

GENOTOXIC EFFECT OF TWO *ACANTHAMOEBA*
LINES ON HeLa CELLS

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**GENOTOXIC EFFECT OF TWO *ACANTHAMOEBA* LYSATES ON HeLa
CELLS**

By
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A research report submitted in partial fulfillment of the requirements for the award of
the degree of Bachelor of Science (Biological Sciences)

**DEPARTMENT OF BIOLOGICAL SCIENCES
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**PENGAKUAN DAN PENGESAHAN LAPORAN
PITA I DAN II**

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: **GENOTOXIC EFFECT OF TWO *ACANTHAMOEBA* LYSATES ON HELA CELLS** oleh **ASHIMAH EFFENDY BINTI ASHRAFF**, no. matrik: **UK12384** telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan memperolehi Ijazah **SARJANA MUDA SAINS (SAINS BIOLOGI)**, Fakulti Sains dan Teknologi, Universiti Malaysia Terengganu.

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DECLARATION

I hereby declare that this research report entitled Genotoxic Effect of Two *Acanthamoeba* Lysates on HeLa Cells is the result of my own research except as cited in the references.

Signature : 
Name : Ashimah Effendy bt Ashraff
Matrix No : UK12384
Date : 13 MAY 2008

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ABSTRACT

Genotoxic effect of *Acanthamoeba* lysates was studied on human cervical cancer cell line. Lysates were obtained from *Acanthamoeba polyphaga* which are freshwater isolate and *Acanthamoeba* (HKL isolate) which is a clinical isolation from corneal scrapings of keratitis patient. These lysates were treated on HeLa cells by using MTT assay. The mitochondrial dehydrogenases in living cells reduced the MTT substances to a blue-magenta colored formazan crystals. The number of intact alive cells was directly proportional to the amount of crystal formed. Results obtained from the study indicated that the concentration of lysates that inhibited 50% (IC₅₀) of the cancer cells population for *Acanthamoeba polyphaga* and *Acanthamoeba* (HKL isolate) lysates are 85.0 µg/mL and 3.0 µg/mL, respectively while the IC₂₅ values are 42.5 µg/mL and 1.5 µg/mL, respectively. The genotoxic effect of these lysates on HeLa cells by their IC₅₀ and IC₂₅ values were studied by using Comet assay. From the DNA damage observed, *Acanthamoeba* (HKL isolate) lysate showed severe genotoxic effect compared to the lysate of *Acanthamoeba polyphaga*. The percentage of the DNA damage for IC₅₀ treatment with *Acanthamoeba* (HKL isolate) lysate majority scored 3 with 57% followed by score 4 and score 2 with 38% and 5%, respectively while for IC₂₅ treatment majority scored 1 with 51% followed by score 2, 3, and 0 with 41%, 5%, and 3%, respectively. The percentage of the DNA damage for IC₅₀ treatment with *Acanthamoeba polyphaga* lysate majority scored 2 with 38% followed by score 1, 3, and 4 with 32%, 25%, and 5%, respectively while for IC₂₅ treatment mostly scored 1 and 0 with 37% and 35%, respectively followed by score 2 and 3 with 22% and 6% respectively. These results indicated that *Acanthamoeba* (HKL isolate) lysate has more potential to become anti-cancer agent than *Acanthamoeba polyphaga* lysate.

ABSTRAK

Kesan genotoksik oleh lisat *Acanthamoeba* telah dikaji ke atas sel kanser servix manusia. Lisat diperolehi dari *Acanthamoeba polyphaga* yang merupakan isolat air tawar dan *Acanthamoeba* (isolat HKL) yang diperolehi daripada kornea pesakit yang menghidap keratitis. Kedua-dua lisat ini telah diuji ke atas sel HeLa dengan menggunakan kaedah MTT. Dehidrogenes mitokondria dalam sel-sel hidup bertindakbalas dengan menukarkan bahan MTT kepada kristal formazan biru-magenta. Bilangan sel hidup adalah berkadar terus dengan jumlah kristal yang terbentuk. Berdasarkan keputusan yang diperolehi dari kajian ini, kepekatan lisat yang dapat merencat pertumbuhan sel sebanyak 50% daripada populasi sel kanser (IC_{50}) bagi lisat *Acanthamoeba polyphaga* dan *Acanthamoeba* (isolat HKL) ialah sebanyak 85.0 $\mu\text{g/mL}$ dan 3.0 $\mu\text{g/ml}$ masing-masing manakala bagi nilai IC_{25} pula ialah sebanyak 42.5 $\mu\text{g/mL}$ dan 1.5 $\mu\text{g/mL}$ masing-masing. Kesan genotoksik kedua-dua lisat ini ke atas sel HeLa oleh nilai IC_{50} dan IC_{25} masing-masing telah dikaji menggunakan kaedah Comet. Berdasarkan kerosakan DNA yang diperhatikan, lisat *Acanthamoeba* (isolat HKL) menunjukkan kesan yang lebih teruk berbanding lisat *Acanthamoeba polyphaga*. Peratus kerosakan DNA bagi rawatan IC_{50} oleh lisat *Acanthamoeba* (isolat HKL) majoriti ialah skor 3 dengan 57% diikuti oleh skor 4 dan 2 dengan 38% dan 5% masing-masing manakala bagi rawatan IC_{25} majoriti ialah skor 1 dengan 51% diikuti oleh skor 2, 3, dan 0 dengan 41%, 5%, dan 3% masing-masing. Peratus kerosakan DNA bagi rawatan IC_{50} oleh lisat *Acanthamoeba polyphaga* pula ialah majoriti skor 2 dengan 38% diikuti oleh skor 1, 3, dan 4 dengan 32%, 25%, dan 5% masing-masing manakala bagi rawatan IC_{25} kebanyakannya skor 1 dan 0 dengan 37% dan 35% diikuti oleh skor 2 dan 3 dengan 22% dan 6% masing-masing. Keputusan kajian ini menunjukkan bahawa lisat *Acanthamoeba* (isolat HKL) lebih berpotensi untuk digunakan sebagai agen anti-kanser berbanding lisat *Acanthamoeba*.