



Molluscicidal effect of some red and green marine algae on *Lymnaea natalensis*

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ABSTRACT

Marine algae are economically important aquatic photosynthetic organisms act as a source of highly bioactive metabolites that might be useful against predators and parasites. In the present study, aqueous and ethanol extracts of four algal species of marine algae; *Pterocladia capillacea* (*P. capillacea*), *Jania rubens* (*J. rubens*), *Ulva lactuca* (*U. lactuca*), and *Enteromorpha intestinalis* (*E. intestinalis*) were tested for their lethal effect on *Lymnaea natalensis* (*L. natalensis*) snail, the intermediate host of the trematode parasite; *Fasciola gigantica*, (*F. gigantica*). Preliminary experiments were done to determine the molluscicidal toxicity of different concentrations of aqueous and ethanol algal extracts against the studied snail. Five concentrations of aqueous and ethanol extracts; 100, 200, 300, 400 and 500 ppm for each algal species were used. LC₅₀ for each extract was calculated daily till the end of the experiment. It is clear from the results that molluscicidal effect of algal extracts is concentration dependent. The most potent extracts as proved by calculated LC₅₀ are *P. capillacea* and *J. rubens* aqueous extract (red algae); 111.3 and 127 ppm respectively and *U. lactuca* ethanolic extract (green algae) 121 ppm. The current study introduces marine algae of promising molluscicidal activity on *L. natalensis* snail. *P. capillacea* and *J. rubens* aqueous extracts and *U. lactuca* ethanolic extract are the most potent and highly significant ones in the net reproductive rate and reduction. Further investigations for isolation of active principals of the algal potent extracts for evaluation as molluscicides and or anthelmintics are necessary required.

INTRODUCTION

Marine algae are economically important as a source of crude oil and as sources of food and number of pharmaceutical and industrial products for humans. An important role in medicine and rich in secondary metabolites and include several natural products (Iwamoto *et al.* 2001). During the last five decades, numerous novel compounds have been isolated from marine organisms and many of these substances have been demonstrated to possess interesting biological activities The phytochemical screening of the marine algae revealed the presence of saponins, alkaloids, glycosides, flavonoids, tannins, phenols, steroids, terpenoids, nitrogenous compounds and volatile

constituents which are of great medicinal value and used in drugs (El Gamal, 2010). The intermediate host of worldwide zoonotic trematode parasite *Fasciola gigantica* (*F. gigantica*) in Egypt, *Lymnaea natalensis* (*L. natalensis*) snail, has received a great attention because of its role in transmission of fasciolosis in man and animals. The snail control is considered not only complementary but essential in breakage *Fasciola* life cycle (Shaapan *et al.* 2015).

Marine organisms as red, green and brown algae have shown to be one of the most promising sources of new bioactive compounds. (Blunt *et al.* 2013; Mayer *et al.* 2013). Algal metabolism concerns the biological and transport processes by which the algae take up nutrients and convert them into materials needed for growth, reproduction and defense of the organisms. It gives rise to a range of unique compounds, including secondary metabolites, some of which have toxicity to other organisms (Raven *et al.* 2012). The preliminary phytochemical screening of the algal species revealed the presence of steroids, phenolic groups, saponins, tannins, flavonoids, carbohydrates, carboxylic acid, coumarins and xantho-proteins in green algae, *Ulva lactuca*, (*U. lactuca*) (Jeeva *et al.* 2012). Analysis of volatile constituents led to identification of 27 components from *U. lactuca*. One of the major components is thymol which constitutes 18.24% of volatiles (Elmanawy *et al.* 2005). It was found that the green algae (*Ulva lactuca*), and the two red algal, *Jania rubens* and *Pterocladia capillacea* (*J. rubens* and *P. capillacea*) extracts contained flavonoids and phenolics (Yacout *et al.* 2011). The presence of steroids, cardiac glycosides and saponins were detected in *Enteromorpha intestinalis* with absence of tannins, flavonoids and alkaloids (Rathish *et al.* 2009). The secondary metabolites of the collected red alga *Pterocladia capillacea* from the Mediterranean Coast of Egypt were isolated. Their chemical structures were elucidated and also diisooctyl phthalate, cholesterol, stigmasterol, linoleic acid and isodomoic acid were isolated from *P. capillacea* (Aboutabl *et al.* 2010). The presence of alkaloids in aqueous extract of *P. capillacea* is previously proved by (El Kassas and Attia 2014). The byproducts of the blue-green alga, *Spirulina platensis*, has a lethal effect against adult *Biomphalaria alexandrina* snails, reduced or stopped their oviposition, and has effect on hatchability of snail's eggs and viability of the free living larval stages of the parasite (miracidia and cercariae), hence minimize the snail populations available for the parasite transmission. Therefore, it may be a potential source of effective compounds for control of *Schistosoma mansoni* (Mostafa and Gawish, 2009).

A decrease of growth regardless age in juveniles and adults, and a reduction of fecundity in adults *Lymnaea stagnalis* snails were recorded after ingestion of toxic *Planktothrix agardhii* (Cyanobacteria, Phormidiaceae). The Survival and locomotion of the snails were not affected. Reduction of growth and fecundity continued to be observed even after feeding of non-toxic food for 3 weeks (Lance *et al.* 2007).

The objective of the current research is to assess the molluscicidal effect of some marine algae; red and green ones against *Lymnaea natalensis* snails for controlling fascioliasis.

MATERIALS AND METHODS

The experiments were done in Parasitology and Animal Disease laboratory, National Research Center, Giza .Egypt.

Snails

Lymnaea natalensis snails were collected from Abou Rawash, Giza and kept in aquaria (35 x 25 x 10 cm) filled with 0.5 L dechlorinated tap water each aquarium and

fed on fresh leaves of lettuce. The water was changed daily and dead snails were removed, live snails were maintained reared two weeks before use in experiments according to the methods described by Van der Steen, *et al.* (1968). A control group was prepared in dechlorinated water. Snails were exposed to the tested materials at room temperature 27 °C and the tested snails were examined daily to assess activity, physiological and mortality changes.

Tested algae

Four different species of marine algae; two species are belong to family Chlorophyceae (green algae); *Ulva lactuca* and *Enteromorpha intestinalis* and two species of family Rhodophyceae (red algae); *Pterocladia capillacea* and *Jania rubens* were used. The algal samples were collected from exposed rocky site near the western edge of Abou-Qir bay, Alexandria, Egypt about 100 meters from the sea shore and at about 1 meter depth. The selection of the four species of algae was based on previous studies (Awad., 2000; El Sayed *et al.* 2001 (Revise high light with the Ref. list??); Hutson *et al.* 2012). The tested algae were thoroughly washed by sea water to remove extraneous matters, then dried in shade and finely grounded. Aqueous and ethanol extracts of the four different species of marine algae were prepared according the procedure of Singaba *et al.* (2006). Identification of the algal species was carried out according to methods described by Chaumont (1993) and Barsanti and Gualtieri (2014) and was also checked by Dr. Manal El-Naggar, Professor of Microbiology and Head of Microbiology Department; National Institute of Oceanography and Fisheries, Alexandria, Egypt.

Preliminary experiment for determination of molluscicidal toxicity

The evaluation of the molluscicidal toxicity of the extracts was conducted according to the world health organization (WHO) guideline application (WHO, 1983). A stock solution of 1000 ppm was prepared on the basis of w/v using dechlorinated water. A series of exploratory experiments were conducted using different concentrations to determine the toxicity range of the algal extracts against the tested snails. In the current experiment, five concentrations of aqueous and ethanol extract, each (100, 200, 300, 400 and 500 ppm) for each algal species were used for snails (Miyasato *et al.* 2012). Ten snails were transferred to a glass aquarium containing 50 ml dechlorinated tap water as control and three replicates each of ten snails were used for each mentioned algal concentration. For each treatment, the mortality percentages were calculated. This experiment was assayed for the algal extract activity on *L. natalensis* snails. Determination of snail fecundity was performed by collection of egg masses produced by snails. The aquaria were lined with plastic sheets for easy collection of egg masses and the numbers of eggs in each egg mass were counted by the use of light microscope. The total number of eggs laid by treated and control snails were recorded daily. The egg-laying capacity was expressed here in the form of the number of eggs/snails/day and this was determined by dividing the total number of laid eggs in any day by the total number of living snails of this day (Czech *et al.* 2001). In order to calculate the net reproductive rate of *L. natalensis* snails throughout their continuous exposure to different concentrations of experimental materials, the following parameters will be considered. Time of exposure in days, survival rate which is the living snails at any given day as a proportion from the original snails, fecundity which means the number of eggs/snails/day and multiplication of the previous two parameters give the product of each day. The summation of the reproductive product of the exposed snails throughout the experiment period gives the still survived snail (Ro). The reduction

percentage (Red) was calculated according to (Osman *et al.* 2014) by using the following equation:

$$\text{Reduction in reproductive rate} = \frac{\text{Control} - \text{treated}}{\text{Control}} \times 100$$

Which means the dead destructive snails

Determination of LC₅₀ for different extracts

The LC₅₀ for each aqueous and ethanol algal extract was calculated daily till the end of the experiment according to method adopted by Dahms and Hellio (2009).

RESULTS

Molluscicidal effect of the algal extracts on snail activates *Lymnaea natalensis*

Treated snails with potent extract was showed lowest values of net reproductive rate (Ro) for survived snails were 0.56, 0.66, 0.88 and 0.89 in *P. capillaceae*, *U. lactuca*, *J. rubens* and *E. intestinalis* aqueous extracts, respectively with algal extract concentrations 300, 500, 500 and 500 ppm, respectively. The highest values of Ro were 3.63 and 4.01 in *U. lactuca* and *E. intestinalis* ethanol extracts, respectively with algal extract concentrations 400 and 500 ppm, respectively. At the same time, Ro values of *J. rubens* and *P. capillaceae* ethanol extracts revealed 1.96 and 2.85, respectively with algal extract concentrations 500 ppm. The still survived snail Ro of control non-treated snail was 95.9. The time of snail exposure to different algal concentrations ranged between 3-7 days at 300 -500 ppm concentrations. The lowest time of exposure was 3 days for *J. rubens*, *U. lactuca* and *E. intestinalis* aqueous extracts. The highest time of exposure was 6, 6 and 7 days for *P. capillaceae*, *U. lactuca* and *E. intestinalis* ethanol extracts, respectively. At the same time *J. rubens* ethanol extract had 4 days of exposure (Table 1).

Table 1: Effect of some red and green algae spp on *L. natalensis* snail activities (R0 & Red) in relation to time of exposure

Algal Extract	Concentration ppm	Time of exposure (days)	Snail activities	
			Ro	Red
<i>P. capillaceae</i> Aqueous	300	5	0.56	98.5
<i>P. capillaceae</i> Ethanol	500	6	2.85	92.1
<i>J. rubens</i> Aqueous	500	3	0.88	97;7
<i>J. rubens</i> Ethanol	500	4	1.96	95.2
<i>U. lactuca</i> Aqueous	500	3	0.66	98.3
<i>U. lactuca</i> Ethanol	400	6	3.63	92.9
<i>E. intestinalis</i> Aqueous	500	3	0.89	97.7
<i>E. intestinalis</i> Ethanol	500	7	4.01	90.8
Control		10	95.9	3.66

Ro: the net reproductive rate of the still survived snails throughout the experimental period (still survived snails)

Red: reduction in reproductive rate (dead destructive snails)

LC₅₀ value of the algal extracts on *Lymnaea natalensis* snails

The LC₅₀ value of each aqueous and ethanolic extract corresponding to each tested alga are showed in table 2. The LC50 value clears and evaluates the molluscicidal effect of each alga against *L. natalensis* snails particularly through field application. The most potent is *P. capillaceae* aqueous extract (111.295) followed by *U. lactuca* ethanolic extract (121.237). The lowest molluscicidal effects are *E.*

intestinalis ethanolic extract (382.569) followed by *U. lactuca* and *E. intestinalis* aqueous extracts (371.365 & 370.182, respectively) (Table 2).

Table 2: LC₅₀ values of aqueous and ethanol extracts of the marine studied algae

Extract	Marine algae			
	<i>P. capillacea</i>	<i>J. rubens</i>	<i>U. lactuca</i>	<i>E. intestinalis</i>
Aqueous	111.295	127.128	371.365	370.182
Ethanol	180.221	150.303	121.273	382.569

DISCUSSION

The use of molluscicides is regarded as an important strategy in the control of the snail hosts of fasciolosis (Mello-Silva *et al.* 2006). Unlike the synthetic drugs, the uses of molluscicides to control snails prevent reinfection of people after treatment (WHO, 2002). Therefore, the search for new molluscicides is still ongoing although there are little reports on molluscicidal natural products from marine organisms to control the population of intermediate host snails of fasciolosis (Miyasato *et al.* 2012).

In the present study, the crude ethanol and water extracts from four different algal species, *P. capillacea*, *J. rubens* (Rhodophyta), *U. lactuca* and *E. intestinalis* (Chlorophyta), were screened for their biological activity against *L. natalensis* snails. It is obvious in the present study that aqueous extract of different experimental algae are more effective than ethanolic one. Also highest dilution, 500 ppm was effective in all algal extracts except *P. capillacea* aqueous one at 300 ppm. Moreover, this extract had the most prominent lowest R₀ (0.56) and highest Red (98.5) and can play as an important molluscicide. The lowest molluscicidal effects are 3.63 and 4.01 in *U. lactuca* and *E. intestinalis* ethanolic extracts, respectively. The LC₅₀ value was encouraged and revealed that most potent is *P. capillacea* aqueous extract (111.295) besides *U. lactuca* ethanolic extract (121.237). The lowest LC₅₀ are *E. intestinalis* ethanolic extract (282.569) followed by *U. lactuca* and *E. intestinalis* aqueous extract (371.365 & 370.182), respectively. These obtained result may be attributed to the presence of saponins, alkaloids and mineral salts that facilitate and accelerate the rate of penetration of algal byproducts through snail's membrane, hence increase their harmful effects and decrease snail fecundity in accordance to Tadros *et al.* (2008), who stated that low concentrations of saponins fractions increased mortality of *B. alexandrina* snails, miracidia, cercariae and adult worm of *Schistosoma mansoni* and decreased hatchability and egg production. Similarly, Mostafa and Gawish (2009), attributed this harmful effect to the content of the phenolic compounds, saponins, alkaloids and mineral salts. Also, embryo toxicity and reduced viability of eggs was observed due to exposure to these algal byproducts (Oliveira-Filho *et al.* 2010). Moreover, survival and snail population were reduced in an infested region. The reduced fecundity of *L. natalensis* that observed in the current results after exposure to marine algae also agree with the results proved suppressed fecundity of snails treated with *Cladophora glomerata* algae (Ishac and Bishai, 1968). The authors stated that alga secretes substance (acrylic acid), which causes high mortality, low fecundity and low hatchability of affected snails. This explanation was also adopted by Bakry, (2009), who indicated that *S. platensis* secretes organic substances or metabolic products such as phycobiline, phenols, terpenoids, steroids, polysaccharides and saponins.

Meanwhile, the ethanol and aqueous extracts of *J. rubens* had no molluscicidal activity against *B. alexandrina* (Soliman *et al.* 1994) which disagrees with the result

of the current study since *P. capillacea* aqueous extract, *U. lactuca* ethanolic extract and *J. rubens* aqueous extracts had a potent molluscicidal activity on *L. natalensis*. This conflict is probably attributed to snail species where the difference in susceptibility of the two snails to the lethal effect of the same extract could be attributed to the natural resistance of different snail's genera and that the molluscicides may vary in their toxicological effects according to the species of the snails' used (Bakry and Hamdi, 2007; Osman *et al.* 2014).

CONCLUSIONS

Collectively, the four tested marine algae showed variable molluscicidal and anthelmintic potentials against *L. natalensis* snails (intermediate host of *F. gigantica*). Aqueous extract of *P. capillacea* showed the most potent molluscicidal effect while ethanol extract of *E. intestinalis* and *U. lactuca* showed the most lowest molluscicidal impact. The current promising results merit further investigations for isolation of active principals of the potent algal extracts and its experimental field application as molluscicides and or *in vivo* evaluation as anthelmintics.

REFERENCES

- Aboutabl, A.E.; Abou-Zeid, A.H; Sleem, A.A. and El Rafi, H.M. (2010). Secondary metabolites and certain bioactivities of *Pterocladia capillacea* (S. Gmelin) Bornet and Dictyopteris membranacea (Stackhouse) Batters. *Med Aro Plant Sci Biotechnol.*, 4: 41-48.
- Awad, N.E. (2000). Biologically active steroid from the green alga *Ulva lactuca*. *Phytotherapy Research: Int J Dev Pharmacolo Toxicolo Eval Nat Prod Deriv.*, 14: 641-643.
- Bakry, F.A. and Hamdi, S.A.H. (2007). Molluscicidal activity of latex aqueous solution of *Euphorbia acetone* and *Euphorbia granulate* against the intermediate hosts of Schistosomiasis and Fascioliasis. *Journal of Union Arab Biologist*, 27: 101-126.
- Bakry, F.A. (2009). Use of some plant extracts to control *Biomphalaria alexandrina* snails with emphasis on some biological effects. *Pesticide biochemistry and physiology*, 95: 159-165.
- Barsanti, L. and Gualtieri, P. (2014). *Algae: anatomy, biochemistry, and biotechnology*. CRC press, Taylor. & Francis Group, Boca Raton, FL, USA. ISBN-13: 978-1-4398-6733-4.
- Blunt, J.W.; Copp, B.R.; Keyzers, R.A.; Munro, M.H. and Prinsep, M.R. (2013). *Marine natural products*. *Natural product reports*, 30: 237-323.
- Chaumont, D. (1993). *Biotechnology of algal biomass production: a review of systems for outdoor mass culture*. *J. Appl Phycol.*, 5: 593-604.
- Czech, P.; Weber, K. and Dietrich, D. R. (2001). Effects of endocrine modulating substances on reproduction in the hermaphroditic snail *Lymnaea stagnalis*. *Aquatic Toxicology*, 53: 103-114.
- Dahms, H.U. and Hellio, C. (2009). Laboratory bioassays for screening marine antifouling compounds. In *Advances in marine antifouling coatings and technologies*, pp: 275-307.
- El Gamal, A.A. (2010). Biological importance of marine algae. *Saudi Pharmaceutical Journal*, 18: 1-25.

- El Kassas, H.Y. and Attia, A.A. (2014). Bactericidal application and cytotoxic activity of biosynthesized silver nanoparticles with an extract of the red seaweed *Pterocladia capillacea* on the HepG2 cell line. *Asian Pac J Cancer Prev.*, 15: 1299-1306
- El Sayed, H.; Choudhary, M.I.; Kandil, S.H.; El Nemr, A.; Gulzar, T. and Shobier, A.H. (2011). Studies on the constituents of the green alga *Ulva lactuca*. *Chemistry of Natural Compounds*, 47: .335.
- Elmanawy, I.M.; Hafez, S.S.; Yassin, M.; El-adel, H.M. and Eraqi, I.S. (2005). Phytochemical investigation and anti-microbial activity of *Ulva lactuca*. *Egypt J. Phycol.*, 6: 28
- Hutson, K.S.; Mata, L.; Paul, N.A. and De Nys, R. (2012). Seaweed extracts as a natural control against the monogenean ectoparasite, *Neobenedenia* sp., infecting farmed barramundi (*Lates calcarifer*). *Int J parasitol*, 42: 1135-1141.
- Ishac, M.M. and Bishai, H.M. (1968). the possible role of the algae *Cladophora glomerata* on the biological control of *Biomphalaria boissyi*, the snail host of *Schistosoma mansoni*. *Hydrobiologia*, 32: 168-180.
- Iwamoto, C.; Yamada, T.; Ito, Y.; Minoura, K. and Numata, A. (2001). Cytotoxic cytochalasans from a *Penicillium* species separated from a marine alga. *Tetrahedron*, 57: 2904–2997.
- Jeeva, S.; Marimuthu, J.; Domettilla, C.; Anantham, B. and Mahesh, M. (2012). Preliminary phytochemical studies on some selected seaweeds from Gulf of Mannar, India. *Asian Pac. J. Trop. Biomed*, 2: S30-S33.
- Lance, E.; Paty, C.; Bormans, M.; Brient, L and Gérard, C. (2007). Interactions between cyanobacteria and gastropods. *Aquatic Toxicology*, 4: 389-396.
- Mayer, A.; Rodríguez, A.D.; Tagliatalata-Scafati, O. and Fusetani, N. (2013). Marine pharmacology in 2009–2011: Marine compounds with antibacterial, antidiabetic, antifungal, anti-inflammatory, antiprotozoal, antituberculosis, and antiviral activities; affecting the immune and nervous systems, and other miscellaneous mechanisms of action. *Marine drugs*, 11: 2510-2573.
- Mello-Silva, C.C.; Vasconcellos, M.C.D.; Pinheiro, J. and Rodrigues, M.D.L.D.A. (2006). Physiological changes in *Biomphalaria glabrata* Say, 1818 (Pulmonata: Planorbidae) caused by sub-lethal concentrations of the latex of *Euphorbia splendens* var. *hislopii* NEB (Euphorbiaceae). *Memórias do Instituto Oswaldo Cruz*, 101: 3-8.
- Miyasato, P.A.; Kawano, T.; Freitas, J.C.; Berlinck, R.G.D.S.; Nakano, E. and Tallarico, L.F. (2012). Molluscicidal activity of some marine substances against the snail *Biomphalaria glabrata* (Mollusca, Planorbidae). *Parasitol Res.*, 110: 1873-1879.
- Mostafa, S.S. and Gawish, F.A. (2009). towards to control *Biomphalaria alexandrina* snails and the free living larval stages of *Schistosoma mansoni* using the microalga *Spirulina platensis*. *Austr J Bas. Appl Sci.*, 3: 4112-4119.
- Oliveira-Filho, E.C.; Geraldino, B.R.; Coelho, D.R.; De-Carvalho, R.R. and Paumgarten, F.J. (2010). Comparative toxicity of *Euphorbia milii* latex and synthetic molluscicides to *Biomphalaria glabrata* embryos. *Chemosphere*, 81: 218-227.
- Osman, G.Y., Mohamed, A.Z.; Sheir, K.S.; Hassab EL Nabi, S.E. and Allam, A.S. (2014). Molluscicidal activity of Mirazid on *Biomphalaria alexandrina* snails: biological and molecular studies. *Inter J. Adv. Res.*, 2: 977-989.

- Rathish, N.; Yogeshkumar, V. and Sumitra, C. (2009). Preliminary phytochemical analysis of some marine algae of western coast of Gujarat. *Plant Archives*, 9: 173-174.
- Raven, J.A.; Giordano, M.; Beardall, J. and Maberly, S.C. (2012). Algal evolution in relation to atmospheric CO₂: carboxylases, carbon-concentrating mechanisms and carbon oxidation cycles. *Phil. Trans. R. Soc. B*, 367: 493-507.
- Shaapan, R.M.; Toaleb, N.I. and Abdel-Rahman, E.H. (2015). Significance of a common 65 kDa antigen in the experimental fasciolosis and toxoplasmosis *J Par Dis*. 39: 550-556.
- Singaba, A.N.B.; Ahmed, A.H.; Sinkkonen, J.; Ovcharenko, V. and Pihlaja, K. (2006). Molluscicidal activity and new flavonoids from Egyptian *Iris germanica* L (var. *alba*) *Zeitschrift für Naturforschung C*, 61: 57-63.
- Soliman, F.M.; El Tohamy, S.F.; Fathy, M.M. and Ramadan, A. (1994). Phytochemical and biological investigation of *Jania rubens* (L.) Lamx. Amino acids, proteins, nitrogenous bases and biological screening. *J. Drug Res. Cairo*, 21: 155-155.
- Tadros, M.M.; Ghaly, N.S. and Moharib, M.N. (2008). Molluscicidal and schistosomicidal activities of a steroidal saponin containing fraction from *Dracaena fragrans* (L.). *J Eryp Soc Parasitol.*, 38: 585-598.
- Van der Steen, W.J.; Van den Hoven, N.P. and Jager, J.C. (1968). A method for breeding and studying freshwater snails under continuous water change, with some remarks on growth and reproduction in *Lymnaea stagnalis* (L.). *Neth J Zool.*, 19: 131-139.
- WHO (World Health Organization) 1983 World Health Organization Report of the scientific working group on plant molluscicides. TDRrSCH-SWG 4Ž .r83.3, 1983:1.
- WHO (World Health Organization) (2002). TDR strategic direction for research: schistosomiasis. In: World Health Organization, Genève.
- Yacout, G.; Ghareeb, D. A.; Elguindy, N. M. and Elmoneam, A. A. A. (2011). Phytochemical constituents and bio screening activities of Alexandria Mediterranean Sea Green and Red Algae. *J Enviro Sci Heal Part B*, 38: 463-478.