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Review

Review of anti-inflammatory, immune-modulatory and wound healing properties of molluscs

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ABSTRACT

Ethnopharmacological relevance: This review focuses on traditional and contemporary anti-inflammatory uses of mollusc–derived products summarising all the *in vitro*, *in vivo* and human clinical trials that have tested the anti-inflammatory activity of molluscan natural products. Inflammatory conditions, burns and wounds have been an ongoing concern for human health since the early era of civilisation. Many texts from ancient medicine have recorded the symptoms, signs and treatments for these conditions. Natural treatments are well-documented in traditional European medicine, Traditional Chinese Medicine (TCM), Siddha and ancient Mediterranean and African traditional medicine and include a surprisingly large number of molluscan species.
Materials and methods: An extensive review of the *Materia Medica* and scientific literature was undertaken using key word searches for “mollusc” and “anti-inflammatory” or “immunomodulatory” or “wound healing”.
Results: Molluscs have been used in ethnomedicine by many traditional cultures to treat different aspects of inflammatory conditions. We found 104 different anti-inflammatory preparations from a variety of molluscan species, of which 70 were from the well-documented Traditional Chinese Medicine (TCM). This traditional use of molluscs has driven the testing for inflammatory activity in extracts from some species in the phylum Mollusca, with 20 *in vitro* studies, 40 *in vivo* animal studies and 14 human clinical trials performed to substantiate the anti-inflammatory and wound healing activity of molluscs. Some of these studies have led to the approval of mollusc-derived products to be used as over-the-counter (OTC) nutraceuticals, like Lyprinol® and Biolane™ from the New Zealand green lipped mussel *Perna canaliculus*.
Conclusion: Natural products provide important leads for the development of pharmaceuticals, including anti-inflammatory agents. Only a small proportion of the molluscan traditional medicines have been tested to confirm their anti-inflammatory activity and most screening studies have tested crude extracts from molluscs without any chemical characterisation. This highlights the need for further research to strategically identify the anti-inflammatory compounds in molluscan medicines to provide leads for novel anti-inflammatory drugs in the future.

Abbreviations: TCM, Traditional Chinese Medicine; OTC, over-the-counter; i.v., intravenous; i.p., interaperitoneal; p.o., orally; FDA, Food and Drug Administration; NSAIDs, non-steroidal anti-inflammatory drugs; PG, prostaglandin; TNFα, tumor necrosis factor alpha; IL, interleukin; COX, cyclooxygenase; WoRMS, World Register of Marine Species; ROS, reactive oxygen species; NFκB, nuclear factor kappa B; NO, nitric oxide; iNOS, induced nitric oxide synthase; LOX, lipoxygenase; LPS, lipopolysaccharide; PUFAs, polyunsaturated fatty acids; GLME, green lipped mussel extract; HFCM, *hannai* fermented with *C. militaris* mycelia; 5-HETE, 5-hydroxyeicosatetraenoic acid; ORAC, oxygen radical absorbance capacity; AA, arachidonic acid; 5-HT, 5-hydroxytryptamine; ETA, eicosatetraenoic acid; AIA, adjuvant-induced arthritis; PLA2, phospholipase A2; HMLE, hard-shelled mussel lipid extract; PT, partial thickness; RA, rheumatoid arthritis; OA, osteoarthritis; PCT, placebo-controlled trial; NSD, no statistical difference; SD, statistically different; HRBC, human red blood cell; FITC, Fluorescein isothiocyanate; ZKC, Zhikang Capsule; TIMPs, tissue inhibitors of metalloproteinases; MMP, matrix metalloproteinase; CIA, collagen-induced arthritis; IFN-γ, interferon gamma; CINC 1, cytokine-induced neutrophil chemoattractant 1; LTB4, leukotriene B4; TXB2, thromboxane B2; DTH, delayed type hypersensitivity reaction; PFC, plaque forming cell; DNFB, dinitrofluorobenzene; FLH, *Fissurella latimarginata* hemocyanin; TRAP, tartrate-resistant acid phosphatase; ACP, acid phosphatase; ALP, alkaline phosphatase; HpH, *Helix pomatia* hemocyanin; TT, tetanus toxoid

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1. Introduction

Many natural products are sourced from the marine environment due to its phenomenal biodiversity. Marine invertebrates (Porifera, Echinodermata, Cnidarian, Mollusca, Arthropoda) have to date provided a substantial diversity of natural products, including terpenes, alkaloids, aliphatic hydrocarbons, steroids, carbohydrates, amino acids and peptides (Leal et al., 2012). These marine-derived natural products have an extensive array of therapeutic properties, including anticoagulant, antimicrobial, wound healing and immune modulating, antioxidant, anticancer, anti-inflammatory, antihypertensive, and other medicinal properties (Perdicalis et al., 2013; Senthilkumar and Kim, 2013). A number of marine natural products have provided important leads for drug development and many are now used in the formulation of novel drugs (Leal et al., 2012; Riguera, 1997; Senthilkumar and Kim, 2013). For example, Ziconotide, first isolated from the cone snail *Conus magus* (Linnaeus, 1758) venom effectively blocks N-type voltage gated calcium channels (Schroeder et al., 2004) and is effective for the treatment of chronic pain. The drug has now been Food and Drug Administration (FDA) approved and has been commercialised under the name of Prialt® (Atanassoff et al., 2000; Svenson, 2013).

The Mollusca is a phylum of marine invertebrates that are of particular interest as a source of new potential drugs leads. Molluscs encompass 7% of living animals on the planet making them the second largest animal phylum with estimated 100–200 thousand species, of which more than 52 thousand have been described and named (Benkendorff, 2010; Bouchet and Duarte, 2006). The phylum Mollusca also includes eight different classes: Gastropoda, Bivalvia, Scaphopoda, Cephalopoda, Polyplacophora, Monoplacophora, Caudofoveata and Solenogastres (Benkendorff, 2010; Ponder and Lindberg, 2008) which illustrates a significant evolutionary divergence over the past 500 million years. Associated with this vast biological diversity is significant chemical diversity, as molluscs use secondary metabolites to communicate and defend themselves against predators and pathogenic invaders (Benkendorff, 2014). As marine invertebrates, molluscs lack acquired immunity and essentially depend on their innate immunity and bioactive compounds to protect against microbial pathogens (Dang et al., 2015; Hooper et al., 2007) and heal wounds in the microbially-rich marine environment.

Molluscs have been a significant focus in the search for biologically active secondary metabolites, with > 1,145 natural products isolated from molluscan species in the last three decades (Benkendorff, 2010, 2014). Two molluscan derived natural products have been clinically tested and approved by the Food and Drugs Administration (FDA); ziconotide from cone shells for the treatment of severe pain and Brentuximab vedotin for treatment of lymphoma and Hodgkin's disease (Mayer et al., 2010). There are at least 18 other compounds originally found in molluscs and associated cyanobacteria that are currently in clinical trials (Mayer, 2017). However, ~ 52% of the molluscan natural products that have been isolated to date have never been tested for any biological activity (Benkendorff, 2014). Furthermore, < 1% of known molluscan species have been studied for their secondary metabolites, although a large number of molluscan species have been used as a source of traditional medicines (Tables 1, 2), which has provided the stimulus for further research into the therapeutic potential of natural products derived from this phylum.

One of the most common therapeutic applications for molluscs in traditional ethnomedicine appears to be for the treatment of inflammatory conditions (Table 1). Inflammation is associated with and may contribute significantly to the pathogenesis of acute and chronic diseases such as atherosclerosis, obesity, multiple sclerosis, chronic obstructive pulmonary disease, asthma, rheumatoid arthritis, neurodegenerative disease and inflammatory bowel disease (Nathan and Ding, 2010). Inflammation can be described as the rapid response of the body to insults such as injury and infection. The inflammatory reaction is recognised macroscopically by four cardinal signs (which were described by Cornelius Celsus in the first century), that of *calor* (heat), *rubor* (redness), *tumor* (swelling), *dolor* (pain) and loss of function (Alessandri et al., 2013). The process of inflammation generally includes the isolation and removal of the injurious stimuli such as damaged cells, chemical irritants and infection, as well as the initiation of the healing process (De Zoysa, 2012). More specifically the response is a spatially and temporally arranged episode in which cells and mediators collaborate to neutralise and eliminate the damaging stimuli, to allow the restoration of homeostasis (Alessandri et al., 2013; Medzhitov, 2010). Although the inflammatory process promotes the elimination of damaging stimuli, the inflammatory process itself may also contribute to damage of neighbouring tissues and can in some cases increase the severity of pathology (Alessandri et al., 2013; Cara et al., 2000).

Current treatments for inflammatory diseases are primarily based on the use of steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) (Gunawardena et al., 2014), which is often reflective of the severity and responsiveness of the inflammation to the particular therapeutic regime. NSAIDs modulate their effect by preventing the synthesis of prostaglandins (PGs) *via* the inhibition of cyclooxygenase (COX) enzymes, which catalyse the conversion of arachidonic acid to PGs (Auriel et al., 2014; Seibert et al., 1997; Vane and Botting, 1998). However, current NSAID options have been linked to increased blood pressure, greatly increased risk of congestive heart failure, occurrence of thrombosis and they also can predispose patients to serious gastrointestinal erosion (Aisen et al., 2003; McMurray and Hardy, 2002). These side effects are common to almost all NSAIDs to some degree (Vane and Botting, 1998). Because anti-inflammatory drugs are among the most consumed pharmaceuticals, with over 70 million prescriptions and 30 billion tablets of NSAIDs sold over the counter each year (Maroon et al., 2006), there is an urgent need to search for safer sources of anti-inflammatory drugs. Steroidal anti-inflammatory drugs also have many disadvantages including immunosuppressing effects, as well as the resistance of many diseases to steroidal anti-inflammatory drugs (Barnes, 2006, 2010). Natural products have traditionally provided important leads for the development of pharmaceutical drugs and there is evidence that they could be a potential source of anti-inflammatory agents that could provide the benefit of greater activity and less side effects.

The aim of this review is to explore the traditional use of molluscs as a means for controlling inflammatory conditions, as well as critically analysing evidence from *in vitro* and *in vivo* studies, and human clinical trials, supporting the further investigation of molluscan derived extracts and natural products for anti-inflammatory, immune-modulatory and wound healing properties. This timely review of anti-inflammatory properties of molluscan natural products should help identify priority targets for future bioassay-guided isolation and development of novel potential anti-inflammatory agents.

2. Materials and methods

An extensive review of the scientific literature on molluscs with anti-inflammatory activities, immunomodulatory and wound healing activities was undertaken by searching bibliographic databases: MEDLINE/PubMed, Scopus, Web of Knowledge and Google Scholar. The keywords used in the search were 'anti-inflammatory' or 'immunomodulatory' or 'wound healing' AND 'mollusc'. Reference lists of published research articles were also checked for relevant data. Research articles were selected for inclusion if they tested an extract/s or compound/s isolated from species in the phylum Mollusca for anti-inflammatory or wound healing activity. Studies using *in vitro* assays for anti-inflammatory activity included inhibition of reactive oxygen species (nitric oxide), oxidative enzymes (nitric oxygen synthase, lipoxygenase, cyclooxygenase), cytokines (tumor necrosis factor alpha, interleukins, nuclear factor kappa B), immunoglobulin G or prostaglandins. *In vitro* papers testing for immune-modulation included

Table 1
Traditional use of molluscs in therapeutic preparations relevant to inflammation and wound healing summarising remedies used from around the world from ancient to current times. NA = Not available.

Country/Region	*Species and family*[a]	*Part used*	*Preparation*	*Therapeutic application*	*Reference*
Gastropoda					
Medieval Eastern Mediterranean	*Chicoreus ramosus* Linnaeus, 1758[b] *Lentigo lentiginosus* Linnaeus, 1758[c]	Operculum	NA	Skin diseases; wounds in the stomach; arthritis; eye and ear diseases; treatment of uterus; diseases	(Lev, 2007; Lev and Amar, 2008)
		Snail shell		Skin diseases, wounds in the stomach, arthritis	(Lev, 2007; Lev and Amar, 2008)
	Muricidae such as *Chicoreus virgineus* Röding, 1798[d]	Operculum	Smell the aromatic substance or smoke produced while placing the operculum on slowly burning charcoal	Rheumatism or arthritis; stomach problem (wounds in stomach); skin diseases; eye and ear diseases; tumors; treatment of uterus diseases	(Lev, 2007; Lev and Amar, 2008)
Islamic Nations (Middle ages)	*Aplysia depilans* Gmelin, 1791 (Aplysiidae)	NA	NA	Dyspnoea (breathing difficulties), dry cough, haemoptysis (coughing up blood)	(Meyerhof and Sobhy, 1932)
Ancient Greco-Roman (Dioscorides, Oribasius and Galen)	*Hexaplex trunculus* Linnaeus, 1758, *Bolinus brandaris* Linnaeus, 1758 and *Stramonita haemastoma* Linnaeus, 1767[e] (Muricidae)	Flesh and ashes of burned shell	Burned flesh along with shell	Wound healing; treatment of cracked skin; healing parotid gland swelling; anti-inflammatory properties	(Voultsiadou, 2010)
Ancient Greece and early Byzantium	*Hexaplex trunculus* Linnaeus, 1758, *Bolinus brandaris* Linnaeus, 1758 and *Stramonita haemastoma* Linnaeus, 1767[e] (Muricidae)	Flesh and shell	Flesh and ashes of burned shell	Anti-inflammatory; sore healing; menstrual cycle abnormalities	(Voultsiadou, 2010)
	Charonia tritonis Linnaeus, 1758 (Ranellidae)	Shell	Ashes of burned shell	Strengthens body's immune system; sore and wound healing	(Voultsiadou, 2010)
		Shell	Burned shell with salt	Remedy for burns and scalds	(Voultsiadou, 2010)
		Shell	Shells burned with salt	Remedy for burns and scalds	(Voultsiadou, 2010)
India	*Filopaludina* sp. Habe, 1964 (Viviparidae)	Foot	Soup prepared from the snail's foot	Asthma; arthritis; joint pain; rheumatism	(Prabhakar and Roy, 2009)
	Filopaludina bengalensis Lamarck, 1822[f] (Viviparidae)	Whole snail	Snails are collected from pond and are kept in clean fresh water in an earthen pot for night and the water is used like eye drop.	Conjunctivitis	(Prabhakar and Roy, 2009)
	Monetaria moneta Linnaeus, 1758[g] (Cypraeidae)	Shell	Burned shell powder	Asthma	(Krishna and Singh, 2012)
Latin America	*Pomacea lineata* Spix, 1827 (Ampullariidae)	NA	NA	Asthma, boils, ulcers	(Alves and Alves, 2011)
North east Brazil	*Littoraria angulifera* Lamarck, 1822[i] (periwinkle snail) (Littorinidae)	Flesh	Ingestion of the cooked flesh; ingestion of the crude flesh	Chesty cough	(Alves and Rosa, 2007a)
Jeju Island, Korea	*Omphalius rusticus* Gmelin, 1791 (Tegulidae)	Shell	Topical application of shell powder	Knee pain	(Kim and Song, 2013)
Europe	Not specified	Operculum	Ashes of calcified operculum	Healing cut veins	(Rätsch and Müller-Ebeling, 2013)
Zimbabwe	Not specified	Hozhwa (Snail) shells	Powdered shell heated and applied directly	Treatment for tropical ulcers	(Galfand et al., 1993)
Bivalvia					
Ancient Greece	*Chlamys* Röding, 1798 spp. and *Pecten* spp. O. F. Müller, 1776 (Pectinidae)	Flesh	Fresh or preserved in salt	Cystitis	(Voultsiadou, 2010)
Ancient Greece and early Byzantium	*Mytilus galloprovincialis* Lamarck, 1819 (Mytilidae)	Shell	Ashes of burned shell	Sores and wounds	(Voultsiadou, 2010)
	Ostrea edulis Linnaeus, 1758 (Ostreidae)	Shell	Ashes of burned and pulverised shell mixed with honey	Treatment of wounds and sores	(Voultsiadou, 2010)
India	Black-lip pearl Oyster (*Pinctada margaritifera* Linnaeus, 1758 (Pteriidae)	Pearl	Ashes of pearl with honey	Asthma and phthisis (tuberculosis)	(Gopal et al., 2008)
Latin America	*Crassostrea rhizophorae* Guilding, 1828 (Ostreidae)	Shell	Powdered	Osteoporosis; pneumonia; stomach ache; flu; pain relief; tuberculosis	(Alves and Alves, 2011; Alves and Rosa, 2007b)
	Neoteredo reynei Bartsch, 1920 (Teredinidae)	Shell	Powdered	Tuberculosis	(Alves and Alves, 2011)
	Lyrodus pedicellatus Quatrefages, 1849[h]	Shell	Powdered	Tuberculosis	(Alves and Alves, 2011)
	Anomalocardia flexuosa Linnaeus, 1767[i] (Veneridae)	Shell	Powdered	Asthma; flu; stomach ache	(Alves and Alves, 2011)
	Megalobulimus oblongus Müller, 1774 (Strophocheilidae)	Shell	Powdered	Asthma	(Alves and Alves, 2011)
	Cassis tuberosa Linnaeus, 1758 (Cassidae)	Shell	Powdered	Asthma	(Alves and Alves, 2011)
North east Brazil	*Crassostrea rhizophorae* Guilding, 1828 (Ostreidae)	Flesh and shell	Ingestion of powdered shell with food; ingestion of the cooked flesh	Osteoporosis	(Alves and Rosa, 2007a)

(continued on next page)

Table 1 (*continued*)

Country/Region	*Species and family*[a]	*Part used*	*Preparation*	*Therapeutic application*	*Reference*
	Anomalocardia flexuosa Linnaeus, 1767[i] (clam) (Veneridae)	Flesh and shell	Ingestion of powdered shell with food; ingestion of the cooked flesh	Asthma	(Alves and Rosa, 2007a)
	Mytella guyanensis Lamarck, 1819 (Mytilidae)	Flesh and shell	Ingestion of powdered shell with food; ingestion of the cooked flesh	Osteoporosis	(Alves and Rosa, 2007b)
Central Chaco, Argentina	*Anodontites trapesialis* Lamarck, 1819 (Mycetopodidae)	Shell	Shell ash applied externally	Skin wounds and injuries	(Martinez and Barboza, 2010)
Jeju Island, Korea	*Mytilus unguiculatus* Valenciennes, 1858[k] (Mytilidae)	Whole animal	Ingestion of decocted whole animal	Fever	(Kim and Song, 2013)
Cephalopoda					
Ancient Greece and early Byzantium	*Octopus vulgaris* Cuvier, 1797 (Octopodidae)	Flesh	Boiled or roasted flesh	Many gynaecological diseases and conditions	(Voultsiadou, 2010)
North east Brazil	*Cephalo. Loligo* sp. Lamarck, 1798 (squid) (Loliginidae)	Shell	Tea made from boiling the shell	Asthma	(Alves and Rosa, 2007a)

[a] The species name is only listed when a single species is identified for use in the traditional medicine. All those only listed at the family level use multiple species within the family. Species are based on the accepted name in the World Register of Marine Species (WoRMS Editorial Board, 2017).
[b] Listed in the publication as *Murex inflatus* Lamarck, 1822.
[c] Listed as *Strombus lentiginosus* Linnaeus, 1758.
[d] Listed as *Murex angulíferus* Lamarck, 1822.
[e] Listed as *Thais haemastoma* Linnaeus, 1767.
[f] Listed as *Bellamya bengalensis* Lamarck, 1822.
[g] Listed as *Cyprraea moneta* Linnaeus, 1758.
[h] Listed as *Teredo pedicellata* Quatrefages, 1849.
[i] Listed as *Anomalocardia brasiliana* Gmelin, 1791.
[j] Listed as *Littorina angulifer* Lamarck, 1822.
[k] Listed as *Mytilus coruscus* Gould, 1861.

phagocytosis assays using macrophages or neutrophils, and haemolysis using red blood cells, but papers that just screened for antibacterial activity were not included.

In vivo preclinical trials for anti-inflammatory activity were included if they used an antigen or adjuvant to stimulate the immune system in animal models for delayed-type hypersensitivity, paw oedema, ear oedema, experimentally induced arthritis, colitis, pyrexia, as well as trials on animals suffering from arthritis. Immuno-modulatory animal models were included that investigated carbon clearance or utilised immunisation followed by investigation of the immune response. Animal models for wound healing included hot-plate latency assay, skin burn and surgical wounds. Additional investigation of pain response was only included if contained within the same paper as any of the experiments listed above (*e.g.* tail immersion, hot plate and tail flick reaction time, induced writhing model). Research articles using mollusc extracts in human clinical trials were included for any form of arthritis and asthma, muscle injury and wound healing in burns patients.

Books and monographs, including review articles and regionally specific ethnomedical research articles (published in English) were used as a source of information about the historical use of molluscs in anti-inflammatory, immunomodulatory and wound healing medicines. The Chinese Compendium of *Materia Medica* was also searched for applications of mollusc in TCM and the Chinese *Marine Materia Medica* (Guan and Wang, 2009) was translated to English by the second author. English translations of the Arabic and Medieval Eastern Mediterranean *Materia Medica* (Lev and Amar, 2008) were also screened for any use of molluscs for conditions that could be related to inflammation.

Nomenclature of the mollusc species included in this review is corrected according to the World Register of Marine Species (WoRMS Editorial Board, 2017). All unaccepted names of species found in the literature have been replaced with the accepted names with reference to the name used in the original publication in footnotes.

3. Traditional use of molluscs for inflammatory conditions and wound healing

3.1. Overview

Molluscs have been involved in the everyday life of humans in many cultures throughout history, providing a source of shells, food, dyes and medicine (Benkendorff, 2010), in addition to the use of molluscs for magical-religious purposes (Léo Neto et al., 2012). Shelled molluscs are considered as healthy food and some species could be value-added as functional or medicinal foods, as they are still an important element in a variety of traditional natural medicines (Benkendorff et al., 2015; China State Administration Traditional Chinese *Materia Medica* Editorial Board, 1999; Lev and Amar, 2008). Molluscan natural medicines have been used in an extensive array of therapeutic applications such as for control of inflammation and in wound healing (Tables 1, 2).

3.2. Historical use of Molluscan natural products in ethnomedicine

Molluscs were an essential part of the ethnomedicine of many cultures throughout history. For example they were used to treat inflammation in Medieval Eastern Mediterranean, Ancient Greco-Roman and European communities and are still used by Zimbabwean, Indian and Latin American people (Table 1). There is also evidence that different parts of molluscs were used as remedies to treat a range of inflammatory diseases. For example, the operculum from Strombidae and Muricidae were used in the eastern Mediterranean medicine to treat a range of conditions including skin diseases, stomach ulceration and arthritis by smelling the aromatic substances in the smoke of the slowly burned opercula (Benkendorff et al., 2015; Lev and Amar, 2008) (Table 1), whilst in other parts of

Table 2
Traditional Chinese Medicines from molluscs as listed in the Chinese Marine *Materia Medica* (Guan and Wang, 2009) for use in conditions related to inflammation and/or wound healing.

Chinese Medicine	CLASS/ Family[a]	Part used	Preparation	Therapeutic application
Gastropoda				
Bao Yu	Haliotidae	flesh	Boil and eat the flesh or decoct and ingest (fresh: 6–9 g each time or sun-dried: 15–50 g each time)	Hectic fever; menstrual disorders
Jia Qi	Nacellidae	shell	Decoct and ingest (10–15 g each time)	Conjunctive congestion with swelling and pain, scrofula (inflammation of lymph nodes)
Jia Xiang	Turbinidae	operculum	Decoct and ingest (5–15 g each time), pulverised and homogenised with water for oral taking (3–9 g),	Abdominal swelling and pain
Zhui Luo Yan	Turritellidae	operculum	Decoct and ingest (15–30 g each time)	Conjunctive congestion with swelling and pain
Shi She	Vermetidae (*Thylacodes adamsii* Mörch, 1859)[b]	shell and flesh	Decoct and ingest (5–15 g each time), pulverised and homogenised with vinegar to apply externally	Carbuncle and swelling
Xie Shou Luo	Potamididae (*Pirenella cingulata* Gmelin, 1791)[c]	shell and flesh	Decoct and ingest (5–15 g each time)	Stomatitis (inflammation of the mouth); recurrent aphthous ulcer; gingivitis (swelling and aching of gums)
Feng Luo Ke	Strombidae	shell	Decoct and ingest (15–25 g each time)	Tuberculosis of lymph node; gastric and duodenal ulcer; furuncle carbuncle
Yu Luo Ke	Naticidae	shell	Decoct and ingest (15–50 g each time)	Tuberculosis of lymph nodes; gastric and duodenal ulcer; furuncle carbuncle
Zi Bei	Cypraeidae	shell	Decoct and ingest (15–25 g each time)	Pyretic dysentery; children with fever; heat-toxicity; conjunctive congestion with swelling and pain; acute and chronic sinusitis (inflammation of sinuses with pus and blood)
Bai Bei	Cypraeidae	shell	Decoct and ingest (5–15 g each time)	Typhoid fever; heat-toxicity; pyretic dysentery; acute and chronic sinusitis;chancre (syphilus lesion) and vulval sore
Rou Se Bao Bei	Cypraeidae (*Lyncina carneola* Linnaeus, 1758)[d]	shell	Ground into powder, decoct and ingest (5–15 g each time)	Heat-toxicity; pyretic dysentery; acute and chronic sinusitis; high fever
Yan Qiu Bei	Cypraeidae (*Erosaris erosa* Linnaeus, 1758)	shell	Ground into powder, decoct and ingest (5–15 g each time)	Phlegm; tuberculosis of lymph nodes; conjunctive congestion with swelling and pain
Guan Luo Ke	Harpidae, Cassidae	shell	Ground into powder, decoct and ingest (15–50 g each time)	Tuberculosis of lymph nodes; gastric and duodenal ulcer
Chun Luo Ke	Tonnidae	shell	Ground into powder or ustulate (scorch) the shell, decoct and ingest (15–30 g each time)	Tuberculosis of lymph nodes
Pi Ba Luo Ke	Ficidae	shell	Ground into powder, decoct and ingest (15–25 g each time)	Children with fever; night sweating; chronic tracheitis
Qian Xian Luo Ke	Ranellidae, Personidae	shell	Ground into powder, decoct and ingest (15–50 g each time)	Furuncle carbuncle; tuberculosis of lymph nodes; gastric and duodenal ulcer
Wa Luo Ke	Bursidae (*Bufonaria rana* Linnaeus, 1758)[e]	shell	Decoct and ingest (15–50 g each time)	Furuncle carbuncle; gastric and duodenal ulcer
Gu Luo	Muricidae (*Murex* spp., *Nassa francolina* Bruguière, 1789)	shell	Decoct the shell and ingest. Ustulate the shell, ground into powder and apply externally.	Clear heat; carbuncle; furuncle; otitis medium and ulcer of lower limb.
Liao Luo	Muricidae (*Mancinella* Link, 1807; *Thais* Röding, 1798; *Purpura* Bruguière, 1789 spp.)	shell	Decoct the shell (15–50 g) and ingest; used for making pills or medicinal powder; Ustulate the shell, ground into powder and apply externally.	Clear heat; swelling and ulcer on the body surface and scrofula
Ji Luo	Muricidae (*Chicoreus ramosus* Linnaeus, 1758)	shell	Decoct the crushed shell; Ustulate (scorch) the shell, ground into powder and apply externally.	Clear heat; scrofula (infection of the lymph nodes); Stomach and duodenal ulcer
Hai Luo	Muricidae (*Rapana* Schumacher, 1817 spp).	flesh	Boil and eat the flesh	Chest and abdomen heat and pain
Hai Luo Ke	Muricidae (*Rapana* spp.)	shell	Decoct the shell and ingest, used as medicinal powder; Ustulate the shell, ground into powder, mixed with sesame oil and apply externally.	Stomach and duodenal ulcer; scrofula
Hai Luo Yan	Muricidae (*Rapana* spp.)	operculum	Decoct the operculum (10–20 g) and ingest; Ustulate the shell, ground into powder and apply externally.	Swelling and ulcer on the body surface
He Ji Luo	Muricidae (*Chicoreus brunneus* Link, 1807)	shell	Decoct the shell and ingest.	Scrofula
Hong Luo	Muricidae (*Rapana rapiformis* Born, 1778)	shell	Decoct the shell (15–25 g) and ingest.	Scrofula
La Luo	Muricidae (*Indothais gradata* Jonas, 1846[f], *Reishia luteostoma* Holten, 1803)[g]	shell	Decoct the crushed shell (15–25 g)	Clear heat; scrofula; phlegm and cough; swelling and ulcer on the body surface
E Luo	Buccinidae, Nassariidae	shell	Ground into powder and ingest, or decoct and ingest (15–25 g each time); Ustulate (scorch) the shell and ground into powder and apply externally.	Gastric and duodenal ulcer; common skin infections; furuncle carbuncle
Xiang Luo	Buccinidae (*Neptunea cumingii* Crosse, 1862), Nassariidae (*Phos senticosus* Linnaeus, 1758)	shell	Decoct and ingest (15–25 g each time); apply externally	Tuberculosis of lymph nodes; gastric and duodenal ulcer; scald

(continued on next page)

Table 2 *(continued)*

Chinese Medicine	CLASS/ Family[a]	Part used	Preparation	Therapeutic application
Jiao Luo Yan	Melongenidae	operculum	Ground into powder, decoct and ingest (5–15 g each time); apply externally	Aching and limp; ulcer of lower limb
Jiao Luo Ke	Melongenidae	shell	Ustulate (scorch) the shell, ground into powder and ingest	Gastric and duodenal ulcer
Fei Luo Ke	Olividae	shell	Decoct and ingest (20–50 g each time)	Hectic fever
Xi Lei Luo Ke	Fasciolariidae	shell	Decoct and ingest (15–30 g each time)	Phlegm; gastric and duodenal ulcer
Yu Luo Ke	Conidae	shell	Decoct and ingest (15–25 g each time); Ustulate (scorch) the shell, ground into powder and ingest (3–6 g)	Tuberculosis of lymph; gastric and duodenal ulcer
Hai Fen	Alpysiidae	egg masses	Decoct and ingest (30–60 g each time);	Tuberculosis of lymph nodes
Bivalvia				
Wa Leng Zi	Arcidae, Noetiidae, Limidae	shell	Decoct and ingest (9–15 g each time); ground into powder for infusion (1.5–3 g); Ustulate (scorch) the shell and ground into powder and apply externally.	Phlegm in hypochondrium; ulcerative gingivitis; bleeding wound; burn and scald
Qing Han	Arcidae *(Barbatia obliquata* Wood, 1828)	shell	Decoct the crushed shell and ingest (15–50 g each time)	Menstrual disorders; gastric and duodenal ulcer
Bi Na Han	Arcidae (*Anadara inaequivalvis* Bruguiere 1789)[h]	shell	Decoct the crushed shell and ingest (15–50 g each time)	Tuberculosis of lymph; gastric and duodenal ulcer
Ban Niu Zhuan Han	Arcidae (*Trisidos semitorta* Lamarck, 1819)	shell	Decoct the crushed shell and ingest(15–50 g each time)	Gastric and duodenal ulcer
Jiang Yao Ke	Pinnidae	shell	Decoct and ingest (15–25 g each time)	Eczema
Zhen Zhu	Pteriidae	pearl	Ground into powder and ingest (0.3–1 g each time) or apply externally.	Conjunctive congestion; ulcers in the mouth and on the tongue; sores and ulcers; ruptured abscess resistant to healing; burn; scald
Zhen Zhu Mu	Pteriidae	prismatic layer, hypostracum	Ground into powder and apply externally.	Ulcer and pyogenic infections, eczema and pruritus
Ding Li	Malleidae	shell	Ground into powder and apply externally (5–15 g each time).	Eczema; furuncle and phyma
Qian Ge	Pteriidae (*Isognomon* spp.)	adductor muscle	Decoct and ingest (15–25 g each time); or ground into powder and ingest	High fever; tuberculosis of lymph nodes; gastric and duodenal ulcer
Hai Yu	Placunidae, Anomiidae	flesh and shell	Boil flesh and ingest, ground shell into powder for infusion and ingest	Eczema; arthrosis like crane knees/ osteoarthritis (flesh only)
Mu Li	Ostreidae, Grypaeidae	shell	Ground into powder and apply externally	Eczema and ulcer
Mu Li	Ostreidae, Grypaeidae	flesh	Smash flesh and apply externally	Tuberculosis of lymph nodes; swelling and aching of gum
Man Yu Ge Ke	Lucinidae, Carditidae, Semelidae, Glossidae	shell	Ground into powder and ingest, or decoct and ingest.	Tuberculosis of lymph nodes; gastric and duodenal ulcer; scald
Ke	Mactridae (*Mactra chinensis* Philippi, 1846)	shell	Decoct the crushed shell and ingest (25–50 g each time), ground into fine powder and apply externally	Gastric and duodenal ulcer; keratitis (inflammation of the cornea); skin ulcer
Ge Li Fen	Mactridae, Astartidae	Shell	Decoct the and ingest (50–100 g each time), ground into powder and ingest or apply externally	Gastric and duodenal ulcer; retention of phlegm and asthmatic cough; carbuncle and swelling; scrofula; eczema; scald
Xi Shi She	Mactridae *(Mactra antiquata* Spengler, 1802[i], Psammobiidae *(Haitula diphos* Linnaeus, 1771)[j]	Shell	Ustulate (scorch) the shell, decoct and ingest.	Tuberculosis of lymph nodes
Ying Ge Ke	Tellinidae	shell	Ustulate (scorch) the shell, ground into powder and ingest	Tuberculosis of lymph nodes, gastric and duodenal ulcer
Fu Ge Ke	Donacidae	shell	Ustulate (scorch) the shell, decoct and ingest.	Tuberculosis of lymph nodes, gastric and duodenal ulcer
Dou Fu Ge Ke	Donacidae (*Donax faba* Gmelin, 1791)	shell	Decoct and ingest (15–50 g each time)	Tuberculosis of lymph nodes
Zi Yun Ge	Psammobiidae	shell	Decoct and ingest (15–50 g each time)	Tuberculosis of lymph nodes, gastric and duodenal ulcer
Ge Ke	Veneridae	shell	Decoct the crushed shell and ingest (10–15 g each time), ground into powder and apply externally	Phlegmatic heat and cough; scrofula; ecthyma; eczema; scald; ruptured abscess resistant to healing
Fu Wen Ge Ke	Veneridae *(Meretrix lamarckii* Deshayes, 1853)	shell	Decoct and ingest (6–15 g each time), ground into powder and apply externally	Scrofula; eczema; scald
Jiang Hu Bu Mu Ge	Veneridae *(Leukoma jedoensis* Lischke, 1874)[k]	shell	Decoct and ingest (10–15 g each time), ground into powder and apply externally	Ecthyma
Ju Chi Ba Fei Ge	*Veneridae (Protapes gallus* Gmelin, 1791)[l]	shell	Decoct and ingest (10–15 g each time)	Scrofula
Hai Ge Ke	Veneridae	shell	Decoct and ingest (10–25 g each time), ground into powder and apply externally	Scrofula; asthma; eczema; scald; furuncle
Ge Zai	Veneridae (*Ruditapes* spp.)	flesh and shell	Boil and eat flesh, decoct and ingest, ustulate (scorch) the shell, ground into powder and apply externally	Ecthyma; asthmatic cough
Ying Ke Ge	Veneridae (*Mercenaria mercenaria* Linnaeus, 1758)	Shell	Decoct and ingest (10–15 g each time)	Dyspnoea (breathlessness) with cough; scrofula; gastric and duodenal ulcer
Ke Li Jia Fu Ge	Veneridae *(Gafrarium dispar* Holten, 1802)	shell	Decoct and ingest (5–15 g each time)	Gastric and duodenal ulcer
He Ai Ba Fei Ge	Veneridae (*Paphia amabilis* Philippi, 1847)	shell	Decoct and ingest (10–15 g each time)	Phlegmatic heat and cough; tuberculosis of lymph nodes; gastric and

(continued on next page)

Table 2 *(continued)*

Chinese Medicine	CLASS/ Family[a]	Part used	Preparation	Therapeutic application
				duodenal ulcer
Lv Lang Ke	Glauconomidae (*Glauconome chinensis* Gray, 1828)	shell	Decoct and ingest (15–50 g each time)	Tuberculosis of lymph nodes; gastric and duodenal ulcer
Sha Hai Lang	Myidae (*Mya arenaria* Linnaeus, 1758)	shell	Decoct and ingest (15–30 g each time)	Tuberculosis of lymph nodes; gastric and duodenal ulcer
Cephalopoda				
Qiang Wu Zei	Sepiidae	flesh	Boil and eat flesh	Rheumatic lumbago; ulcer of lower limb; ulcerative carbuncle; furuncle and phyma
Hai Piao Xiao	Sepiidae	cuttlebone	Decoct and ingest, ground into powder and apply externally	Stranguria with turbid discharge; eczema; skin ulcer with pus; ruptured abscess resistant to healing; trachoma (chronic inflammation of the mucous membranes of the eyes)
Zhang Yu	Octopodidae	flesh	Decoct and ingest, smash and apply externally	Sore and pyogenic infections; ruptured abscess resistant to healing
Polyplacophora				
Hai Shi Bie	Acanthochitonidae (*Acanthochiton rubrolineata* Lischke, 1873)	whole	Ground into powder and infusion, or make capsule or tablet for oral taking (2–6 g each time)	Asthma; pulmonary tuberculosis
Cuo Shi Bie	Ischnochitonidae	whole	Ground into powder and infusion for oral taking (1–3 g each time)	Asthma; pulmonary tuberculosis

[a] The species name is only listed when a single species is identified for use in the Chinese medicine. All those only listed at the family level use multiple species within the family. Species are based on the accepted name in the World Register of Marine Species (WoRMS Editorial Board, 2017).
[b] The original genus quoted in China Marine Herbal is *Serpulorbis imbricatus* Dunker, 1860.
[c] Listed as *Cerithidea cingulate* Gmelin, 1791.
[d] Listed as *Cypraea carneola* Linnaeus, 1758.
[e] Listed as *Bursa rana* Linnaeus, 1758.
[f] Listed as *Thais gradata* Jonas, 1846.
[g] Listed as *Thais luteostoma* Holten, 1803.
[h] Listed as *Scapharaca binakayanensis* (*Arca binakayanensis* Faustino, 1932).
[i] Listed as *Coelomactra antiquate* Spengler, 1802.
[j] Listed as *Sanguinolaria diphos* Linnaeus, 1771.
[k] Listed as *Prorothaca jedoensis* Lischke, 1874.
[l] Listed as *Paphia gallus* Gmelin, 1791.

Europe the ash of the opercula was used to heal severed veins rather than smelling the smoke of the opercula (Rätsch and Müller-Ebeling, 2013). According to the medieval *Materia Medica* of eastern Mediterranean (Lev and Amar, 2008) (Table 1) snail shells have also been used by various cultures throughout the world to treat wounds in the stomach, arthritis and skin diseases. Similarly, the shell of the mollusc *Monetaria moneta* (Linnaeus, 1758) (Table 1) has been used as a treatment for a number of diseases including asthma in India (Krishna and Singh, 2012), whilst in Zimbabwe snail shells were used to treat topical ulcers (Galfand et al., 1993). The fisher people in the Bihar region of India also use preparations from different parts of molluscs as a remedies for inflammation. For example, this group prepare a soup from the foot of the freshwater snail *Bellamya* sp. to treat asthma, arthritis, joint pain and rheumatism (Prabhakar and Roy, 2009). Although the specific parts or preparation methods for the molluscs used in Latin American ethnomedicines are not listed for each species, there is some evidence for the use of shells and powdered preparations for the treatment of asthma, skin ulcers, influenza, stomach pain, osteoporosis, pneumonia, pain relief and tuberculosis (Alves and Alves, 2011). There are at least 34 documented species of molluscs that were used in ethnomedicine from different cultures as remedies for inflammation (Table 1).

Traditional Chinese Medicine (TCM) is one of the oldest and best documented systems of ethnomedicine in the world. It still has a significant impact on the healthcare system of the people of China and Chinese communities outside China (Seong, 2015). Marine creatures and their products represent a crucial part in the TCM. Molluscs in particular, significantly contribute to the anti-inflammatory remedies available in TCM (Table 2). Molluscs in TCM are used to treat variety of inflammatory conditions including eczema, menstrual disorders, non-resolving ruptured abscesses, osteoarthritis, asthma, as well as for healing burns and scalds (Table 2). Natural product use of molluscs in TCM ranges from the use of the whole mollusc, shells, flesh, operculum, egg masses and even the pearl (Table 2). The mollusc shells are the most used part to treat inflammation with more than 50 preparations documented as useful anti-inflammatory remedies (Table 2). Other parts of mollusc which are often used as anti-inflammatory in TCM include flesh, with flesh from 8 different species used (Table 2).

The range of different mollusc species used in TCM include four different classes with two species of Polyplacophora, three Cephalopoda, 31 Bivalvia and 34 Gastropoda. Veneridae spp. is the most common used bivalve group within anti-inflammatory preparations (Table 2). Applications include ingesting the decocted shell or applying the ground shell, which is used to treat fever, phlegm and cough, scrofula, ecthyma, eczema, scald, ruptured abscess resistant to healing, gastric and duodenal ulcer. The most frequently used gastropod species used in the TCM for anti-inflammatory preparation were the muricids, with 9 anti-inflammatory remedies, which also include ingesting decocted shells and applying the ustulated powdered shells. Muricidae treatments are used for fever, carbuncle, furuncle, otitis medium, and ulcers of the lower limb, stomach and duodenum. Some preparations containing Muricidae flesh and operculua were used to treat chest and abdomen infection, pain, swelling and skin ulcers (Table 2). The ethnobiological history provides a repository of medicinal efficacy and experience as a sound source for further investigation. It is possible that scientific and evidence-based studies will provide a basis for the anecdotal reports on the use of traditional mollusc medicines and may help to substantiate their use.

4. *In vitro* studies on the anti-inflammatory and immunomodulatory activity of molluscan natural products

4.1. Overview

The prevalent use of molluscan natural products in ethnomedicine has drawn the attention of scientists in the last few decades to test defined molluscan products in the laboratory to verify the activity. There have been 13 different studies (mostly in the last 10 years) on the *in vitro* anti-inflammatory activity of gastropod molluscs (Table 3). On the other hand, seven studies have focused on just two different species of bivalve molluscs (Table 3).

4.2. Gastropoda

Muricidae feature in a number of records of traditional medicines (Table 1) and are also well known for their bioactive secondary metabolites (see recent review by Benkendorff et al., 2015), which include the brominated indole precursors to the dye Tyrian purple (Benkendorff, 2013; Cooksey, 2001). This dye is dominated by 6,6 dibromoindigo, although the purple secretion from some muricids has been shown to contain a mixture of both brominated and non-brominated indigo and indirubin (Cooksey, 2001). Indirubin has been found to prevent the increase of reactive oxygen species (ROS) from macrophages (Man et al., 2012). Brominated derivatives of indirubin have also shown anti-inflammatory activity in RAW264.7 and rat microglia cell culture (Kim and Park, 2012). With RAW264.7 cells in particular, indirubin was reported to inhibit the release of inflammatory cytokines interleukin (IL) IL-6 and IL-1β (Kim and Park, 2012). In addition, isatin, an oxidation product produced during Tyrian purple formation and a precursor of indirubin, has also been shown to have anti-inflammatory activity, as demonstrated in a lipopolysaccharide (LPS) and interferon gamma (IFNγ)-stimulated RAW264.7 model by: inhibiting the production of nitric oxide (NO); prostaglandin 2 (PGE2); inducible nitric oxide synthase (iNOS); cyclooxgenase 2 (COX-2) and tumor necrosis factor-alpha (TNFα) (Matheus et al., 2007). Recent studies have confirmed that extracts of the hypobranchial glands and egg masses of the Australian Muricidae *Dicathais orbita* Gmelin, 1791, along with the brominated indole precursors of Tyrian purple effectively inhibit NO, PGE2, TNFα and the translocation of the prototypical proinflammatory signalling molecule nuclear factor kappa B (NFκB) (Ahmad et al., 2017). The oxidation product 6-bromoisatin is of particular interest as a bioavailable multifunctional compound with demonstrated efficacy and safety in animal models for colon cancer prevention (Esmaeelian et al., 2014; Benkendorff et al., 2015), in addition to preventing acute lung inflammation in a recent mouse model after oral gavage (authors unpublished data). Indole anti-cancer drug leads have regularly shown promise for the treatment of a range of diseases including inflammation (Gul and Hamann, 2005). However, these indole compounds are only found in the hypobranchial glands and reproductive material of Muricidae (Benkendorff et al., 2015) and are present in trace amounts in the operculum and flesh and their presence in the shells is uncertain, which are the parts mostly used in traditional anti-inflammatory applications (Table 1). Consequently, Muricidae natural medicines could be optimised to include standard concentrations of these bioactive compounds along with other well characterised components in the shell of flesh that may improve the bioavailability and activity.

4.3. Bivalvia

Bivalves provide a source of food in many cultures, as well as being a source of natural remedies. These molluscs are rich in polyunsaturated fatty acids (PUFAs) which are 'healthy' lipids and reportedly found to have anti-inflammatory activity. For example, New Zealand green lipped mussel (*Perna canaliculus)* extracts have been the focus of much scientific research on their anti-arthritic activity, following observation that the coastal Maori population who consumed this mussel regularly suffered less from arthritis than the inland population (Halpern, 2000; Sankaran and Mouly, 2007). These observations lead to a series of formal studies that ultimately resulted in a range of lipid extracts from this mussel being commercialised and that are now available over the counter, including the clinically tested anti-inflam-

Table 3
In vitro anti-inflammatory, wound healing and immune-modulatory activity of extracts and compounds isolated from molluscs. The type of activity in specific assays is explained with the statistically significant concentrations relative to the negative controls (solvent or delivery agent).

Species	Geographic distribution	Extract or compound	Type of assay and concentrations used	Biological activity	Statistically significant concentrations	Reference
Gastropoda						
Haliotis discus hannai Ino, 1953 (disk abalone)	Eastern Asia (including Japan)	Extract from *H. discus hannai* fermented with *C. militaris* mycelia (HFCM-5)	LPS- stimulated RAW264.7 macrophages treated with 0.05, 0.1 or 0.2 mg/mL	a) Inhibited the production of NO in RAW264.7 macrophages. b) Decreased TNFα and IL-6 in a dose-dependent manner.	0.05, 0.1 or 0.2 mg/mL	(Joung et al., 2014)
		Abalone intestine digest	LPS- stimulated RAW264.7 macrophages treated with 50, 100, 250 or 500 μg/mL of the intestine digest	a) Supressed the production of NO *via* iNOS. b) Reduced the generation of TNFα, IL-6, IL1β.	50, 100, 250 or 500 μg/mL	(Qian et al., 2012)
Haliotis diversicolor Reeve, 1846	China	Shell powder	a) LPS-stimulated RAW264.7 treated with 1, 2, or 5 mg/mL of shell powder to evaluate the iNOS expression. b) Phagocytosis of FITC coated beads by RAW264 treated with treated with 1, 2, or 5 mg/mL of shell powder.	a) Decreased (iNOS) expression. b) Enhanced the functions of macrophages.	1, 2 and 5 mg/mL	(Chen et al., 2016)
Neverita didyma Röding, 1798[a] (the bladder moon snail)	Mozambique South Africa Madagascar	Acetone and methanol extracts of whole body	Human red blood cell (HRBC) membrane stabilisation method. 0.5 mL of 10% v/v HRBC suspension added to 3 mL of saline or distilled water medium before treated with 0.2, 0.4, 0.6, 0.8, or 1.0 mL	Both acetone and methanol extracts decreased the percentage of haemolysis with the acetone extract exhibited higher activity than methanol extract.	0.2, 0.4, 0.6, 0.8, and 1.0 mL	(Ravi et al., 2012)
Volegalea cochlidium Linnaeus, 1758[b]	Indo-West Pacific	Acetone and methanol extracts of whole body	Human red blood cell (HRBC) membrane stabilisation method. 0.5 mL of 10% v/v HRBC suspension added to 3 mL of saline or distilled water medium before treated with 0.2, 0.4, 0.6, 0.8, or 1.0 mL	Both acetone and methanol extracts decreased the percentage of haemolysis with the acetone extract exhibited higher activity than methanol extract.	0.2, 0.4, 0.6, 0.8, and 1.0 mL	(Ravi et al., 2012)
	Indo-West Pacific	The whole body ether extract	Tested for effects on *Candida albicans* phagocytosis by human neutrophils using slide method. The human neutrophils monolayer treated with 50, 100, 250, 500, 750, 1000, 2000, 5000 or 10,000 ng of the ether body extract.	a) Stimulated the phagocytosis in low concentrations. b) Suppressed the phagocytosis in higher concentration *in vitro*.	NA	(Ponkshe and Indap, 2002)
Euchelus asper Gmelin, 1791	Indo-West Pacific	The whole body ether extract	Tested for effects on *Candida albicans* phagocytosis by human neutrophils using slide method. The human neutrophils monolayer treated with 50, 100, 250, 500, 750, 1000, 2000, 5000 or 10,000 ng of the ether body extract.	a) Stimulated the phagocytosis in low concentrations. b) Suppressed the phagocytosis in higher concentration *in vitro*.	NA	(Ponkshe and Indap, 2002)
Filopaludina bengalensis Lamarck, 1822[c]	India	Footpad lipid extract	LPS-stimulated macrophage treated with 20 or 40 μg/mL	a) Inhibited reactive oxygen species (ROS), TNFα, and NO production. b) Inhibited the NFκB p65 translocation.	20 and 40 μg/mL	(Bhattacharya et al., 2014)
Aplysia fasciata Poiret, 1789	Gulf of Mexico Mediterranean Sea North Atlantic Ocean Red sea	Fatty acids	a) LPS- Stimulated RAW 264.7 treated with 15.6 −250 μg/mL of fatty acids from *A. fasciata*. b) Inhibition of lipoxygenase measured by non-cellular enzymatic assay based on linoleic acid. Concentrations of fatty acids used were: 2–4 mg/mL.	a) Decreased NO levels. EC_{50} 77 ± 7 μg/mL b) Inhibited the lipoxygenase. EC_{50} 3.06 ± 0.22 μg/mL	a) EC_{50} 77 ± 7 μg/mL b) EC_{50} 3.06 ± 0.22 μg/mL	(Pereira et al., 2015)
Aplysia punctata Cuvier, 1803	Mediterranean Sea North Atlantic Ocean Red sea	Fatty acids	a) LPS- Stimulated RAW 264.7 treated with 15.6 −250 μg/mL of fatty acids from *A. puncata*. b) Inhibition of lipoxygenase measured by non-cellular enzymatic assay based on linoleic acid. Concentrations of fatty acids used were: 2–4 mg/mL.	a) Decreased NO levels. EC_{50} 72 ± 12 μg/mL b) Inhibited the lipoxygenase EC_{50} 1.75 ± 0.09 mg/mL	a) EC_{50} 72 ± 12 μg/mL b) EC_{50} 1.75 ± 0.09 mg/mL	(Pereira et al., 2015)
Dicathais orbita Gmelin, 1791	Australia	Chloroform extract of the hypobranchial gland	a) LPS-stimulated RAW264.7 treated with 0.08, 0.4, 2, 10 or 50 μg/mL	a) Inhibited the production of NO EC_{50}: 30.8 μg/mL.	a) 10 and 50 μg/mL for NO assay.	(Ahmad et al., 2017)

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Table 3 *(continued)*

Species	Geographic distribution	Extract or compound	Type of assay and concentrations used	Biological activity	Statistically significant concentrations	Reference
			b) Calcium ionophore stimulated 3T3 ccl-92 fibroblast treated with 0.08, 0.4, 2, 10 or 50 μg/mL	b) Downregulated the production of TNFα in RAW264.7 $EC_{50:}$ 43 μg/mL. c) Inhibited the translocation of NFκB in RAW264.7. d) Downregulated the production of PGE2 in 3T3 fibroblasts $EC_{50:}$ 34.2 μg/mL.	b) 0.4, 2, 10 and 50 μg/mL for TNFα assay. c) 0.08, 0.4, 2, 10 and 50 μg/mL for PGE2 assay. d) 40 μg/mL in NFκB translocation assay	
		6-Bromoisatin	a) LPS-stimulated RAW264.7 treated with 0.08, 0.4, 2, 10 or 50 μg/mL b) Calcium ionophore stimulated 3T3 ccl-92 fibroblast treated with 0.08, 0.4, 2, 10 or 50 μg/mL	a) Inhibited the production of NO EC_{50}: 30.8 μg/mL. b) Downregulated the production of TNFα in RAW264.7 $EC_{50:}$ 43 μg/mL. c) Inhibited the translocation of NFκB in RAW264.7. d) Downregulated the production of PGE2 in 3T3 fibroblasts $EC_{50:}$ 34.2 μg/mL.	a) 0.4, 2, 10 and 50 μg/mL in NO assay. b) 0.08, 0.4, 2, 10 and 50 μg/mL in TNFα assay. c) 0.08, 0.4, 2, 10 and 50 μg/mL in PGE2 assay. d) 40 μg/mL in NFκB translocation assay.	(Ahmad et al., 2017)
Muricidae		Indirubin, a minor pigment in Tyrian purple and indirubin derivatives	a) LPS- Stimulated RAW 264.7 treated with. b) LPS-stimulated primary rat brain microglia 0.5, 1, 2, or 4 μM of Indirubin-3′-oxime.	a) Indirubin supressed the effect of extracellular ATP on macrophages b) Indirubin-3-oxime derivatives inhibited the release of IL-1β, TNFα and IL-6. c) Indirubin-3′-oxime inhibits inflammatory activation of rat brain microglia.	Indirubin: NA.Indirubin-3′-oxime: a) 4 μM for NO. b) 2 μM for TNFα, IL-β1, IL-6 and PGE2. c) 2 and 4 μM for NFκB.	(Jung et al., 2011; Kim and Park, 2012; Man et al., 2012)
Bivalvia						
Perna canaliculus Gmelin, 1791 (New Zealand green-lipped mussel)	New Zealand	Lyprinol (stabilised supercritical fluid fraction)	a) Arachidonic acid and calcium ionophore-stimulated human polymorphonuclear (PMN) leukocytes. b) LPS-stimulated human macrophages.	a) Inhibited the production of leukotrein B-4 by human PMN. b) Unfractionated lipid extract inhibited the production of PGE2 by human macrophages EC_{50} 1.2 μg/mL.	NA	(Whitehouse et al., 1997)
		An homologous series of novel omega 3 polyunsaturated fatty acids (omega-3 PUFA)	a) Arachidonic acid and calcium ionophore-stimulated human neutrophils treated with three dilutions of HPLC fractions 1:100, 1:1000 or 1:10,000.	Inhibited lipoxygenase products of the arachidonic acid pathway.	Three fractions showed apparent inhibition at: 1:100, 1:1000 and 1:10,000.	(Treschow et al., 2007)
		Biolane™ GLME	Biolane GLME digested with pepsin and pancreatic enzyme tested for its activity to prevent different osteoarthritic mechanism	a) Inhibited the biosynthesis of cholesterol, COX2, TNFα and PGE. b) Antioxidant activity. c) Anti-platelet aggregation activity. d) Fibrinolytic activity.	NA	(Cheras, 2005; Cheras et al., 2005)
		Freeze dried green-lipped mussel powder (Perna®)	a) LPS-stimulated human THP-1 monocytes treated with increasing concentrations of *Perna* extract: 0.0001, 0.001, 0.01, 0.1 or 1 mg/mL. b) Treated the peripheral blood neutrophils with increasing (100, 200 or 400 μg/mL) concentrations of *Perna* extract to measure superoxide burst.	a) Inhibited TNFα and IL-12p40 production in THP-I. b) Reduced neutrophil superoxide burst activity *in vitro*.	a) 0.1 and 1 mg/mL for TNFα and IL-12p40. b) 100, 200 and 400 μg/mL for superoxide inhibition.	(Lawson et al., 2007)
		Hydrochloric acid extract of the freeze-dried powder of *Perna canaliculus*	a) IgG antibody modulation V2E9 hybridoma cells treated with containing 0 μg, 5 μg, 10 μg, 15 μg, 20 μg, and 25 μg protein). b) Cytokine bioassays using V2E9, THP-1, L-929, U-937, A375. S2, Jurkat E6–1, EL-4, CTLL-2, LS174T, and 7TD1 cell lines.	Decreased IgG production.	a) For IgG suppression: 10 μg, 15 μg and 20 μg b) For cytokines bioassays: 5 μg, 10 μg, 15 μg,	(Mani and Lawson, 2006)

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Table 3 *(continued)*

Species	Geographic distribution	Extract or compound	Type of assay and concentrations used	Biological activity	Statistically significant concentrations	Reference
		Tween-20 extract of the freeze-dried powder of *Perna canaliculus*	a) IgG antibody modulation V2E9 hybridoma cells treated with containing 0 μg, 5 μg, 10 μg, 15 μg, 20 μg, and 25 μg protein). b) cytokine bioassays using V2E9, THP-1, L-929, U-937, A375. S2, Jurkat E6–1, EL-4, CTLL-2, LS174T, and 7TD1cytokine bioassays cell lines	a) Decreased IgG production b) Decreased the production of IL-2 and IL-6 c) Inhibited COX-1 and COX-2 cyclooxygenase activity	5 μg, 10 μg, 15 μg,20 μg and 25 μg	(Mani and Lawson, 2006)
Anadara kagoshimensis Tokunaga, 1906[d]	North-western Pacific	A novel polypeptide fraction (P2)	LPS-induced RAW264.7 treated with 15, 50 or 150 μg/mL of P2 fraction for NO inhibition assay and 1.33, 4 or 12 μg/mL for cytokines assays.	a) Inhibited the production of NO in LPS-stimulated RAW264.7 macrophage. b) Inhibited the secretion of IL-6 and TNFα in human cervical cancer HeLa cells. c) Downregulated the IL-8 d) Inhibited the COX-2 and iNOS-related pathways.	a) NO: 150 μg/mL b) IL-6: 4 and 12 μg/mL. c) TNFα: 1.33, 4 and 12 μg/mL. d) 12 μg/mL for IL-8, COX-2 and iNOS.	(Wu et al., 2014)

[a] Listed in the publication as *Natica didyma* Röding, 1798)
[b] Listed as *Hemifusus pugilinus* Born, 1778.
[c] Listed as *Bellamya bengalensis* Lamarck 1822)].
[d] Listed as *Arca subcrenata* (Lischke, 1869 (Ark shell).

matory nutraceutical called Lyprinol®. An *in vitro* study was performed to assay the ability of Lyprinol to inhibit the production of leukotrienes and 5-hydroxyeicosatetraenoic acid (5-HETE) by human neutrophils (Halpern, 2000). In this study, human neutrophils were sourced from human blood and incubated with increasing concentrations of Lyprinol, followed by stimulation with arachidonic acid (AA) and calcium ionophore, to trigger the 5-lipooxygenase (5-LOX) inflammatory pathway. The results showed significant inhibition of leukotrienes and 5-HETE synthesis. The NZ green lipped mussel extract (GMLE) has been shown to contain a unique omega-3 fatty acid, eicosatetraenoic acid (ETA), which appears to act as dual inhibitor of AA oxygenation by both the COX and LOX pathways (Bierer and Bui, 2002). A comparative study was also performed in Australia by Cheras et al. (2005) examining the activity of Biolane™ (another GMLE) and other well-known anti-arthritic agents such as chondroitin sulfate, glucosamine sulfate and Lyprinol. The study compared the ability of these anti-arthritic agents to inhibit different osteoarthritic mechanisms ranging from cholesterol biosynthesis inhibition, PGE inhibition, inhibition of COX-2 expression, TNFα inhibition, oxygen radical absorbance capacity-antioxidant (ORAC), anti-platelet aggregation activity and fibrinolytic activity. All the agents tested were initially digested with pepsin and pancreatic enzymes to simulate the *in vivo* digestive processes. Biolane showed broad activity with positive inhibitory results in all of the assays compared to the other tested anti-arthritic agents (Cheras, 2005; Cheras et al., 2005). Lyprinol® was also found to inhibit PGE2, cholesterol biosynthesis, phospholipase A2 (PLA2) and platelet aggregation (Cheras, 2005; Cheras et al., 2005). These observations provide evidence supporting the oral use of the GMLE for control of some inflammatory conditions.

There are only a few studies that have identified the bioactive compounds involved in anti-inflammatory activity from mollusc extracts and consequently there is a need for more bioassay guided fractionation and structure elucidation to determine the mode of action of these compounds. A large proportion of the *in vitro* studies listed in Table 3 have tested methanol, acetone or ether extracts, although a few studies have tested fatty acids extracts and only one study has tested the anti-inflammatory activity of a polypeptide from *Anadara kagoshimensis* Tokunaga, 1906 in lipopolysaccharide (LPS)-stimulated RAW264.7 (Wu et al., 2014). Consequently, there is need for further characterisation of methanol/ether extracts as they may lead to the identification of novel active ingredients and provide lead molecules for development of new anti-inflammatory drugs.

5. *In vivo* animal models using molluscan extracts for wound healing and inflammatory diseases

5.1. Overview

The promising anti-inflammatory results of molluscs are not only demonstrated in *in vitro* assays, but in fact the *in vivo* results are even more promising. Many studies in the last few decades have focused on *in vivo* anti-inflammatory activity of mollusc extracts in different animal models (Table 4). Out of the 40 *in vivo* studies on anti-inflammatory activity of the molluscs listed, there were 18 studies focused on gastropods, 18 on 8 different species of bivalves, and only 4 studies on cephalopods (Table 4). Extracts from the NZ green lipped mussel (*Perna canaliculus*) were the most examined using *in vivo* assays (Table 4).

5.2. In vivo anti-inflammatory models

Carrageenan is an inflammatory agonist standard as it induces paw oedema and is the most commonly used technique to screen the anti-inflammatory responses in animal models (Winter et al., 1962). This inflammatory agent is commonly used in experimental pharmacology because it causes inflammation by inducing histamine, 5-hydroxytryp-

tamine (5-HT) and prostaglandin (PG). This model has been used to test anti-inflammatory agents *in vivo* on 10 different mollusc extracts and all showed positive ant-inflammatory activity relative to appropriate delivery controls (Table 4). Some other animal models have also been used to test the anti-inflammatory activity of mollusc extracts and compounds *e.g.* rodent models for arthritis including adjuvant-induced and collagen-induced arthritis. These models allow study of arthritis *in vivo* by measuring many arthritis-specific factors, as well as general inflammatory factors. All these are well-established models to study anti-inflammatory activity and have been used to investigate the *in vivo* activity of mollusc extracts across a wide range of different doses (Table 4).

Following on from the promising *in vitro* anti-inflammatory results with use of *Perna canaliculus* extracts, *in vivo* studies have been conducted to examine if these finding could be replicated in animal models using green-lipped mussel extract (GLME) (Table 4). Miller and Ormrod (1980) reported that intraperitoneal (i.p.) injection of a crude GMLE significantly reduced the foot pad oedema in a carrageenan-induced oedema model in rats. Years later, in 1993, the same research group tested a glycogen extract from the same mollusc using the rat model, although but this time they used intra venous (i.v.) infusion of the extract and reported a significant reduction in the size of the foot pad oedema and suppression of neutrophil sequestration to the site of inflammation (Miller et al., 1993). In another study, Lawson et al. (2007) found that prophylactically treating Wistar rats with freeze dried GLM powder (Perna) effectively reduced the incidence, onset, and severity of collagen-induced arthritis. In addition, furan fatty acids derived from the GLM were found to inhibit adjuvant-induced arthritis in Wistar rats in a dose dependent manner (Wakimoto et al., 2011). In another study to assess the effect of stabilisation of the lipid extracts on their activity, rats were subjected to experimentally-induced inflammatory swelling before feeding with stabilised or non-stabilised GLME for 4 days (the days 10–13). The rats fed GLME stabilised with tartaric acid showed complete inhibition (100%) of swelling compared to use of non-stabilised GLME, which inhibited the swelling by 14%. This study demonstrated the importance of tartaric acid stabilisation of the lipid extracts which was observed to extend, as well as enhance the anti-inflammatory activity of the GLME (Halpern, 2000).

In vivo studies on a lipid extract from the Indian green mussel *Perna viridis* (Linnaeus, 1758) have shown that this related freshwater species, also contains anti-inflammatory activity, with the lipid extract significantly reducing carrageenan-induced oedema in rats. The extract was subsequently commercialised as anti-inflammatory product under the name of Cadalmin™ GMe (contains powder of freeze dried *P. viridis* as an active ingredient) (Chakraborty, 2012; Chakraborty et al., 2013a, b). Aqueous extract from the footpad of the Indian fresh water bivalve *Lamellidens marginalis* (Lamarck, 1819) also showed significant healing effects when fed to male albino rats with adjuvant-induced arthritis (AIA). The treated rats showed reduced paw oedema, paw weight and ankle diameter, and the bivalve extract significantly restored the levels of serum IL1β, IL6, CINC1, TNFα, IL10 and lysosomal enzyme levels, as well as reducing the levels of neutrophil infiltration (Chakraborty et al., 2010).

The anti-inflammatory properties or bioactive extracts from fresh water snails are also of interest to natural product researchers. The supernatant of homogenised tissue derived from the fresh water snail *Filopaludina bengalensis* (Lamarck, 1822) was used in an *in vivo* study to examine anti-osteoporotic and anti-osteoarthritic activity (Sarkar et al., 2013). Osteoarthritic and osteoporotic Wistar male/female albino rats received the *F. bengalensis* extract orally for 15 days. The results suggested a significant improvement in urinary (hydroxyproline/glucosamine/calcium/phosphate/creatinine) and serum parameters (serum acid phosphatase/alkaline phosphatase/Tartrate Resistant Acid Phosphatase (TRAP)), calcium/creatinine, and cytokines (TNFα/IL-1β/Cytokine-Induced Neutrophil Chemoattractant-1 (CINC-1)) in rats that received the snail extract (Sarkar et al., 2013), supporting the anti-osteoarthritis activity of the fresh water molluscs. Further purification of the high molecular weight protein fraction (VB-P4) from the flesh extract of this fresh water gastropod also demonstrated significant anti-osteoporosis activity in an experimentally induced osteoporotic model in rats. The results showed significant decrease in the calcium, creatinine and phosphate in urine and decreased levels of calcium and creatinine in serum, in addition to significantly decreased levels of TNFα, IL-12, IL-6 and PGE2 relative to the positive osteoporosis control (Sarkar et al., 2015b). The effects of the snail protein were comparable to the standard anti-osteoporosis drug treatment of vitamin D_3, arachitol (200 mg kg^{-1}) and calcium (1500 mg kg^{-1}). Another protein fraction of the aqueous flesh extract from the same mollusc (VB-P5) demonstrated anti-osteoarthritic, anti-nociception, anti-inflammatory activity in Wistar albino rat models. Feeding the rats the purified fraction (VB-P5) prevented ankle/knee swellings, decreased the urinary glucosamine, calcium, phosphorous, and creatinine levels, suppressed the serum ACP, ALP and TRAP enzymes, as well as serum calcium and creatinine, decreased the levels of TNFα, IL-12, IL-12 IL-1β and PGE2, reduced the size of paw and ear oedema and increased the reaction time of hot plate and tail flick model (Sarkar et al., 2015a). All these results support the anti-inflammatory activity of this Indian fresh water snail.

Cephalopods are not an exception when it comes to the anti-inflammatory activity of molluscs. Several studies have been carried out to support the anti-inflammatory properties of cephalopod products (Table 4). For example, assays of ink extract from *Sepia officinalis* (the common cuttlefish) demonstrated anti-inflammatory activity in Swiss mice by inhibiting acetic acid-induced writhing and increasing the latency period of mice on the hot-plate, with the LD_{50} above 2000 mg/Kg i.p. (Soliman and Fahmy, 2013). Another study using *Sepia pharaonis* (Pharaoh Cuttlefish)-derived liver oil showed a major reduction in both carrageenan-induced and formalin-induced paw oedema (Joseph et al., 2005). *Ommastrephes bartramii* Lesueur, 1821 (Neon flying squid) also suppressed the enhanced capillary permeability and inhibited both leucocyte emigration and protein exudation into the pouch fluid in the granuloma pouch model in rats (Mimura et al., 1987) (Table 4). The results of these studies provide evidence to support the anti-inflammatory activity of cephalopods, in addition to gastropods and bivalves.

5.3. *In vivo* wound healing assays

Wound healing from skin burn and cut models have also been used to assess the wound-healing activity of mollusc extracts (Table 4), where defined diameter temperature controlled skin heater or sterilised blades are used to induce local cutaneous injuries on the dorsum skin. In addition, delayed hypersensitivity models where rodents are injected with an antigen-adjuvant have been used to induce delayed-type hypersensitivity demonstrating many measurable inflammatory factors. Immunomodulation is also measured using the carbon clearing model in rat/mice (Table 4). In this model rat/mice are injected with a carbon particle suspension days after the administration of test compound/extract. Small aliquots of venous blood drawn periodically are lysed with acetic acid before the amount of carbon in the blood is measured colourimetrically at 660 nm, which indicates the phagocytic activity (Ponkshe and Indap, 2002). Furthermore, animal models have been used to test the anti-pyretic activity of mollusc extracts. In this model, rodents are injected with a certain amount of yeast mixture to stimulate inflammation and consequently high temperature and the anti-pyritic activity is assessed by measuring the temperature of the animal. Analgesic activity has also been tested *in vivo* in a well-established animal model where the animal is triggered by a thermal or physical stimulus to show sensitivity of animals to the stimulus used, indicating the degree of pain.

The *in vivo* wound healing properties of mollusc extracts have been studied following evidence associated with the traditional use of some

Table 4
In vivo anti-inflammatory, wound healing and immune-modulatory activity of extracts and compounds isolated from molluscs in a) animal models and b) human clinical trial. The active dose is used to describe the dose at which a statistically significant effect was observed relative to the negative controls.

Species	Geographic distribution	Extract or compound	Type of animal model	Biological activity	Active dose	Reference
Gastropoda						
Helix pomatia Linnaeus, 1758 (terrestrial snail)	Local to Central and Southeast Europe but now moved by human to Asia and the Americas	Hemocyanin (HpH)	Female 8-week old Balb/c mice immunised with 16 μg, 40 μg, and 100 μg/mouse HpH combined with tetanus toxoid (TT) (i.p.)	a) Increased number of anti-TT IgG producing plasmocytes b) Induced a significant increase of B and T cell proliferation.	a) 16 μg, 40 μg and 100 μg/mouse b) 100 μg/mouse	(Gesheva et al., 2015)
Filopaludina bengalensis Lamarck, 1822[a]	India	Footpad lipid extract	Antigen-adjuvant induced delayed-type hypersensitivity	a) Decreased paw oedema. b) Decreased NO level, serum TNFα level and CINC 1 level c) Decreased splenic $CD4^+/CD8^+$ ratios. d) Increased the level of T_{reg} cells.	20 and 40 μg/mL	(Bhattacharya et al., 2014)
		Extra-pallial fluid	a) Carrageenan (s.c.) –induced Paw Oedema in male Wistar rats (150–160 g body weight) received 1.8, 3.6 or 7.2 μg/g, body weight, of extract orally. b) Freund's adjuvant (into the sub plantar pad)-induced Poly-arthritis in Wistar rats received the same doses above. c) Eddy's Hot Plate Method on male Swiss mice (25–30 g body weight) received oral dose of 3.6, 7.2 or 14.4 μg/mL of the extract. d) Tail Immersion Method on Swiss mice received oral dose of 3.6, 7.2 or 14.4 μg/mL of the extract.	a) Lowered paw inflammation in carrageenan-induced rats and in Freund's adjuvant-induced rats. b) Showed analgesic dependency in Eddy's hot plate and tail immersion in mice.	a) 1.8, 3.6 and 7.2 μg/g in. b) 1.8, 3.6, 7.2 and 14.4 μg/g.	(Adhikari et al., 2015)
Haliotis diversicolor Reeve, 1846	China	Shell powder	full-thickness burn injury in male Wistar rat treated topically with 0.2 mL of 600 mg/mL or 200 mg/mL daily	a) Decreased neutrophil infiltration. b) Promoted wound healing noticeably after 14 days and was mostly healed on day 28. The wound	600 mg/mL and 200 mg/mL	(Chen et al., 2016)
Filopaludina bengalensis Lamarck, 1822[b]	India	10% Aqueous flesh homogenate (VBE)	a) Bilateral overectomy-induced osteoporotic and bacterial collagenase injection-induced osteoarthritic Wistar rats were fed orally with 1 g/kg or 2 g/kg VBE for 15 days.	a) Prevented ankle/knee swellings. b) Decreased the urinary markers. c) Suppressed the serum enzymes and cytokines (TNFα and IL-1β).	1 g/kg and 2 g/kg	(Sarkar et al., 2013)
		Purified protein fraction VB-P4 from the aqueous flesh extract	Experimentally induced-osteoporotic model in Wistar female albino rats treated with an i.p. dose of 400 μg/100 g or 200 μg/100 g VB-P4 for 15 days.	a) Restored the bone structural architecture. b) Decreased the urinary glucosamine, calcium, phosphorous, and creatinine levels. c) Suppressed the serum ACP, ALP and TRAP enzymes as well as serum calcium and creatinine. d) Decreased the levels of TNFα, IL-12, IL-12 IL-1β and PGE2.	400 μg/100 g and 200 μg/100 g	(Sarkar et al., 2015b)
		Purified protein fractions VB-P4/VB-P5 from the aqueous flesh extract	a) Bacterial collagenase induced-osteoarthritic model in Wistar male albino rats. b) Hot plate and tail flick model in male Swiss mice received 400 μg/100 g of VB-P4 or VB-P5i.p. c) Xylene induced ear oedema in male Swiss mice received 400 μg/100 g of VB-P4 or VB-P5 i.p.	a) Prevented ankle/knee swellings. b) Decreased the urinary glucosamine, calcium, phosphorous, and creatinine levels. c) Suppressed the serum ACP, ALP and TRAP enzymes as well as serum calcium and creatinine. d) Decreased the levels of TNFα, IL-12, IL-12 IL-1β and PGE2.	Both fractions VB-4 and VB-5 were 400 μg/100 g.	(Sarkar et al., 2015a)

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Table 4 *(continued)*

Species	Geographic distribution	Extract or compound	Type of animal model	Biological activity	Active dose	Reference
			d) Carrageenan-induced paw oedema in male Swiss mice received 400 μg/100 g of VB-P4 or VB-P5 i.p.	e) Reduced the size of paw and ear oedema. f) Increased the reaction time of hot plate and tail flick model.		
Tectus tentorium Gmelin, 1791[c]	India	100% Acetone fraction of crude extract purified by silica gel column	a) Analgesic effect in Swiss mice hot plate model and acetic acid-induced writhing model treated with 25 or 50 mg/kg body weight orally (p.o) of the extract. b) Carrageenan-induced rat paw oedema on albino rats treated with 25 or 50 mg/kg (p.o) of the extract.	a) Inhibited acetic acid-induced abdominal constrictions. b) Inhibited the writhing. c) Decreased paw thickness.	25 and 50 mg/kg	(Chellaram et al., 2012)
		100% Acetone fraction of crude extract purified by silica gel column	Carrageenan-induced paw oedema in rats received 100 and 200 mg/kg (p.o.).	Decrease the paw thickness.	100 and 200 mg/kg	(Chellaram and Edward, 2009b)
Drupella margariticola Broderip, 1833[d]	Indian ocean; Japan; Madagascar; Seychelles; Tanzania	100% Acetone column purified extract	Carrageenan-induced paw oedema in rats received (p.o.) dose of 50 or 100 mg/kg of the extract.	Decreased in the paw thickness.	50 and 100 mg/kg	(Chellaram and Edward, 2009a)
Babylonia zeylanica Bruguière, 1789	India	Benzene: methanol extracts	a) Carrageenan induced paw oedema in Wistar albino rats received orally 100 or 200 mg/kg of the extract. b) Tail-immersion method for estimation of pain. c) Yeast induced pyrexia.	a) Reduced the paw oedema in rats. b) Decreased the pain in analgesic activity. c) Reduced the yeast induced pyrexia raised body temperature.	a) 100 and 200 mg/kg. b) 200 mg/kg. c) 100 and 200 mg/kg.	(Santhi et al., 2012)
Rapana venosa Valenciennes, 1846[e] (veined rapa whelk)	Western Pacific Ocean (and invasive in some other regions in the northern hemisphere)	Hemocyanin (RtH)	Mice immunised multiple times with RtH incorporated with influenza A hemagglutinin (i.p.). each mice received 250, 100 or 40 μg of RtH.	Induce strong humoral immune response and could be used as adjuvant.	40, 100 and 250 μg/mouse	(Tchorbanov et al., 2008)
	Western Pacific Ocean (and invasive in some other regions in the northern hemisphere)	Amino acids	Skin burns in Wistar rats treated with amino acids at a concentration of 0.3 mg/kg twice a day.	Accelerate skin wounds healing *via* enhancement of dermal and epidermal neoformation	0.3 mg/kg	(Badiu et al., 2010)
		Lipid extract	Skin burns in Wistar rats treated topically with 0.2 mg/kg lipid extract twice a day.	very efficient in healing induced skin burns	0.2 mg/kg	(Badiu et al., 2008)
*Fissurella latimarginata*Sowerby, 1835	Pacific ocean	Hemocyanin (FLH)	C57BL/6 female mice were immunized with 200 μg of FLH and then with FLH coupled with 2, 4-dinitrofluorobenzene (DNFB) subcutaneously (s.c.).	Produced high robust specific humoral immune response: a) Increased IFN-γ and higher numbers of tumor-infiltrating CD4+ lymphocytes. b) Rapidly increased the generation of IL-6, IL-12, IL-23 and TNFα by dendritic cells.	200 μg/mice	(Arancibia et al., 2014)
Purpura persica Linnaeus, 1758	India	100% Chloroform purified extracts (in 0.5% (W/v) suspension of Sodium Hydroxide)	a) Carrageenan-induced oedema in Wistar albino rats treated with 100 and 200 mg/kg, p.o. purified chloroform extract. b) Tail-immersion method. c) Brewer's yeast-induced pyrexia in albino rats received 100 and 200 mg/kg, (p.o.)	a) Reduced carrageenan induced oedema. b) Reduced the pain in analgesic activity. c) Reduced the high temperature of the yeast-infected rats. d) No toxicity up to 800 mg/Kg dose.	a) 100 and 200 mg/kg. b) 100 and 200 mg/kg. c) 100 and 200 mg/kg.	(Santhi et al., 2011)
Euchelus asper Gmelin, 1791	Indo-West Pacific	The whole body ether extract	a) Carbon clearance methods in Swiss albino mice. b) Delayed type Hypersensitivity (DTH) reaction. c) Plaque Forming Cell (PFC) assay.	a) Immunosuppressive activity *in vivo*. b) Stimulated DTH. c) Reduced the number of plaque forming cells.	40, 80 and 160 mg/kg	(Akerkar et al., 2009; Ponkshe and Indap, 2002)
Volegalea cochlidium Linnaeus, 1758[f]	Indo-West Pacific	The whole body ether extract	a) Carbon clearance methods in Swiss albino mice received 40, 80 or 160 mg/kg of the extract (i.p.).	a) Immunosuppressive activity *in vivo*. b) Increased the plaque forming cells.	40 and 80 mg/kg in carbon clearance method.	(Akerkar et al., 2009; Ponkshe and Indap, 2002)

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Table 4 *(continued)*

Species	Geographic distribution	Extract or compound	Type of animal model	Biological activity	Active dose	Reference
			b) Delayed type Hypersensitivity (DTH) reaction. c) Plaque Forming Cell (PFC) assay.			
Bivalvia						
Perna canaliculus Gmelin, 1791 (New (Zealand green-lipped mussel)	New Zealand	Furan fatty acid (F6) (active component of Lyprinol)	Adjuvant-induced arthritis in Wistar rats received 1, 5 or 10 mg/kg F6 for 5 days.	Showed dose dependent inhibition of adjuvant-induced arthritis in Wistar rats	10 mg/kg	(Wakimoto et al., 2011)
		A crude fraction of the New Zealand GLM	A carrageenan-induced inflammatory oedema of the rat hind footpad. These rats received 500 mg/kg of the crude preparation (i.p.) or (p.o.).	Effectively reduced the footpad oedema but only if injected (i.p.).	500 mg/kg	(Miller and Ormrod, 1980)
		Lyophilized *Perna canaliculus* powder (Perna®)	Collagen-induced arthritis (CIA) in female Wistar rats received 100 mg/kg/day of Perna.	Significant reductions in disease incidence, onset, and severity of CIA in rats.	100 mg/kg/day	(Lawson et al., 2007)
		Freeze-dried, stabilized GLM powder	Dogs suffering from arthritis were fed GLM powder (> 34 kg weighed dogs received 1000 mg/day 34–25 kg received 750 mg/d; < 25 kg received 450 mg/d).	Significantly reduced: a) Total arthritic scores. b) Scores for joint pain and joint swelling.	a) For > 34 kg weighed dogs = 1000 mg/day b) 34–25 kg = 750 mg/d; c) < 25 kg = 450 mg/d.	(Bierer and Bui, 2002)
		Glycogen extracts	Carrageenan induced foot pad oedema in female Dark Agouti rats received 5, 10, 15, 20 or 25 mg (i.v.) injection of the glycogen extract.	a) A) Decreased foot pad oedema. b) Reduced the infiltration of neutrophils.	5, 10, 15, 20 and 25 mg/rat	(Miller et al., 1993)
		Super fluid extracted, lipid-rich extract of Lyprinol®	Adjuvant-induced polyarthritis in Wistar and Dark Agouti rats. Collagen (II)-induced autoallergic arthritis in Wistar and Dark Agouti rats.The rats received 5 mg/rat Lyprinol (p.o.).	Inhibited arthritis development.	5 mg/rat	(Whitehouse et al., 1997)
		GLM lipid extract (Seatone)	Adjuvant-induced Arthritis in female Wistar rats received 300 mg/kg of Seatone.	a) Reduced the thickness of rear paw b) Reduced the inflammatory score in the fore paw	300 mg/kg	(Whitehouse et al., 1999)
		Lyprinol®	Dextran sulfate sodium- induced colitis model of male C57BL/6 mice 5 mg/mouse of Lyprinol *via* oral gavage for 13 days.	Reduced the loss of body weight. Reduced disease activity index. Reduced crypt area losses. Reduced cecum and colon weight.	5 mg/mouse	(Tenikoff et al., 2005)
		Lipid extract (GMLE)	Adjuvant-induced (AIA) and collagen-induced arthritis (CIA) in rats received 100 mg/kg of HMLE for 30 days.	a) Reduced the swelling of paw oedema. b) Suppressed the inflammatory mediators (LTB4, PGE 2, and TXB2). c) Suppressed pro-inflammatory cytokines (IL-1, IL-6, INFγ-, and TNFα) and MMPs (MMP1, MMP13) promoted anti-inflammatory cytokines (IL-4, IL-10) and TIMPs (TIMP1) productions.	100 mg/kg	(Li et al., 2014)
Perna viridis Linnaeus, 1758	Indian ocean, South pacific ocean, Coral sea, and Caribbean Sea	Cadalmin™ GMe	Histamine-induced paw oedema.	Showed significant decrease in histamine-induced oedema.	NA	(Chakraborty, 2012)
		Methanol and aqueous/ethanol extract of the whole body tissue.	a) Carrageenan induced paw oedema. b) Formalin-induced chronic paw oedema. c) Dextran-induced acute paw oedema. Male Swiss Albino mice received (100, 500 or 1000 mg/kg) of the extracts (p.o.) for 7 days in all assays listed above.	a) Showed significant decrease in carrageenan-induced oedema. b) Inhibited the formalin-induced inflammation. c) Methanol extract inhibited the dextran-induced paw oedema.	a) And b) 100, 500 and 1000 mg/kg. b) 500 and 1000 mg/kg MeOH extract only.	(Sreejamole et al., 2011)

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Table 4 *(continued)*

Species	Geographic distribution	Extract or compound	Type of animal model	Biological activity	Active dose	Reference
Lamellidens marginalis Lamarck, 1819	India	Aqueous extract of footpad	Adjuvant-induced Arthritis in male Albino rats received 500 mg/kg/day and or 1 g/kg/day of extract for 13 days.	a) Reduced paw diameter, ankle diameter and paw weight. b) Restored serum IL1_, IL6, CINC1, TNFα, IL10 and lysosomal enzyme levels. c) Restored the level of neutrophils infiltration.	500 and 1000 mg/kg	(Chakraborty et al., 2010)
Parreysia cylindrical Annandale & Prashad, 1919	India	Processed shell powder	a) Carrageenan induced paw oedema in Wistar albino rats received 100 or 200 mg/L (p.o.). b) Analgesic activity model (sensitivity to warm water) in Wistar albino rats received an oral dose of 100 mg/mL or 200 mg/mL. c) Wound healing properties in albino rats by inducing 2 cm wound using sterilised blades. Rats then treated topically with ointment prepared from 15% shell powder in egg albumin.	a) Reduced the carrageenan paw oedema. b) Decreased the pain in an analgesic activity. c) Scar formed on 2 cm wound in thigh region in 8 days. d) No toxicity up to 800 mg/Kg dose.	a) 100 and 200 mg/mL. b) 100 and 200 mg/mL c) 15% of shell powder in egg albumin.	(Swapna, 2015)
Mytilus unguiculatus Valenciennes, 1858[g] (Hard-shelled mussel)	Subtropical Western Pacific Ocean	Lipid extract (HMLE)	Adjuvant-induced (AIA) and collagen-induced arthritis (CIA) in rats received 100 mg/kg of HMLE for 30 days.	a) Reduced the swelling of paw oedema. b) Suppressed the inflammatory mediators (LTB4, PGE (2), and TXB2). c) Suppressed pro-inflammatory cytokines (IL-1, IL-6, INFγ-, and TNFα) and MMPs (MMP1, MMP13) promoted anti-inflammatory cytokines (IL-4, IL-10) and TIMPs (TIMP1) productions.	100 mg/kg	(Li et al., 2014)
Mytilus galloprovincialis Lamarck, 1819 (Mediterranean mussel)	Global in temperate to polar waters (Northern and Southern Hemisphere)	Amino acids	Skin wound healing in Wistar rats topically treated with 0.3 mg/kg of amino acids.F	Accelerated skin wound healing *via* enhancement of dermal and epidermal neoformation.	0.3 mg/kg	(Badiu et al., 2010; Soliman and Fahmy, 2013)
		Lipid extract	Skin burns in Wistar rats treated topically with 0.2 mg/kg lipid extracts 2 time/day for 22 days.	Very efficient in healing induced skin burns.	0.2 mg/kg	(Badiu et al., 2008)
Coelatura aegyptiaca Cailliaud, 1827 (Egyptian freshwater mussel)	Northeastern Africa Northern Africa Western Africa	Fresh mussel extract	a) Hot plate latency assay. b) Acetic acid-induced writhing test and formalin induced paw licking in mice.Mice treated with 200 mg/kg of the extract (i.p.).	a) Reduced the paw licking times. b) Slightly increased the latency time of mice in the hot plate.The LD_{50} was above 2000 mg/Kg i. p.	200 mg/kg	(Soliman and Fahmy, 2013)
Pinctada imbricate Röding, 1798[h]	China	Pearl used (Zhikang Capsule (ZKC) component)	Dextran Sodium Sulfate-induced colitis in C57BL/6 mice fed on 170, 340 or 680 mg/kg ZKC.	● Supressed TNFα, IFN-γ, IL-1β, and IL-12. ● Promoted anti-inflammatory mediators (IL-4 and IL-10).	680 mg/kg	(Fei and Xu, 2016)
Cephalopoda						
Sepia officinalis Linnaeus, 1758 (the common cuttlefish)	Mediterranean Sea, North Sea, and Baltic Sea.	Ink extract	a) Hot plate latency assay. b) Acetic acid-induced writhing test and formalin induced paw licking in mice.Mice treated with 200 mg/kg of the extract (i.p.).	a) Inhibited the acetic acid-induced writhing. b) Increased the latency period of mice in the hot-plate.	200 mg/kg	(Soliman and Fahmy, 2013)
Sepia pharaonic Ehrenberg, 1831 (Pharaoh cuttlefish)	Indian Ocean eastern; Indian Ocean western; Pacific northwest; Pacific western central	Liver oil	Carrageenan and formalin induced paw oedema in male albino Sprague Dawley rats fed on 1% cuttlefish liver oil for 45 days	Decreased both carrageenan-induced and formalin-induced paw oedema.	1% in the diet for 45 days	(Joseph et al., 2005)
Ommastrephes bartramii Lesueur, 1821[i] (Neon flying squid)	Subtropical and temperate waters of the Pacific, Atlantic, and Indian Oceans	Low-molecular-weight melanoprotein from *Ommastrephes bartramii* (Fr.SM II)	Carrageenan-induced rat paw oedema. Inducing mouse ear with xylene. Carboxymethyl cellulose pouch method. Mice/rats were injected (i.v.) with 10 or	Reduced the paw oedema. Suppressed the enhanced capillary permeability caused by xylene. Inhibit both leucocyte emigration and protein exudation into the pouch fluid.	10 and 20 mg/kg in the paw oedema assay 20 mg/kg in enhanced capillary permeability induced by xylene assay	(Mimura et al., 1987)

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Table 4 (*continued*)

Species	Geographic distribution	Extract or compound	Type of animal model	Biological activity	Active dose	Reference
			20 mg/kg of (Fr.SM II).		in mice and pouch method in rats	
Sepiella inermis Van Hasselt, 1835[j]	China	Inner shell (Zhikang Capsule (ZKC) component)	● Dextran Sodium Sulfate-induced colitis in C57BL/6 mice fed on 170, 340 or 680 mg/kg ZKC.	● Supressed TNFα, IFN-γ, IL-1β, and IL-12. ● Promoted anti-inflammatory mediators (IL-4 and IL-10). ● Preserved the colon appearance.	Only 680 mg/kg dose was showed significant effect in all assays.	(Fei and Xu, 2016)

[a] Listed in the publication as *Bellamya bengalensis* Lamarck 1822.
[b] Listed as *Viviparous bengalensis* Lamarck, 1822 (fresh water snail).
[c] Listed as *Trochus tentorium* Gmelin, 1791.
[d] Listed as *Drupa margariticola*(Broderip, 1833
[e] Listed as *Rapana thomasiana* Crosse, 1861.
[f] Listed as: (*Hemifusus pugilinus* Born, 1778).
[g] Listed in the publication as *Mytilus coruscus.*
[h] Listed in the publication as *Pinctada martensii* Dunker, 1880.
[i] Listed as *Ommastrephes bartrami* Lesueur, 1821.
[j] Listed in the publication as *Sepiella maindroni* Rochebrune, 1884.

species. The anti-pyretic and wound healing properties of the processed shell of *Monetaria moneta* Linnaeus, 1758 were confirmed in a study undertaken by Immanuel et al. (2012). The processed shell was administered orally to albino rats injected with a yeast mixture to stimulate inflammation. The rats showed rapid recovery to normal body temperature (36 °C) 3 h post treatment compared to untreated controls which remained at 38.5 °C for up to 5 h. To test wound healing activity, the powder of the shell was used as an ointment over the 2 cm wound in the thigh region and the scar formed in 8 days, compared to the control rats which showed very minor improvement in the wound (from 2 cm to 1.6 cm) even after 9 days (Immanuel et al., 2012). The results were highly indicative of the effective wound healing properties associated with the shell of a gastropod commonly used in traditional medicines (Table 1). Lipid extracts from the muricid *Rapana venosa* Valenciennes, 1846 have been also demonstrated to significantly improve the healing of induced skin burns in Wistar rats, by reducing the healing time by 10 days compared to the control untreated rats (Badiu et al., 2008). The *in vivo* data revealed improved neoformation of the hypodermal, epidermal and dermal layers of the skin in a rat skin burn model (Badiu et al., 2008). The lipid extract used in the assay contained a mixture of polyunsaturated fatty acids, vitamin E, sterol and aromatic compounds (Badiu et al., 2008), although the most active compound/s that improved healing was not identified. Amino acid extracts from *Rapana venosa* were also reported to accelerate healing time in a burned skin in Wistar rats by improving the neoformation of dermal and epidermal tissue layers (Badiu et al., 2010). The bivalve *Mytilus galloprovincialis* Lamarck, 1819 also demonstrated significant wound healing properties in the same model at the same effective dose (Badiu et al., 2010). These studies support the wound-healing properties associated with the flesh of Muricidae and bivalve, although in traditional use, the flesh was typically burned prior to use and it remains uncertain whether that burnt flesh or shells would retain the bioactive compounds.

6. Human clinical studies

Only a small number of human clinical trials (14) have been undertaken to test the anti-inflammatory efficacy of molluscan products (Table 5). Most of these (13 clinical trials) have focused on NZ green lipped mussel extracts (GLME) including Lyprinol® and Biolane™/ Seatone® following on from the promising anti-inflammatory activity *in vitro* and in animal models (Tables 3, 4). These clinical studies support the efficacy and the safety of GMLE for use in humans (Gibson, 2000; Halpern, 2000; Lyprinol, 2017; Whitehouse et al., 1997). One of the earliest studies was a double-blind parallel control trial undertaken in Glasgow's Homeopathic hospital involving 66 outpatients who were scheduled to undergo surgery due to osteoarthritis and/or rheumatoid arthritis that was unresponsive to the conventional therapeutic regimes such as NSAIDs (Gibson et al., 1980). In brief, a total of 38 patients with osteoarthritis and 28 with rheumatoid arthritis were divided into two groups. Group 1 received a daily dose of GMLE and group 2 received a daily dose of powdered dry fish meal as a placebo. The trial was conducted for 3 months with regular checks for pain, grip strength, joint stiffness, functional efficiency, limbering up time and time taken to walk 15 m. Overall 68% of rheumatoid arthritis and 39% of osteoarthritis patients treated with mussel extract showed improvement, as indicated by a significant reduction in pain and joint stiffness and consequently improving the ability of patients to cope with life and the enhancement of general health. However, 10% of patients given the extract demonstrated a transient worsening of these symptoms, although no other side effects were detected in this study. This study showed the potential of the GLME as a life style nutraceutical supplement or as a possible alternative to other therapies for the treatment of rheumatoid arthritis and osteoarthritis. Nevertheless there may be a need to refine the dosage or treatment regime to address the deterioration in a smaller number of patients. In another double blind study involving 53 patients with gonarthrosis (the arthritis of

Table 5
Human clinical trials on anti-inflammatory and wound healing molluscan natural products.

Species	Geographic distribution	Extract or compound	Type of clinical trial and study design	Outcome and results	Reference
Gastropoda					
Cornu aspersum Müller, 1774 (Common garden snail)	native to the Mediterranean region	Elicina® a cream contains 80% of mucus from the snail *Cornu aspersum*	Applied on 27 patients with deep partial thickness (PT) facial burns and its effect compared to 16 patients treated using a well-known burn ointment MEBO The treatments were applied twice daily for 14 days.	Accelerated the healing process significantly compared to MEBO (burn surface epithelialization 11 ± 2 days vs 15 ± 3 days, $p < 0.001$) and significantly reduced the pain scored on the fourth day post-burn ($p < 0.001$).	(Tsoutsos et al., 2009)
Bivalvia					
Perna canaliculus Gmelin, 1791 (GML)	New Zealand	Powdered *Perna canaliculus* or Lyprinol®	54 Osteoarthritis (OA) patients consumed 2 capsules of Lyprinol twice a day for 8 weeks.	80% of patients reported significant pain relief. 39% showed improvement in joint function 87% of patients and 90% of doctors reported improvement in global assessment.	(Cho et al., 2003)
		Powdered *Perna canaliculus* or Lyprinol®	Double-blind parallel comparison study included 30 patients with classical rheumatoid arthritis (RA) and 30 patients with osteoarthritis. Each category divided into two groups group A (received 1150 mg/day mussel powder) and group B (received 210 mg/day Lyprinol®) for 90 days. Then, all patients received the lipid extract (Lyprinol) for another three months.	76% of RA and 70% of OA demonstrated significant improvement in the prospective of pain, swelling, stiffness and improving functional index in both RA and OA as compared to the baseline assessments. There was NSD between the GMLE and Lyprinol treatment.	(Gibson and Gibson, 1998)
		(Lyprinol®)	Placebo controlled study involved 46 patients with atopic asthma consumed 2 capsules of Lyprinol® (each capsule contains 50 mg of ω-3 PUFAs (eicosapentaenoic acid and docosahexaenoic acid) and 100 mg plive oil)) or placebo containing only 150 mg olive oil for 8 weeks.	Lyprinol® was suggested to relief the symptoms of asthma compared to the placebo control but there was no statistical difference (NSD).	(Emelyanov et al., 2002)
		Seatone® (freeze dried GLM powder- same as Biolane™)	Double-blind crossover randomised PCT involved 6 patients with rheumatoid arthritis consumed Seatone (no dosage information) or placebo for 6 weeks and then patients crossover to the other treatment for further 6 weeks.	NSD between Seatone and placebo treatments in all the observed parameters of RA.	(Highton and McArthur, 1975)
			Double-blind crossover randomised, placebo controlled trial involved 30 patients with rheumatoid arthritis consumed 900 mg/day Seatone or dried fish placebo for 8 weeks.	NSD between Seatone and placebo treatments in all the observed parameters of RA despite some improvements in some parameters.	(Huskisson et al., 1981)
			Double-blind randomised PCT involved 35 patients with rheumatoid arthritis consumed 920 mg/day Seatone or dried fish placebo for 3 months followed by 1180 mg/day for	NSD between Seatone and placebo treatments in all measures.	(Larkin et al., 1985)

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Table 5 *(continued)*

Species	Geographic distribution	Extract or compound	Type of clinical trial and study design	Outcome and results	Reference
			another 3 months.		
			Double-blind randomised PCT involved 53 Gonarthrosis (arthritis of the knee) patients received Biolane (2100 mg/day) or placebo for 6 months.	SD in most of the measures including visual analogue of pain score, global assessment, and the functional state of arthritis condition. NSD in the other measures (articular mobility; use of walking stick; time to walk set distance or maximum walking distance; pain intensity) between Seatone and placebo treatment.	(Audeval and Bouchacourt, 1986)
		Perna canaliculus GLME	Double blind randomised placebo controlled trial involved 47 patients with rheumatoid arthritis consumed 1050 mg/day mussel extract for 12 weeks compared to a placebo control.	There was improvement in the patient's status. However, NSD noticed between the treatment with mussel extract and the placebo.	(Caughey et al., 1983)
			120 Patients in the 60–70 age group with osteoarthritis of the knee. Consumed 2–3 capsules of 500 mg GLME/day for one year.	Practitioner's assessment suggested that 63 of patients had made an excellent improvement, 38 good improvement and 19 with no change. All patients in this study consumed the same treatment, hence, no negative control group.	(Kendall et al., 2000)
			Double-blind, parallel comparison placebo controlled study included a total of 38 patients with osteoarthritis and 28 with rheumatoid arthritis received a daily dose of 300 mg of GMLE or placebo control three times daily for 90 days.	68% of rheumatoid arthritis and 39% of osteoarthritis patients showed improvement; 10% demonstrated a transient worsening of the symptoms. However, NSD compared to the placebo.	(Gibson et al., 1980)
		PCSO-524™ Lipid extract	A total of 71 children aged 6–13 years with asthma were enrolled in a 16-week, single centre, double-masked, placebo-controlled. Children consumed 2 capsules of Lyprinol® or placebo daily.	Improved the percentage of children reporting little or no trouble with their asthma at three months of treatment (97% compared to 76% $p = 0.057$).	(Lello et al., 2012)
		PCSO-524™ Lipid extract (Lyprinol®)	Pain relief changes in people with Osteoarthritis taking PCSO-524™ (1200 mg per day) compared to patients taking fish oil for 12 weeks.	Significantly relieved the pain and discomfort in 100% of patients. Significantly improved the health and disease condition of patients treated with lyprinol (88%) compared to fish oil (59%) ($p < 0.05$)	(Zawadzki et al., 2013)

knee) patients, the treatment group received 2100 mg of GLME/day for six months, which was compared to a control group which received a matching dose of placebo (Audeval and Bouchacourt, 1986). According to the results of this study, GLME was effective in preventing deterioration and enhancing the repair mechanisms, as well as providing both analgesic and anti-inflammatory benefits (Audeval and Bouchacourt, 1986). In several other double blind randomised control trials for rheumatoid arthritis using lower doses of GLME, positive effects were sometimes recorded, but there were no significant differences when compared to the placebo control (Caughey et al., 1983; Highton and McArthur, 1975; Huskisson et al., 1981; Larkin et al., 1985). This suggests that the efficacy of GMLE for treatment of arthritic may depend on the dose, duration and the specific formulation or batch.

In a further year-long pilot study performed in USA involving 120 patients in the 60–70 age group, also with gonarthrosis, the patients were given a dose of 3 capsules of GLME (500 mg GLME) daily with food (Kendall et al., 2000). At the end of the study the results were subjectively assessed by patients and separately by a medical practitioner. In brief, patient assessments showed that 95 patients reported excellent symptom improvement, whilst 16 reported good improvement and 9 reported no change to the symptoms. However, practitioner assessments suggested that 63 of patients had demonstrated excellent improvement, 38 good improvement and 19 with no change (Kendall et al., 2000). Once again the study reported a low incidence of side effects and when this observation is added to the overall positive outcomes from these clinical trials it would seem that with an optimised treatment regime and formulation, GLME could be of significant value for at least some patients suffering from chronic and disabling inflammatory disorders such as arthritis (Gibson et al., 1980; Halpern, 2000).

Lyprinol® has also been investigated for its potential efficacy for the treatment of asthma (Table 5). A double-blind randomised, placebo controlled clinical trial performed by Emelyanov et al. (2002) used the lipid extract to treat patients with atopic asthma. The study involved 46 patients who were given 2 capsules of Lyprinol® per day or a placebo containing olive oil (Emelyanov et al., 2002). In brief, Lyprinol® was found to relieve the symptoms of asthma compared to the placebo control, although the difference between the treatment with Lyprinol and the placebo was not statistically significant for the treatment of the atopic asthma. However, in a separate double blind placebo controlled study in 73 children with asthma for 16 weeks, the refined GLME product PCSO-524™ did result in statistically significant improvements relative to the controls (Lello et al., 2012) Another form of GMLE, (Biolane®) was also tested for its ability to heal the injury of soft tissues in otherwise healthy people (Lambert et al., 1998). This study was carried out on well trained cyclists who were given Biolane GLME 3 weeks prior to the induction of muscle injury with outcomes compared to a control group of cyclists who were given a placebo. The treatment group showed increased peak power and accelerated recovery of peak power after injury compared to the control group. This was an unprecedented study to reveal the effect of Biolane® on restoring damaged tissues.

Despite some conflicting results, all the clinical studies mentioned above have led to acceptance of the GLME as an effective anti-inflammatory agent. It is available now as an over-the-counter drug in different formulations. There are two main formulations of NZ GLME that have been optimised for stability and demonstrate more reliable *in vivo* efficacy for relieving inflammatory disorders, 1) Lyprinol® which contains 50 mg of PCSO-524® (lipid extract from *P. canaliculus*), 100 mg of a proprietary oleic acid blend and 0.225 mg of vitamin E, and 2) Biolane® which contains the *P. canaliculus* extract manufactured from farmed mussels in peak condition using a quality controlled process (Table 5). However, less refined GML products, such as those based on the freeze-dried mussel power have less statistical support for differences between the GLME treated group and the placebo treated group in clinical trials, although health improvement was noticed among some of the participants (Table 5).

The only other human clinical trials that have been undertaken with mollusc extracts examined the effect of a formulated snail mucus product on wound healing in burn victims (Table 5). Elicina® (Locafar, Chile) is a cosmetic skin repair cream which is composed of 80% of the mucus secretions from the brown snail *Cornu (Helix) aspersum* Muller, 1774 (common garden snail). In a clinical study, the cream was applied twice daily on adult patients with deep partial thickness facial burns for a maximum period of 14 days or until full epithelialisation (Tsoutsos et al., 2009). This cream healed the facial burns in around 11 days, significantly faster than the well-known burn ointment MEBO which took around 15 days for healing. The snail cream not only accelerated the healing process, but also reduced the pain caused by the burns. The results suggest that the cream maybe an effective alternative treatment in open wound management of partial thickness burns in adults (Tsoutsos et al., 2009). However, further double blind randomized control trials are required to test the suite of different snail mucus formulations that are currently available on the open market and promoted for their skin healing properties.

7. Nutraceutical and cosmeceutical development

There are few commercialised molluscan products currently available. Extensive preclinical and clinical studies have led to the acceptance of the GLME as an anti-inflammatory nutraceutical under the name of Lyprinol® (Halpern, 2000, 2008; Lyprinol, 2017; Treschow et al., 2007; Whitehouse et al., 1997). Lyprinol® is also marketed as an over-the-counter oral treatment for rheumatism and arthritis and fractions derived from Lyprinol can also be used to relieve moderate-asthma in children (Lyprinol, 2017). Biolane™ is also marketed as a quality controlled nutraceutical with anti-inflammatory activity and the well supported activity may be due to the preparation not containing any further additives other than powdered GLM (Cheras et al., 2005). The Indian green mussel extract *P. viridis* (Linnaeus, 1758) has also been commercialised as an anti-inflammatory OTC under the name Cadalmin™ (Chakraborty, 2012).

Overall it would appear that mollusc extracts with a long history of human ethnomedical consumption are more likely to be relatively safe for use as nutraceuticals. Further research to identify the active constituents in the ethnomedical preparations from molluscs that are used specifically for anti-inflammatory treatments could facilitate strategic development of scientifically validated products that can be value-added as nutraceuticals or pharmaceuticals. However, safety should never be assumed for concentrated natural products, requiring industry investment for comprehensive preclinical and clinical trials.

Products that are not safe for human oral consumption may still have potential for topical application in skin repair and wound healing if they do not cause tissue damage. Cosmeceuticals do not have to undergo the same rigorous level of clinical testing for safety and efficacy as do pharmaceuticals and/or nutraceuticals. However, efficacy under-pinned by evidence-based science will be an essential requirement for marketing mollusc-based products with specific efficacy statements. In recent years there has been a proliferation in the range of snail creams available for skin care, suggesting a need for improved quality control measures. Allantoin and glycolic acid are two bioactive ingredients in mollusc mucus that contribute to its use as constituents in cosmetic skin creams. El Mubarak et al. (2013) validated a method for simultaneous detection of these compounds and demonstrated that there was significant variability in the levels of these compounds between different cosmetic creams, which is likely to reflect the efficacy of snail mucus content and potentially the quality of the product. This further demonstrates the importance of improved quality control for OTC cosmeceutical and nutraceuticals. Overall more research is required on molluscan bioactive compounds to identify the bioactive agents, optimise methods for their detection and quantification in extracts and formulations for standardisation, as well as validation of their *in vivo* efficacy and to confirm their safety. With a greater level of clinical testing, the products can be considered for registration as

therapeutic agents (*e.g.* by US Food and Drugs Administration, European Medicines Agency or Therapeutic Goods Administration in Australia) thus leading to not only wider commercial value but also providing safer and more effective options than NSAIDs.

The safety of using the traditional or natural medicine should never be assumed due to the toxicity of some natural products. In some traditional medicine systems, traditional methods of preparation could reduce or remove the toxic components. Mostly the shells are used for mollusc species known to produce toxic venoms *e.g.* cone snails and neogastropods. Flesh from Muricidae species in particular are only used after boiling in TCM which may effectively break down the muscle relaxing choline esters produced in their hypobranchial glands (Benkendorff et al., 2015; Roseghini et al., 1996). Buccinidae is another family of neogastropod molluscs that have a relatively large salivary glands containing significant quantities of tetramine, which blocks nicotinic acetylcholine receptors *e.g.* *Neptunea* Röding, 1798 spp. (Fujii et al., 1992; Modica and Holford, 2010; Watson-Wright et al., 1992). Reports have demonstrated that people can become intoxicated as a result of consuming these species (Modica and Holford, 2010) but only one traditional medicine from *Neptunea cumingii* (Crosse, 1862) was identified here for anti-inflammatory applications (Table 1) and this involved a decoction of the shell, so is unlikely to contain tetramine from the salivary gland. However, the TCM using the sea hare (*Aplysia* Linnaeus, 1767) eggs to treat tuberculosis of lymph nodes (Table 2) is of concern because consumption of *Aplysia* eggs is known to cause liver damage and they contain toxic glycoprotein compounds (Hino et al., 1994; Johnson and Willows, 1999; Kicklighter et al., 2005). Nevertheless, the lack of other soft-bodied gastropods *i.e.* Nudibranchia species in the traditional medicines is most likely because many of them contain toxic compounds that are derived from their diets on sessile invertebrates including sponges, ascidians and cnidarians (Benkendorff, 2010, 2014). Caution is also required for filter feeding bivalves and their predators, which can accumulate environmental toxins including heavy metals (Bryan et al., 1977; Lau et al., 1998; Usero et al., 2005) and paralytic shell fish toxins from toxic algal blooms (Callejas et al., 2015; Hallegraeff and Bolch, 2016; Hallegraeff et al., 2003; Lagos et al., 1999). As well as the toxic substances molluscs can also contain human pathogens and there have been many infectious outbreaks associated with consumption of oysters, clams and mussels (Potasman et al., 2002). Consequently any molluscs used as functional food should be subject to shellfish safety and quality assurance programs. The potential for toxic metabolites also suggest the need for identification of the bioactive compounds, safety testing and quality assurance on any nutraceutical or traditional medicine to ensure safety.

8. Conclusion

Mollusc products have been used as anti-inflammatory remedies and ethnomedicines for centuries by a vast range of ethnocultural groups. In this review we have found some 104 different anti-inflammatory remedies in the literature. Only a few of these preparations have been clinically tested or had their ant-inflammatory activity examined by evidence based scientific methods. However, to date the few preparations tested for anti-inflammatory activity in either *in vitro* or *in vivo* assays have shown promising results. Lyprinol® and Biolane™ from the New Zealand Green Lipped mussel *Perna canaliculus* and Cadalmin™ GMe from the green mussel *Perna viridis* are prime examples of the strategic development of anti-inflammatory remedies from mollusc species that have a sound ethnomedical history.

This review clearly indicates the significance of molluscan natural products as potential leads for anti-inflammatory nutraceutical and cosmeceutical agents. In addition, this review also indicates that there is a paucity of studies that have investigated the anti-inflammatory activity of molluscs, considering the high diversity of species within this phylum. Furthermore, despite the vast use of molluscan preparations in ethnomedicine and the *in vitro* and *in vivo* anti-inflammatory activity results, only few molluscan natural products have yet been chemically characterised. Therefore, more attention should be paid toward identifying molluscan natural products as a potential source of anti-inflammatory drug leads.

Author contributions

KB and TBA conceptualised the review and undertook database searched for the literature on molluscan anti-inflammatory and wound healing activity. LL translated and interpreted the application of molluscs from the Chinese *Materia Medica*. MK assisted with the interpretation of anti-inflammatory assays and *in vivo* models. TBA wrote the first draft of the paper and all authors provided feedback and approved the final version of the manuscript.

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