UNIVERSITI PUTRA MALAYSIA

EFFECTS OF COLA NUT [*COLA NITIDA* (VENT.) SCHOTT & ENDL.] AQUEOUS EXTRACT ON RAT LIVER DURING HEPATOCARCINOGENESIS

MOHAMMAD KADIVAR

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MASTER OF SCIENCE

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By

MOHAMMAD KADIVAR

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

November 2010

This thesis is dedicated to my lovely parents, Maryam Nowroozalizadeh Shirazi and Mohammad Rahim Kadivar, who taught me the value of education. I am deeply indebted to them for their love, endless support and unwavering faith in me.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chair: **Professor Fauziah Othman, PhD**

Faculty: **Medicine and Health Sciences**

The effect of *Cola nitida* aqueous extract in hepatocarcinogenesis induced male *Sprague Dawley* rat livers, and elemental analysis of the cola nut was studied to investigate the possible anticancer activity. The unprocessed cola nuts were observed for their surface morphological structure under the scanning electron microscope (SEM). Together with the imaging, samples were then elementally analyzed using energy dispersive x-ray microanalysis attached to variable pressure scanning electron microscopy (EDX-VPSEM). SEM study of cola nut illustrates numerous crystals packed in clusters within the cell wall. The elemental analysis results revealed that the cola nut contained high amount of oxygen and carbon, in addition to potassium, phosphorus and magnesium. Potassium, magnesium and phosphorous have been well reported as co-factors of antioxidant enzymes to protect the body from oxygen free radicals. Additionally, these elements play important roles in metabolic mechanisms in the body.

Hepatocarcinogenesis was induced in rat livers according to the modified Solt and Farber method. Diethylnitrosamine (DEN) was injected into the rats at 200 mg/kg body weight to initiate hepatocarcinogenesis and after two weeks this was followed by feeding 0.02% 2-Acetylaminofluorene (AAF) to promote the hepatocarcinogenesis. The DEN/AAF induced rats were treated with 1, 2.5, and 5% (w/v) concentrations of cola nut extract or 0.001, 0.0025, and 0.005% w/v dilutions of glycyrrhizin as a drug control. There were normal and cancer controls; in addition, three groups of normal rats were treated with three concentrations of cola nut to observe the side effect of the cola nut on normal livers.

The body and liver weight profile results of this study showed no significant difference between treated groups compared to normal and cancer controls. The similarity in body weight gain and relative liver weight results might occur because of the short length of the *in vivo* experiment (eleven weeks).

The supplementation of cola nut extract decreased the level of plasma and microsomal GGT and GST tumor marker enzymes significantly in DEN/AAF induced liver tissues even better than glycyrrhizin. Additionally, it was revealed that cola nut extract has no effect on the level of GST and GGT enzymes in normal cells.

The histological and ultrastructural examination as well as the lesions scoring results demonstrated that the cola nut extract reduced neoplastic stage of the hepatocarcinogenic liver cells more than glycyrrhizin based on their abnormal

morphology, inflammation, necrosis, and fibrosis. Moreover, rat's normal hepatocytes treated with cola nut extract illustrated normal features.

TUNEL assay results showed the significant increase in the number of apoptotic cells in hepatocarcinogenic liver tissues treated with cola nut extract and glycyrrhizin. These results showed that cola nut did not induce the apoptosis in normal liver cells.

Quantitative real-time RT-PCR results revealed that although the level of alpha-fetoprotein (AFP) mRNA increased in DEN/AAF induced liver cells, but the supplementation of the cola nut and glycyrrhizin decreased it predominantly in hepatocarcinogenic liver cells treated with cola nut extract.

These findings suggest that cola nut might act as a promising anticancer against hepatocarcinogenesis with even higher efficacy compared to glycyrrhizin, without any side effects in normal liver cells.

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

KESAN EKSTRAK AKUEUS COLA NUT [*COLA NITIDA* (VENT.) SCHOTT & ENDL.] TERHADAP HATI TIKUS SEMASA HEPATOCARCINOGENESIS

Oleh

MOHAMMAD KADIVAR

November 2010

Pengerusi: Profesor Fauziah Othman, PhD

Fakulti: Perubatan dan Sains Kesihatan

Kesan ekstrak akueus *Cola nitida* dalam hati tikus *Sprague Dawley* yang diaruh hepatokarsinogenesis, dan analisis elemen terhadap cola nut dijalankan untuk mengkaji kemunglanan aktiviti antikanser yang dimiliki. Cola nut yang belum diproses diperhatikan untuk struktur morfologi permukaan di bawah SEM. Bersama dengan pengimejan, analasis elemen sampel dijalankan menggunakan EDX-VPSEM. Kajian SEM terhadap cola nut menunjukkan pelbagai hablur-hablur bersusun dalam kumpulan di dinding sel. Hasil analisis unsur elemen menunjukkan cola nut mengandungi jumlah oksigen dan karbon, yang tinggi di samping kalium, fosforus dan magnesium. Kalium, magnesium dan fosforus telah diketahui umum sebagai ko-faktor bagi enzim antioksidan untuk melindungi tubuh dari radikal bebas oksigen. Tambahan pula, unsur-unsur ini memainkan peranan yang penting dalan mekanisme-mekanisme metabolik di dalam tubuh.

Aruhan hepatokarsinogenesis berdasarkan teknik Solt dan Farber yang diubah suai. Sebanyak 200 mg/kg DEN disuntik ke dalam tikus untuk memulakan hepatokarsinogenesis dan selepas 2 minggu diikuti dengan memberi 0.02% AAF dalam makanan untuk penggalakan. Tikus yang diaruh DEN/AAF dirawat dengan 1, 2.5, dan 5% (w/v) kepekatan ekstrak cola nut atau 0.001, 0.0025, dan 0.005% w/v glycyrrhizin sebagai dadah kawalan. Terdapat normal dan kawalan kanser, dan 3 kumpulan tikus normal dirawat dengan tiga kepekatan cola nut yang berbeza untuk melihat kesan sampingan cola nut pada hati normal.

Profil berat badan dan hati menunjukkan tiada perbezaan yang ketara antara kumpulan yang dirawat berbanding dengan normal dan kawalan kanser. Persamaan dalam penambahan berat badan dan hasil berat relatif hati kemungkinannya berlaku kerana jangka masa eksperimen *in vivo* yang singkat (11 minggu).

Penambahan ekstrak cola nut mengurangkan paras plasma dan enzim penanda barah mikrosom GGT dan GST dalam tisu hati yang diaruh DEN/AAF malah lebih baik dari glycyrrhizin. Malah, ekstrak cola nut juga telah terbukti tiada kesan terhadap paras enzim GST dan GGTs dalam sel normal.

Pemeriksaan histologi dan ultrastruktur beserta hasil skor lesi menunjukkan ekstrak cola nut mengurangkan paras neoplastik hati sel hepatocarcinogenik lebih baik daripada

glycyrrhizin. Tambahan lagi, hepatosit normal yang dirawat dengan ekstrak cola nut menggambarkan ciri-ciri normal.

Asai TUNEL menunjukkan peningkatan yang signifikan dalam bilangan sel apoptotik tisu hati hepatokarsinogenik dirawat dengan ekstrak cola nut dan glycyrrhizin. Hasil ini menunjukkan bahawa cola nut tidak mengaruh apoptosis dalam sel hati normal.

Hasil kuantitatif real-time RT-PCR menunjukkan bahawa walaupun paras AFP mRNA meningkat pada sel hati yang diaruh dengan DEN/AAF, penambahan cola nut dan glycyrrhizin mengurangkan "predominantly" dalam sel hati tikus yang diaruh hepatokarsinogenesis yang dirawat dengan ekstrak cola nut.

Hasil kajian ini menunjukkan bahawa cola nut berkemungkinan bertindak sebagai anti kanser dan agen kemopreventif yang lebih baik berbanding dengan glycyrrhizn, tanpa sebarang kesan sampingan dalam sel hati normal.

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I certify that a Thesis Examination Committee has met on 16 November 2010 to conduct the final examination of Mohammad Kadivar on his thesis entitled "Effects of Cola Nut [*Cola Nitida* (Vent.) Schott & Endl.] Aqueous Extract on Rat Liver During Hepatocarcinogenesis" in accordance with the Universities and University College 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.

Members of the Thesis Examination Committee were as follows:

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This thesis was submitted to the senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of **Master of Science**. The members of the Supervisory Committee were as follows:

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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.

MOHAMMAD KADIVAR

Date: 16 November 2010

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