TOXICOLOGICAL ASSESSMENT OF *NANNOCHELOROPSIS OCUVATA* MICROALGAE IN RATS

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By

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Thesis Submitted To School of Graduate Studies, Universiti Putra Malaysia in Fulfilment of the Requirements for the Degree of Master of Science

June 2011
To my beloved wife Bahareh and my children Shervin and Nazanin
Abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Master of Science

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June 2011

Chairman:  Loh Su Peng, PhD

Faculty: Medicine and Health Sciences

*Nannochloropsis oculata* is a green unicell microalgae with valuable ingredients. Despite this, some of them are harmful for humans and animals. The chief route of human and animal exposure to the effects of these microalgae is food, especially that derived from aquatic origins. The major toxicity effects of harmful microalgae are associated with their production of toxins and bioaccumulation of heavy metals. The aims of this study were to determine the mineral composition of Nannochloropsis and to investigate acute and sub-chronic toxicity of *Nannochloropsis oculata*. The mineral content of *Nannochloropsis oculata* was assayed using a Flame Atomic Absorption Spectrometry (AAS) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Mineral analysis showed that *Nannochloropsis oculata* did not bioconcentrate high amounts of heavy metals. In the acute study, twelve Sprague-Dawley rats of both sexes were gavaged with 12 g/kg body weight of
Nannochloropsis oculata one time and then tested for morbidity and mortality in 14 days. Rats had free access to food and water in these two weeks. Blood samples were drawn before treatment and kept as a control for later analysis. Results of the acute study revealed that the oral LD₅₀ of Nannochloropsis oculata in rats was greater than 12 g/kg body weight since no toxicity effects were observed on Nannochloropsis oculata in terms of morbidity signs, plasma biochemical parameters, organ tissue, or body weight gain. In the sub-chronic study, thirty six Sprague-Dawley rats of both sexes were chosen and divided into three groups; a control group and two treatment groups. These groups were provided with diet containing respectively 0, 3, and 6 g/kg body weight of Nannochloropsis oculata every early morning and then allowed free access to normal food and water ad libitum for 60 days. The results of the sub-chronic study demonstrated that in comparison with the control group, no biologically significant effects of Nannochloropsis oculata were observed on organ weights, male body weight gain, or on the plasma biochemical parameters. However, low creatinine and significant differences in body weight gain by female rats were noted in the treatment groups. Histopathology examination of male and female rat livers and kidneys did not disclose any adverse effects. In addition, no significant differences were found in the antioxidant activities between the treatment groups and the control one; the antioxidant activity of plasma was evaluated using the Ferric Reducing Ability of Plasma (FRAP) and ABTS methods. In summary, it could be concluded that exposure of growing rats of both genders to Nannochloropsis oculata at dosages up to 12 g/kg of body weight is non-toxic.
Penilaian ketoksikan mikroalga *Nannochloropsis oculata* dalam tikus

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*Nannochloropsis oculata* merupakan sejenis mikroalga unisel hijau yang kaya dengan khasiatnya. Walaupun manapun, sesetengah daripadanya adalah berbahaya kepada manusia dan haiwan. Laluan utama pendedahan manusia dan haiwan terhadap mikroalga tersebut adalah melalui makanan, terutama sekali yang berasal dari lautan. Kesan utama ketoksikan mikroalga ini adalah dikaitkan dengan penghasilan toksin dan bioakumulasi logam berat. Tujuan kajian ini adalah untuk menentukan komposisi mineral *Nannochloropsis oculata* dan mengkaji ketoksikan akut dan sub-kronik *Nannochloropsis oculata*. Kandungan mineral *Nannochloropsis oculata* ditentukan dengan menggunakan nyalaan AAS dan ICP-MS. Analisa kandungan mineral menunjukkan *Nannochloropsis oculata* tidak mengumpul jumlah logam berat yang tinggi. Di dalam kajian akut, dua belas tikus Sprague-Dawley bagi kedua-dua jantina telah diberikan 12g/kg berat badan *Nannochloropsis*
ooculata kering sekali secara suap paks dan diuji untuk morbiditi dan mortaliti selama 14 hari. Di dalam tempoh 2 minggu tersebut, tikus bebas untuk mendapatkan makanan dan minuman. Sampel darah telah diambil sebelum rawatan sebagai kawalan untuk analisis seterusnya. Keputusan daripada kajian akut ini menunjukkan nilai oral LD$_{50}$ untuk *Nannochloropsis oculata* dalam tikus adalah lebih daripada 12 g/kg berat badan memandangkan tiada kesan ketoksikan diperhatikan yang merangkumi tanda morbiditi, parameter biokimia plasma, tisu organ dan berat badan. Di dalam kajian sub-kronik, tiga puluh enam ekor tikus Sprague-Dawley bagi kedua-dua jantina telah dipilih dan dibahagikan kepada tiga kumpulan; satu kumpulan kawalan dan dua kumpulan rawatan. Kumpulan-kumpulan tersebut telah dibekalkan dengan diet yang mengandungi 0, 3, dan 6 g/kg berat badan *Nannochloropsis oculata*, pada setiap awal pagi dan dibenarkan bergerak bebas untuk mendapatkan makanan dan minuman selama 60 hari. Keputusan daripada kajian sub-kronik terdasarkan perbandingan dengan kumpulan kawalan menunjukkan bahawa tiada kesan biologi yang signifikan oleh *Nannochloropsis oculata* diperhatikan pada berat organ, peningkatan berat badan tikus jantan, mahupun parameter biokimia plasma. Walau bagaimanapun, kreatinin yang rendah dan perbezaan signifikan dalam peningkatan berat badan tikus betina dapat diperhatikan dalam kumpulan rawatan. Pemeriksaan histopatologi ke atas hati dan ginjal tikus jantan dan betina tidak menunjukkan sebarang kesan buruk. Tambahan pula, tiada perbezaan signifikan didapati di dalam aktiviti antioksidan di dalam kumpulan rawatan dengan kumpulan kawalan. Aktiviti antioksidan di dalam plasma ditentukan dengan menggunakan kaedah FRAP dan ABTS. Secara rumusan, ia
dapat disimpulkan bahawa pendedahan tikus bagi kedua dua jantia kepada *Nannochloropsis oculata* pada dos setinggi 12 g/kg berat badan adalah tidak toksik.
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I certify that a Thesis Examination Committee has met on 20 June 2011 to conduct the final examination of Soroush Kafaie on his thesis entited “Toxicological Assessment of *Nannochloropsis Oculata* Microalgae in Rats” in accordance with the Universities and University colleges Act 1971 and the constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the Master of Science.

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DECLARATION

I declare that the thesis is my original work except for quotations and citation which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for other degree at Universiti Putra Malaysia or other institutions.

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Date: 20 June 2011
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