CYTOTOXIC AND GENOTOXIC EFFECTS OF METHANOLIC CRUDE EXTRACTS FROM SELECTED MARINE SPONGES COLLECTED FROM TERENGGANU ISLANDS ON *ACANTHAMOEBA* SPP.

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MASTER OF SCIENCE
UNIVERSITI MALAYSIA TERENGGANU
MALAYSIA

2008

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QL 368 .A5 I3 2008

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Cytotoxic and genotoxic effects of methanolic crude extracts from selected marine sponges collected from Terengganu Island on Acanthamoeba SPP / Ida Muryany Md Yasin.

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Thesis Submitted in Fulfillment of the Requirement for the
Degree of Master of Science in the Faculty of Science and Technology
Universiti Malaysia Terengganu

May 2008

From the bottom of my heart.....Both of you are very special..

Abang and baby Hasan...

Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu in fulfillment of the requirement for the degree of Master of Science.

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IDA MURYANY BT. MD YASIN

May 2008

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Faculty : **Science and Technology**

Acanthamoeba is a free-living amoeba but pathogenic to humans, causing a potentially blinding infection of the cornea (keratitis). Keratitis is an important public health problem developing country. Therefore, research on new drugs for the treatment of this infection is still constitutes an important therapeutic demand. The objective of this study were determined the cytotoxic and genotoxic effects of crude extracts of sponges from *Aaptos sp.*, *Xestospongia sp.* and *Halicondria sp.* collected from Kapas, Perhentian and Redang Islands, Terengganu on trophozoites of two clinical isolates namely *Acanthamoeba castellanii* (IMR isolate) and *Acanthamoeba sp.* (HKL isolate) and one free-living amoeba, *Acanthamoeba polyphaga* strain 1501/3A (but potential of being pathogenic). The cytotoxicity experiments were conducted in 24 well plates for 72 hr at 30°C. IC_{50} concentration obtained ranging from 0.456 to 1.6 mg/mL. Results of each test were reported as amebostatic because all trophozoites did not grow and appear to form young cyst and induce encystation. The statistical analyses (Student t-test) showed there was a significant statistical evidence of differences in percentage of viability cells after treated with all of the crude extracts at 95% confidence interval level ($p<0.05$) compared to the negative control. Observation under light microscopy showed all crude extracts had amoebostatic effect on 30 minutes incubation after treatment of the trophozoites *Acanthamoeba sp.* (HKL isolate) and *A. castellanii* (IMR isolate) and trophoizoites of *A.*

polyphaga strain 1501/3A became encysment after 48 hr of exposured to crude extracts. In other experiments, coverslips were placed in 6-well plates containing mixture of extract and amoeba culture media for *Acanthamoeba* attachment and incubated for 72 hr at 30°C and the morphological observation on the structural damage of the treated and untreated trophozoites were investigated under SEM. Trophozoites displayed abnormal and irregular in shape, in most cases with cystic appearances and became shrunk. Number of acanthapodia and food cup also was reduced compared to untreated cells. The extracts produced disruption of amoeba cell membrane which showed extensive blebbing and smaller size of pores on the cell surface was clearly visible. Apoptosis in *Acanthamoeba* spp. were determined by Acridine Orange Propidium Iodide (AOPI) staining and observed by fluorescence microscopy. Negative control cells wells gave green colour indicating that there was no leakage occurred. Cells treated with crude extracts of sponges showed the leakage of membrane cells after 72 h treatment approved by nuclei of apoptotic cells were substantially fragmented and condensed, whereas nuclei of normal cells were round and appearing. Cells were observed undergoing early and late apoptosis revealed a characteristic by distinct morphological changes such as cytoplasmic condensation and disorganization with dense orange areas, cells shrinkage and smaller in size and also blebbing of plasma membrane under fluorescence microscopy. Statistical analysis was done by ANOVA following Dunnette test showed exposured of *Acanthamoeba* cells to IC_{30} of crude extracts resulted in highly significant ($P<0.05$) increased in apoptosis, compared to negative control. Genotoxicity study was performed by the Alkaline Comet Assay. After cells were electrophorised under alkaline condition, all of the crude extracts significantly induced DNA damage compared to negative control. Majority of the DNA cells was at score 1, 2 and 3. Furthermore, statistical analyses showed, for cells treated with crude extracts there was a significant difference at Score 0, 1, 2 and 3 at the level of ($P < 0.05$) compared to untreated cells. In conclusion, the present study suggested that crude extracts of sponges showed cytotoxic and genotoxic effects against *Acanthamoeba* spp. and have a potential to be a new antiamoebic agent.

Abstrak tesis yang dikemukakan kepada Senat Universiti Malaysia Terengganu sebagai memenuhi keperluan untuk Ijazah Sarjana Sains.

KESAN SITOTOKSIK DAN GENOTOKSIK METANOLIK EKSTRAK KASAR DARI SPONGES MARIN TERPILIH YANG DIPEROLEHI DARI PULAU-PULAU DI TERENGGANU KE ATAS *ACANTHAMOEBA* SPP.

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Mei 2008

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Acanthamoeba adalah ameba hidup bebas tetapi patogenik kepada manusia, berpotensi menyebabkan infeksi kebutaan pada kornea (keratitis). Keratitis adalah masalah kesihatan umum yang penting di negara membangun. Oleh itu, penyelidikan ke atas dadah baru untuk rawatan infeksi ini tetap menjadi keperluan terapeutik yang penting. Objektif kajian ini adalah untuk menentukan kesan sitotoksik dan genotoksik ekstrak kasar sponges dari *Aaptos sp.*, *Xestospongia sp.* dan *Halicondria sp.* yang diperolehi dari Pulau Kapas, Redang dan Perhentian, Terengganu ke atas trofozoit dari dua isolat klinikal iaitu *Acanthamoeba sp.* (HKL isolat) dan *Acanthamoeba castellanii* (IMR isolat) dan satu ameba hidup bebas iaitu *Acanthamoeba polyphaga* strain 1501/3A (tetapi berpotensi menjadi patogenik). Eksperimen sitotoksisiti dilakukan di dalam plat 24 telaga selama 72 jam pada 30°C. Kepekatan IC_{50} yang diperolehi adalah antara 0.456 hingga 1.600 mg/mL. Keputusan setiap eksperimen menunjukkan amebostatik kerana semua trofozoit tidak menunjukkan pertumbuhan dan bertukar menjadi sista muda dan menyebabkan eksistasi. Analisis statistik (Student t-test) menunjukkan perbezaan yang signifikan dalam peratusan viabiliti sel selepas sel dirawat dengan ekstrak kasar pada aras keertian 95% ($p<0.05$) berbanding kawalan negatif. Pemerhatian di bawah mikroskop cahaya

menunjukkan semua ekstrak kasar memberi kesan amebostatik pada 30 minit inkubasi selepas diberi rawatan ke atas trofozoit *Acanthamoeba sp.* (HKL isolat) dan *Acanthamoeba castellanii* (IMR isolat) dan trofozoit *Acanthamoeba polyphaga* strain 1501/3A bertukar kepada ensismen selepas 48 jam pendedahan kepada ekstrak kasar. Dalam eksperimen lain, sisip kaca diletakkan di dalam plat 6 telaga yang mengandungi ekstrak dan media kultur ameba untuk perlekatan *Acanthamoeba* dan inkubasi selama 72 jam pada 30°C dan pemerhatian morfologi pada kemusnahan struktur trofozoit yang diberi rawatan dan tanpa rawatan diperhati menggunakan SEM. Trofozoit menjadi abnormal dan bentuk tidak tetap, kebanyakannya menjadi sistik dan mengecut. Bilangan 'acanthapodia' dan 'food cup' juga berkurang berbanding sel tanpa rawatan. Ekstrak ini menyebabkan kemusnahan pada membran sel ameba di mana ia menunjukkan pembintikan yang teruk dan pengecilan saiz vakuol pada permukaan sel sangat jelas kelihatan. Apoptosis pada sel *Acanthamoeba* telah ditentukan menggunakan pewarnaan Acridine Orange Propidium Iodide dan diperhatikan menggunakan mikroskop fluoresen. Sel kawalan negatif memberi warna hijau yang menunjukkan tiada kebocoran sel berlaku. Sel yang di beri rawatan dengan ekstrak kasar sponges menunjukkan kebocoran pada sel membran ameba selepas 72 jam rawatan dibuktikan melalui nukleus pada sel apoptotik yang fragmen dan kondensasi, sebaliknya nukleas sel normal adalah bulat dan jelas. Sel yang mengalami apoptosis awal dan lambat menunjukkan perubahan morfologi yang jelas seperti kondensasi dan ketidakteraturan sitoplasma yang padat, pengecutan sel dan saiz sel bertambah kecil dan juga pembintikan membran plasma. Analisis statistik menggunakan ANOVA diikuti Ujian Dunnette menunjukkan pendedahan sel *Acanthameba* pada IC_{30} ekstrak kasar adalah signifikan ($p<0.05$) berbanding kawalan negatif. Kajian genotoksisiti adalah menggunakan Asai Komet Beralkali. Selepas sel dielektroforesis dalam keadaan beralkali, semua ektrak kasar menyebabkan kemusnahan DNA secara signifikan berbanding kawalan negatif. Majoriti DNA sel menunjukkan skor 1, 2 dan 3. Analisis statistik menunjukkan sel yang diberi rawatan menunjukkan perbezaan signifikan pada Skor 0, 1, 2 dan 3 ($p<0.05$) berbanding sel yang tidak diberi rawatan. Kesimpulan kajian ini menunjukkan bahawa ekstrak kasar sponges memberi kesan sitotoksik dan genotoksik ke atas *Acanthameba* spp. dan berpotensi untuk menjadi agen antiamebik yang terbaru.