UNIVERSITI PUTRA MALAYSIA

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Abstract of thesis presented to the Department of Cell & Molecular Biology in fulfilment of the requirement for the Bachelor of Science (Hons.) Cell & Molecular Biology

THE ANTI- CANCER EFFECT OF *CLINACANTHUS NUTANS* IN 4T1 BREAST CANCER IN BALB/C MICE

By

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June 2015

Chair: Dr. Nik Mohd Afizan bin Nik Abd. Rahman

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Breast cancer is one of the most common cancers that cause death in the world especially among women. A good treatment should be applied to stop the growth of tumor and effectively regulate the immune system. *Clinacanthus nutans* was reported to have antioxidant properties against cancer cell lines. However, the *in vivo* antitumor effects of methanol extract *C. nutans* toward breast cancer have not been reported yet. The present study is to evaluate the anticancer effect of *C. nutans* methanol extracts and compare the effectiveness between high dose (1000mg/kg) and low dose (200mg/kg) of *C. nutans* leaf extract on breast cancer in Balb/c mice. As described in this study, methanol extract of *C. nutans* has decreased more mitotic cells in low dose compared to high dose of treatment, as evidenced by hematoxylin and eosin staining of the tumor. It modulated the immune system by increasing the population of cytotoxic and helper T cell but suppressed natural killer cell. Furthermore, methanol extract of *C. nutans* elevated the level of interleukin-2 and

interferon gamma more in the low dose compared to the high dose of treatment. It was able to inhibit metastasis more effectively in low dose of treatment, as evidenced by bone marrow smearing and clonogenic assay. Besides, methanol extract of *C. nutans* was also found to possess antioxidant effect by decreasing the NO and MDA levels and it was more effective in the low dose of treatment. On the whole, the low dose of methanol extract of *C. nutans* may serve as an agent in curing breast cancer.

Keywords: *Clinacanthus nutans*, 4T1, cancer, metastasis

Abstrak tesis yang dikemukakan kepada Jabatan Biologi Sel & Molekul

Sebagai memenuhi keperluan untuk Bacelor Sains (Kepujian) Sel Biologi & Molekul

KESAN ANTI-KANSER CLINACANTHUS NUTANS DALAM KANSER 4T1 DI TIKUS BALB/C

Oleh

CHUA SIK JUAN

Jun 2015

Pengerusi: Dr. Nik Mohd Afizan bin Nik Abd. Rahman

Fakulti:

Kanser payudara adalah salah satu kanser yang paling biasa menyebabkan kematian di dunia terutama di kalangan wanita. Rawatan yang baik bukan sahaja harus menghentikan pertumbuhan tumor malah perlu berkesan mengawal sistem imun. *Clinacanthus nutans* dilaporkan mempunyai sifat antioksidan sel kanser. Walau bagaimanapun, kesan antitumor *in vivo* ekstrak metanol *C. nutans* terhadap kanser payudara belum dilaporkan lagi. Kajian ini adalah untuk menilai kesan anti-kanser *C. nutans* ekstrak metanol dan membandingkan keberkesanan antara dos tinggi (1000mg / kg) dan dos rendah (200mg / kg) ekstrak *C. nutans* terhadap kanser payudara di BALB / c tikus. Seperti yang dinyatakan dalam kajian ini, ekstrak metanol daripada *C. nutans* telah menyebabkan penurunan bilangan sel-sel mitosis lebih banyak dalam rawatan dos yang rendah berbanding dengan rawatan dos yang tinggi, seperti yang ditunjukkan oleh pewarnaan hematoxylin dan eosin. Sistem imun telah dimodulasikan dengan peningkatan populasi sitotoksik dan penolong T sel

tetapi menindas sel pembunuh semula jadi. Tambahan pula, dos rendah rawatan ekstrak metanol daripada *C. nutans* dapat meninggikan tahap interleukin-2 dan interferon gamma lebih berkesan berbanding dengan dos rawatan yang tinggi. Dos rendah rawatan juga dapat menghalang metastasis dengan lebih berkesan, seperti yang ditunjukan dalam hasil puncalitan sum-sum tulang dan assay klonogenik. Selain itu, ekstrak metanol daripada *C. nutans* juga didapati mempunyai kesan antioksidan dengan mengurangkan tahap NO dan MDA dan dos rendah rawatan menunjukkan hasil yang lebih berkesan. Secara keseluruhannya, dos rawatan rendah ekstrak metanol daripada *C. nutans* mungkin boleh berfungsi sebagai agen dalam menyembuhkan kanser payudara.

Kata kunci: *Clinacanthus nutans*, 4T1,kanser, metastasis

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Approval

This thesis was submitted to the Department of Cell & Molecular Biology, Faculty of Biotechnology & Biomolecular sciences and has been accepted as fulfilment of the requirement for the degree of Bachelor of Sciences (Hons.) Cell & Molecular Sciences. The member of the Supervisory Committee was as follows:

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CHAPTER 1.0 INTRODUCTION

Cancer is a disease which leading the cause of death worldwide. Based on the statistic provided by World Health Organization, mortality due to cancer were 8.2 million in 2012. Breast, liver, colorectal, lung and stomach cancers are the most common cancers that cause deaths every year. Among the cancers, breast cancer accounted for 521,000 deaths in 2012 which is increasing compared to 2008 for 458,000 deaths (WHO:Cancer, 2014). In developing countries such as Malaysia, cancer appears to be the major health threat. According to the Health Fact 2013 from Malaysian Ministry of Health, 13.63% of deaths due to cancer were recorded in 2012. Among all type of cancers, breast cancer was the most common cancer especially among the females.

Few researches have proved that the level of free radical, the reactive oxygen/ nitrogen species (RONS) played an important role in tumorigenesis (Shi et al., 2012). RONS are generated in many ways and have a role in biological systems. Even at low concentration, RONS act as a defensive agent against infection and function to induce mitogenic response. On the other hand, overproduction of RONS will cause damage to lipids, protein and nucleic acids. This free radical eventually will give rise to genetic instability and promote the formation of cancer (Valko et al., 2006). Antioxidant supplement could scavenge RONS and prevent the tumor growth as evidence has shown that patients with breast cancer were saved by consuming antioxidant supplement (Hu et al., 2012). Chemotherapy treatments for cancer show limited benefit and low efficiency. Those treatments also cause severe side-effects and dose-limiting toxicities (Monsuez et al., 2010). Therefore, traditional herbs are now popular to study their potential to be used as alternate for anticancer treatment.

Clinacanthus nutans (Family: Acanthaceae) or commonly known as "Sabah snake grass" or Belalai Gajah in Malaysia is one of the medical plants in Malaysia with high medical values (Roosita et al., 2008). This species of *Clinacanthus* is small shrubs that can be found in tropical regions of the Asia. It serves as herbal tea in Malaysia. Malaysian always boiled the fresh leaves of the plant with water and consumed it for health purpose. In Thailand, it is known as a traditional medicine that is used for the treatment of skin rashes, varicella- zoster virus (VZV) lesions, insect- and snake- bite and herpes simplex virus (HSV) (Tuntiwachwuttikul et al., 2006). In recent year, a number of researches has shown that the compounds in the *C. nutans* can inhibit the production of viral RNA which has the potential to treat the infection of Type 2 Dengue Virus (Sittiso et al., 2010).

Antioxidant properties of *C. nutans* toward cultured cancer cell lines were previously evaluated using radical scavenging assays. The result showed that *C. nutans* leave extract possesed antioxidant properties against cultured cancer cell lines which propose that it might be an alternative for cancer treatment or prevention (Yong et al., 2013). However, no research has been done about the effect of *C. nutans* leave extract toward breast cancer cells. Therefore, the present research is done with the following objectives:

1. To evaluate the anticancer effect of *Clinacanthus nutans* leaves on breast cancer in Balb/c mice.
2. To compare the effectiveness between high dose and low dose of *Clinacanthus nutans* leaf extract on breast cancer in Balb/c mice.

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