UNIVERSITI PUTRA MALAYSIA

IN VITRO CYTOTOXICITY AND *IN VIVO* ANTITUMOUR PROPERTIES OF KENAF SEED OIL TOWARDS LEUKAEMIA

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BY

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

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IN VITRO CYTOTOXICITY AND *IN VIVO* ANTITUMOUR PROPERTIES OF KENAF SEED OIL TOWARDS LEUKAEMIA

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Faculty : Institute of Bioscience

The current treatments for leukaemia such as chemotherapy and radiation therapy have prolonged the survival rate. However, the adverse effects of these treatments are difficult to handle. Thus, there is a need to seek for other remedies, such as the use of natural products. Natural products such as plants play an important role in the current cancer treatment. The advantage of using plant-derived anticancer agents is that the produced adverse effects are lesser as compared to the synthetic drugs. Kenaf (*Hibiscus cannabinus*) seed oil (KSO) is a rich source of bioactive phytochemicals with high anti-oxidative and cancer chemopreventive properties. Nevertheless, the anti-leukaemia properties of KSO have yet been investigated. This study investigated the anti-leukaemia properties of KSO *in vitro* and *in vivo*. KSO was extracted by

supercritical carbon dioxide fluid extractor (SFE), and evaluated for cytotoxic properties on leukaemia (HL-60, WEHI-3B and K562) and normal (3T3) cells by MTT assay with concentrations ranging from 50 to 800 µg/ml for 72 hours. The morphological changes of KSO-treated leukaemia cells were observed under an inverted light microscope and a fluorescence microscope. The cell cycle profile of KSO-treated leukaemia cells was analysed by flow cytometry. For *in vivo*, acute toxicity and anti-leukaemia properties of KSO were determined. Male BALB/c mice were injected intraperitoneally with WEHI-3B cells and administered orally with KSO at the dose of 0.5, 1.0 and 1.5 g/kg for 14 days. Upon completion, the blood of the mice was examined for the expression of cell surface marker of T cell (CD3), B cell (CD19), monocyte and granulocyte (CD11b) by staining with anti-CD3-FITC, anti-CD19-PE and anti-CD11b-PE antibodies, respectively. The livers and spleens were isolated, weighed and photographed. The spleens were processed for histopathological analysis. The yield of KSO by SFE ranged from 11 to 13% (w/w). KSO was found to be cytotoxic towards all the leukaemia cells in a dose-dependent manner with no effects on 3T3 cells even at the highest concentration employed (800 µg/ml). Oil from SFE at 600 bar 40 ºC (KSO V600/40) was the most cytotoxic towards HL-60, WEHI-3B and K562 cells as compared to other extractions (KSO V600/60, KSO V600/80 and Soxhlet) with the 50% inhibition concentration (IC_{50}) values of 178.78±10.52, 189.43±11.63 and 213.33±15.45 µg/ml, respectively. KSO V600/40-treated leukaemia cells exhibited typical characteristics of apoptosis such as cellular shrinkage, membrane blebbing, chromatin condensation and nuclear fragmentation. Cell cycle analysis revealed that KSO V600/40 at IC_{50} value induced G1 phase cell cycle arrest and significantly increased ($p<0.05$) the sub-G1 apoptotic population in the leukaemia cells. For *in vivo*, acute toxicity study revealed that KSO

V600/40 did not cause any mortality in the healthy normal mice even at the highest dose (5.0 g/kg), suggesting that KSO is non-toxic by oral route. Treatment with KSO V600/40 at 1.0 and 1.5 g/kg increased the population of T cells, but decreased the population of immature monocytes and granulocytes in the blood of WEHI-3B injected BALB/c mice (WEHI-3B/BALB/c mice). Spleen and liver weight of WEHI-3B/BALB/c mice decreased after the treatment with KSO V600/40. Moreover, infiltration of leukaemic cells into the splenic red pulp reduced after the treatment, indicating that KSO V600/40 reduced the severity of leukaemia in WEHI-3B/BALB/c mice. In conclusion, KSO V600/40 showed cytotoxic effect via the induction of G1 phase cell cycle arrest and apoptosis in the leukaemia cells, and reduced the severity of leukaemia in WEHI-3B/BALB/c mice.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGENALPASTIAN KESAN SITOTOKSIK *IN VITRO* DAN ANTITUMOR *IN VIVO* MINYAK BIJI KENAF TERHADAP LEUKEMIA

Oleh

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Rawatan untuk leukemia yang ada pada waktu ini seperti kemoterapi dan terapi radiasi telah meningkatkan tahap kehidupan. Walau bagaimanapun kesan sampingan daripada rawatan berkenaan adalah sukar ditangani. Oleh itu, adalah penting untuk mendapatkan kaedah rawatan yang lain seperti penggunaan bahan semulajadi. Bahan semulajadi seperti tumbuh-tumbuhan memainkan peranan yang penting dalam rawatan kanser pada masa kini. Kelebihan penggunaan agen antikanser berasaskan tumbuh-tumbuhan adalah dapat mengurangkan kesan sampingan berbanding ubatan sintetik. Minyak biji kenaf (*Hibiscus cannabinus*) (KSO) merupakan sumber yang kaya dengan fitokimia bioaktif dengan ciri anti-pengoksidaan yang tinggi dan pencegahan kanser. Namun demikian, ciri anti-leukemia KSO masih belum dikaji. Kajian ini dilakukan bagi menentukan kesan anti-leukemia KSO *in vitro* dan *in vivo*.

KSO diekstrak menggunakan pengekstrak cecair karbon dioksida superkritikal (SFE) dan diuji kesan sitotoksik ke atas sel leukemia (HL-60, WEHI-3B dan K562) dan sel normal (3T3) pada kepekatan dalam julat 50 ke 800 µg/ml selama 72 jam. Perubahan morfologi sel leukemia yang dirawat dengan KSO diperhatikan di bawah mikroskop keterbalikan dan mikroskop pendarflour. Profil kitaran sel leukemia yang dirawat dengan KSO dianalisis dengan sitometri aliran. Bagi kajian *in vivo*, ciri ketoksikan akut dan anti-leukemia KSO ditentukan. Mencit BALB/c jantan disuntik dengan sel WEHI-3B secara intraperitoneal dan KSO diberi secara oral pada dos 0.5, 1.0 dan 1.5 g/kg selama 14 hari. Selepas tamat, darah mencit dikaji untuk kehadiran penanda permukaan sel T (CD3), sel B (CD19), monosit serta granulosit (CD11b) dengan pewarnaan dengan anti-CD3-FITC, anti-CD19-PE dan anti-CD11b-PE, masing-masing. Hati dan limpa mencit diasing, ditimbang dan diambil gambar. Limpa diproses untuk analisis histopatologi. Hasil pengekstrakan KSO daripada SFE adalah di antara 11-13% (berat/berat). KSO didapati sitotosik terhadap kesemua sel leukemia secara bersandar dos tanpa memberi kesan terhadap sel normal 3T3 walaupun pada kepekatan yang paling tinggi (800 µg/ml). Minyak yang terhasil daripada pengekstrakan SFE pada 600 bar 40 ºC (KSO V600/40) memberikan kesan sitotoksik yang paling tinggi terhadap sel HL-60, WEHI-3B dan K562 berbanding estrak yang lain (V600/60, V600/80 dan Soxhlet) dengan nilai IC_{50} 178.78±10.52, 189.43±11.63 dan 213.33±15.45 µg/ml, masing-masing. Sel leukemia yang dirawat dengan KSO V600/40 menunjukkan ciri-ciri apoptosis seperti pengecutan sel, *blebbing*, kondensasi kromatin dan fragmentasi nukleus. Analisis kitaran sel menunjukkan KSO V600/40 pada nilai IC_{50} mengaruh penahanan kitaran sel pada fasa G1 dan meningkatkan populasi apoptosis sub-G1 secara signifikan ($p<0.05$) pada sel leukemia tersebut. Secara *in vivo*, kajian ketoksikan akut menunjukkan KSO

V600/40 tidak menyebabkan sebarang kematian pada normal mencit walaupun pada dos yang paling tinggi (5.0 g/kg). Ini menyarankan bahawa KSO adalah tidak toksik apabila diberi secara oral. Rawatan dengan KSO V600/40 pada 1.0 and 1.5 g/kg meningkatkan populasi sel T, tetapi menurunkan populasi monosit dan granulosit yang tidak matang dalam darah mencit WEHI-3B/BALB/c. Berat limpa dan hati mencit WEHI-3B/BALB/c menurun selepas rawatan dengan KSO V600/40. Sebagai tambahan, infiltrasi sel leukemia ke dalam pulpa merah limpa menurun selepas rawatan, menunjukkan KSO V600/40 mengurangkan kesan teruk leukemia pada mencit WEHI-3B/BALB/c. Secara kesimpulannya, KSO V600/40 mengaruh penahanan kitaran sel pada fasa G1 dan apoptosis pada sel leukemia, dan mengurangkan keterukan leukemia pada mencit BALB/c yang diaruh WEHI-3B.

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DECLARATION

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

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