (Fig. 2) and enalalpril [54].

Fibrinogen-clotting enzymes from the Malayan pit viper (*Calloselasma rhodostoma*) cause open wounds of their victims. In clinical practice, these enzymes are used in medicine to treat haemostatic disorders, including stroke, deepvein thrombosis, myocardial infarction, peripheral arterial thrombosis, priapism, and sickle-cell crisis [51,55].

The protein contortrostatin has been isolated from the venom of the Copperhead (*Agkristrodon contortrix*), a pit viper in the southern United States. The venom of this snake has less neurotoxicity, but leads to severe bleeding and prevents blood clotting. Contortrostatin reveals anticancer activity *in vivo* against breast cancer, ovarian cancer and brain tumors by inhibition of angiogenesis [56]. Contortrostatin-type compounds belong to a class of proteins known as disintegrins, which competitively interact with integrins and thereby block tumor cell adhesion and tumor invasion [57].

The venom of the Common Mamba (*Dendroaspis angusticeps*) causes muscle spasms in the victims of that snake leading to respiratory paralysis. Dendrotoxins in the venom of the snake enhance the release of the neurotransmitter acetylcholine and can be used to treat Alzheimer's disease [58].

Disintegrins are viper peptides of a length of 41 to 84 amino acids residues. They inhibit blood coagulation by binding to fibrinogen receptor, integrin $\alpha_{IIb}\beta_3$, of thrombocytes. Disintegrins are investigated for the treatment of cancer, thrombosis and other diseases [59]. As an example, **echistatin**,the active ingredient of the Saw-Scaled Viper (*Echis carinatus*), inhibits bone destruction in osteoporosis and blood clotting [60].

3.2 Spider and Scorpion Venoms

There are many peptides that were identified from spider venoms. Examples of peptides discovered are psalmotoxin-1, Tx2-6, psalmopeotoxin I, psalmopeotoxin II and many others [61]. In addition to anticancer peptides purified from scorpion venom, antimicrobial peptides have also been characterized from scorpion venom and hemolymph [62, 63]. In the present review, we focus on a few examples only.

The peptide, GsMTx-4, isolated from the venom of the Chilean rose Tarantula (*Grammostola spatulata*) inhibits arrhythmia by targeting myocardial ion channels [64]. GsMTx-4 produced a complete block of stretch-activated channels in outside-out patches. This effect appeared to be specific, since whole-cell voltage-sensitive currents remained non-affected [65]. Similarly, in isolated ventricular cells from a rabbit dilatative cardiomyopathy model, GsMTx-4 produced a near complete block of the volume-sensitive cation-selective current, but did not affect the anion current. In myopathic heart cells with tonically active swell-induced current, GsMTx-4 also reduced the cell size, which implicates a role in volume regulation [65].

The Yellow Israeli scorpion (*Leiurus quinquestriatus quinquestriatus*) belongs to the most dangerous scorpion species of North Africa and Middle East. It attacks preys with a venom that causes convulsions, paralysis and severe pain. Chlorotoxin (36 amino acids residues) was isolated from the venom and blocks small-conductance Cl-channels [66]. It can specifically accumulate in cancer tissue after intraveneous injection. Ig chlorotoxin is coupled to fluorescent dyes, it can be used as contrast agent in cancer imaging techniques to

distinguish malignant from healthy tissues. This allows an improved detection of cancer foci during surgery. In living mice and samples from healthy and malignant human biopsies, the chlorotoxin lights up tumor tissues only a few hours after injection into the blood stream, and this effect lasts for up to 14 days. This dye is particularly suitable for gliomas [67]. This tumor type has a high recurrence rate, because surgeons usually remove as little healthy brain tissue as possible and, therefore, malignant cells may remain at the tumor margins. Matrix metalloproteinase-2 (MMP-2) is specifically up-regulated in gliomas and related cancers, but not in normal brain. Chlorotoxin selectively interacts with MMP-2 isoforms, but not other MMPs. The compound inhibits the enzymatic activity of MMP-2 and causes a reduction in the surface expression of MMP-2, indicating that chlorotoxin is a specific MMP-2 inhibitor with significant therapeutic potential for gliomas and other diseases that invoke the activity of MMP-2 [68]. Chlorotoxin also detects other tumor entities, such as prostate and colon carcinoma, or even small metastases in lymph vessels. Since chlorotoxin has already been proven in several clinical trials as non-toxic to humans, it is a promising candidate for use as a novel contrast agent in the future [69,70].

3.3 Compounds from the Skin Secretion of Amphibians

There is a large biodiversity of bioactive compounds in amphibian skin, including the steroidal samandarines from salamanders, the batrachotoxins, histrionicotoxins, gephyrotoxins, and epibatidine from neotropical frogs (*Dendrobatidae*), the pumiliotoxins, allopumiliotoxins, homopumiliotoxins, and decahydroquinolines from certain genera of anurans from *Dendrobatidae, Mantellidae, Bufonidae*, and *Myobatrachidae*, a variety of izidines (pyrrolizidines, indolizidines, quinolizidines, lehmizidines), pyrrolidines, piperidines, various tricyclics (related in structures to the coccinellines), and spiropyrrolizidines, the pseudophrynamines from one genus of Australian frogs, and a variety of other alkaloids. With the exception of the samandarines and the pseudophrynamines, most alkaloids appear to be derived from dietary sources [71].

One selected example for animal venoms with potential use in medicine are the skin secretions of poison dart frogs [72]. One of the most effective compound classes in these skin secretions are alkaloids. One of them is the epibatidine from small colorful frogs of the *Epipedobates* genus (Fig. 2). It leads to convulsions, paralysis and hypotension. In animal studies, it also showed analgesic effects, which were 120-fold more potent than those of morphine [73]. The toxic and analgesic effects cannot be separated in the original natural product derived from the frogs. It was only after chemical derivatization that the toxic effects could be removed. Epibatidine does not seem to cause addiction in patients, because the compound does not bind to opiate receptors, but to nicotinic acetylcholine receptors in the brain [74,75].

3.4 Toxins from Marine Snails

There are more than 500 species of predatory cone snails of the *Conus* genus [76]. Cone snails are tropical marine mollusks that poison their prey with a complex mixture of active neuropeptides. Most conotoxins are short peptides and consist of 10 to 30 amino acid residues. Most of these cone snail toxins target specific ion channels and receptors [77,78]. Each *Conus* venom contains a unique combination of 50 to 200 neuro-pharmacologically active peptides. However, the structure and function have only been determined for only a small number of these peptides so far. Three classes of targets of these peptides have been

identified: voltage-gated ion channels, ligand-gated ion channels and G protein-coupled receptors (Table 1).

Table 1. Targets and modes of actions of conotoxins [79]

Target	Mode of action
Voltage-gated ion channels	Protraction of inactivation of calcium channels Specific inhibition of sodium channels, natrium channels, and potassium channels
Ligand-gated ion channels	Antagonists of *N*-methyl-d-aspartate and serotonin receptors Competitive and non-competitive nicotinic receptor antagonists
G protein-coupled receptors	Neurotensin and vasopressin receptor agonists

Among the conotoxins that have been isolated from *Conus* species some have demonstrated a considerable clinical potential for pain reduction [80]. One feature of conotoxins making them attractive for therapeutic use is their high efficiency and selectivity. The biological effects of many conopeptides can be divided into three categories: production of excitotoxic shock, paralysis and inhibition of sensory circuits [79]. The basis for the therapeutic use of neurotoxins isolated from cone snails is the specific targeting of components of neural transmission [81,82].

Conus purpurascens contains ω-conotoxin, a peptide that blocks N-type calcium channels, thereby preventing calcium influx and neurotransmitter release in spinal cord synapses. The peptide blocks, thereby, the transmission of pain stimuli and relieves pain even in cases in which morphine is not effective [83].

One of the *Conus* peptides that found its way into clinical trials for pain treatment is ω-conotoxin MVIIA [84,85], an N-type calcium channel blocker isolated from *Conus magus*. Ziconotide® is a synthetic ω-conotoxin that inhibits the entry of calcium ions into neurons. Multiple types of voltage-gated calcium channels have been identified in sensory pathways that play a role for nociceptive signal transductions, but the N-type seems to be the dominant channel and is particularly important at the spinal level [86]. The ω-conotoxins inhibit neuromuscular transmission by preventing the voltage-activated entry of calcium into the nerve ends. Analgesic effects are elicited by inhibiting neurotransmitter release from primary nociceptive afferent fibers. Thereby, the spread of pain signals to the brain is suppressed. Ziconotide® reduces both malignant and non-malignant chronic pain. Clinical studies on ω-conotoxin MVIIA demonstrated that this compound is approximately 1,000-fold times more potent than morphine, but does not display the addictive properties of opiates. Most neurological adverse events with respect to delayed elimination of ziconotides from neural tissues are manageable by dose reduction [87].

Another *Conus* peptide under clinical development is contulakin-G, which is isolated from *Conus geographus.* It is a neurotensin agonist and was considerably more potent to inhibit pain as neurotensin in *in vivo* experiments. It is being under investigation as drug against pain after operations [88,89].

The conotoxin, ACV1, from *Conus victoriae,* has been studied for the treatment of neuropathic pain [90]. ACV1 accelerates the recovery of injured nerves. Rat experiments indicated that ACV1 suppresses the transmission of pain sensations in the nervous system by inhibiting pain transmission at nicotinic acetylcholine receptors. ACV1 is a much smaller

molecule in comparison to ω-conotoxins and unlike ziconotide, which has to be injected into the spine, it can be applied into muscles or fat tissues [90].

3.5 Toxins from Other Marine Animals

The sea squirt (*Ecteinascidia turbinata*) lives in tropical waters and in the Mediterranean Sea. It produces the toxin ecteinascidin 743 (Fig. 2) which destabilizes the DNA of tumor cells and inhibits DNA repair processes. The substance is used to treat soft tissue sarcomas and ovarian cancer when standard therapy fails [91].

A related species, *Aplidium albicans*, produces aplidine (Fig. 2), a substance that inhibits division of cancer cells [92].

Corals are delicate creatures and yet extremely well-fortified. To ward off hungry fish, horn corals of the *Pseudopterogorgia* genus that are native to the Caribbean waters produce pseudopterosin (Fig. 2). The substance exhibits anti-inflammatory properties in humans. The hope is that it could be used to treat skin diseases, such as eczema or psoriasis soon as an alternative to cortisol treatment [93].

The Sea Hare, *Dolabella auricularia*, is a marine snail that feeds on algae. With the aid of bacteria included in these algae, the sea hare generates dolastatin (Fig. 2). This drug is under clinical investigation for cancer treatment [94,95].

4. CONCLUSION AND PERSPECTIVES

It is obvious from traditional folk medicines and modern academic medicine that many animals and animal toxins may be used for therapeutic purposes. The use of animals and their toxins in medicine has long been known. Four thousand years ago, the Chinese have already made powder from toad skin and used it as a heart medication [96]. The Greeks used leeches in medicine 2000 years ago [97]. In 1781, a study was published describing the effects of snake venoms on blood coagulation [98].

These toxins are quite specific in their effect on the body. Many toxins of animal origin include toxins which specifically bind toion channels of excitable and non exitable cells. The extraction of animal toxins sometimes poses a challenge, but represents the initial step for further investigations. While the "milking" of snakes is already an established method, the extraction of other toxins such as spider toxins is still a difficult dangerous procedure requiring anesthetizing the spiders with CO_2 followed by a slight electric shock to release the toxin. On the other hand, anesthesia with CO_2 is not always required. *Tarantula* spiders (family Theraphosidae) which are not especially harmful to humans can also be milked without anesthesia by holding the animal in the hand or some support and applying an electrical stimulus at the basis of the chelicerae [99]. However, only a small amount of venom can be obtained after milking. Sometimes, the production of 100 mg of spider toxin requires “milking” of about 30 animals. This illustrates the need to synthesize the active ingredient purified from animal venoms [100,101].

The future of natural products from animals will depend on cross-disciplinary approaches reaching from organic and medicinal chemistry to synthesize and derivatize animal toxins to pharmacology to unravel cellular and molecular modes of action and medicine to test new animal toxin-derived drugs in clinical trials [102].

An example that this strategy may be successful to develop novel and innovative treatment options has been recently shown for conotoxins. The potassium channel protein, HERG, is not a classical drug target for anticancer drugs. However, the increased HERG expression in cancer cells and the capability of the conotoxin, k-PVIIA, from *Conus purpurascens* to interact with a charged extracellular unit of HERG may indicate that animal toxins may serve as new lead compounds to attack novel cancer cells targets [103].

We have good reason to assume that animal toxins generated over millions of years during evolution of life on earth will drive the development of novel drugs for many diseases with a need for more effective treatments.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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