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PROPERTIES OF VANILLIN AND ITS EFFECTS ON COLORECTAL CANCER

IN VITRO AND *IN VIVO*

By

HO KET LI

Thesis Submitted to the School of Graduate Studies,

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Doctor of Philosophy

PROPERTIES OF VANILLIN AND ITS EFFECTS ON COLORECTAL CANCER *IN VITRO* AND *IN VIVO*

By

HO KET LI

August 2011

Chairman: Professor Maznah Ismail, PhD

Faculty : Institute of Bioscience

Vanillin is responsible for the flavor and smell of vanilla, a widely used flavoring agent. Previous studies showed that vanillin was a good red blood sickle cell inhibitor, anti-microbial agent and anti-mutagen. However, vanillin must be administered at high concentrations and prevented from being oxidized by the upper gastrointestinal tract to be medically effective. Hence, the objectives of this study were: i. to investigate the cytotoxic properties of vanillin on HT-29 colorectal cancer cell line; ii. to assess the negative effect of vanillin when administered at high concentrations *in vivo*; iii. to investigate the chemopreventive properties of vanillin on colorectal cancer and iv. to study the effects of vanillin on expression of selected genes *in vivo*. Methods used to study the cytotoxic effects include cytotoxicity assay, double staining cell morphological analysis, cell cycle analysis, apoptosis test and cell proliferation assay. The negative effect of oral and intra-peritoneal administration of vanillin to Sprague-Dawley rats in unoxidized form at high concentrations (150 mg/kg

and 300 mg/kg) was also investigated. Following the administration, animal behavior was observed and recorded. After 14 weeks of vanillin administration, the effects of vanillin on blood cells, kidney, liver and brain were studied. For the chemopreventive properties of vanillin, rats were injected with azoxymethane (AOM) and subsequently treated with vanillin. Aberrant crypt foci (ACF) counts and multiplicity were recorded. RNA was extracted from colon for DNA repair, apoptosis, cell cycle, anti-inflammation, proto-oncogene, colorectal cancer biomarker and tumor suppressor gene expressions study. Findings from the *in vitro* study showed that vanillin was cytotoxic towards HT-29 and 3T3 cells with the IC_{50} value of 400 µg/ml and 1000 µg/ml respectively. Vanillin also induced apoptosis and cell cycle arrest. Different concentrations of vanillin showed arrest of cell cycle at different checkpoints. G_0/G_1 arrest was noted at lower concentration of vanillin (200 µg/ml) while G_2/M arrest occurred at higher concentration of vanillin (1000 µg/ml). From the *in vivo* study, results showed that treatment of 300 mg/kg of vanillin by intra-peritoneal injection caused the rats to be unconscious without exerting any negative effect on blood cells, kidney and liver. Further analysis with GenomeLab GeXP genetic system on brain tissues showed that the expression of most xenobiotic metabolism, cell progression, tumour suppressor, DNA damage and inflammation genes was maintained at normal level. However, the expressions of a few xenobiotic metabolism, cell cycle arrest and apoptosis genes were up-regulated by 5 % ethanol injection. This shows that 5% ethanol could pose negative effects onto the brain cells. Nevertheless, when 5 % ethanol was injected together with vanillin, the expression of genes was comparable to normal level. Hence, it is postulated that vanillin might have neuro-protective property. For the chemopreventive effect, AOM-injected rats treated with vanillin have significantly higher ($p<0.05$) ACF counts and multiplicity compared to the

control group. The colon gene expression analysis showed that vanillin could enhance recombinational repair and mismatch repair, arrest cell at cell cycle checkpoints, increase the expression of tumour suppressor gene, colorectal cancer biomarker and proto-oncogene. However, vanillin did not induce apoptosis and inflammation in ACF-bearing colon. In conclusion, vanillin could induce cytotoxic effects on colorectal cancer cells. It was not showing negative effects when administered at high concentrations through oral and intra-peritoneal injections. However, the ACF count and multiplicity indicate that vanillin was a co-mutagen instead of chemopreventive agent in AOM-injected rats. Nevertheless, it should be noted that only intraperitoneally injected vanillin would become co-mutagen, orally administered vanillin is not a co-mutagen.

Abstrak tesis dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi keperluan Ijazah Doktor Falsafah

FUNGSI DAN KESAN VANILLIN ATAS KANSER KOLON SECARA *IN VITRO* DAN *IN VIVO*

Oleh

HO KET LI

August 2011

Pengerusi: Profesor Maznah Ismail, PhD

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Vanilin bertanggungjawab untuk memberi rasa dan bau vanila, satu agen perisa yang diguna dengan meluas. Kajian lepas menunjukkan bahawa vanilin merupakan perencat sel darah merah sabit, agen anti-mikrob dan anti-mutagen yang baik. Namun demikian, vanilin perlu diberikan pada kepekatan yang tinggi dan tidak boleh dioksidakan di bahagian perut dan usus untuk kesan perubatannya yang berkesan. Oleh yang demikian, tujuan kajian ini adalah untuk 1. menyelidik kesan sitotoksik vanilin ke atas sel kanser kolon HT-29; ii. menyelidik kesan negatif vanilin apabila diberikan pada kepekatan yang tinggi secara *in vivo*; iii. menyelidik kesan pencegahan vanilin terhadap kanser kolon dan iv. menyelidik kesan vanilin atas penzahiran gen kolon. Kaedah yang digunakan untuk mengkaji kesan sitotoksik vanilin termasuk: Ujian sitotosik, analisis morfologi sel dengan dua-pewarnaan,

analisis kitaran sel, ujian apoptosis) dan ujian pertumbuhan sel. Kesan negatif vanilin apabila diberi kepada tikus Sprague-Dawley dengan cara oral dan suntikan intra-peritoneal pada kepekatan yang tinggi (150 mg/kg dan 300 mg/kg) juga dikaji. Selepas vanilin diberikan, tingkah-laku tikus diperhatikan dan dicatatkan. Selepas diberi vanilin untuk 14 minggu, kesan negative vanillin atas sel darah, ginjal, hati dan otak juga dikaji. Bagi kesan pencegahan kanser kolon, tikus disuntik dengan azoksimetana (AOM) dan kemudiannya dirawat dengan vanilin. Bilangan "aberrant crypt foci" (ACF) dan keserbaragamannya direkodkan. RNA diekstrak dari kolon untuk kajian penzahiran gen termasuk: gen pembaikian DNA, gen apoptosis, gen kitaran sel, gen perencat keradangan, proto-onkogen, biomarker kanser kolon dan gen penindas kanser. Keputusan kajian *in vitro* menunjukkan bahawa vanilin adalah sitotosik terhadap sel HT-29 dan 3T3 dengan IC_{50} 400 µg/ml dan 1000 µg/ml masing-masing. Vanilin juga boleh menyebabkan apoptosis dan perhentian kitaran sel. Vanilin dalam kepekatan yang berbeza akan menghentikan kitaran sel pada fasa yang berbeza. Perhentian sel pada fasa G_0/G_1 boleh dicapai dengan menggunakan vanilla pada kepekatan yang rendah (200 µg/ml) dan perhentian pada fasa G_2/M boleh dicapai dengan menggunakan vanilin pada kepekatan yang tinggi (1000 µg/ml). Daripada kajian kesan negative vanillin, rawatan 300 mg/kg secara suntikan menyebabkan tikus mengalami keadaan tidak sedar tanpa menunjukkan kesan negatif terhadap sel darah, ginjal dan hati. Analisis selanjutnya melalui system genetic GenomeLab GeXP ke atas tisu otak tikus menunjukkan bahawa penzahiran kebanyakan gen metabolime xenobiotik, perkembangan sel, penindas kanser, kerosakan DNA dan keradangan adalah pada paras normal. Walau bagaimanapun, penzahiran beberapa gen metabolime xenobiotik, penahanan kitaran sel dan apoptosis telah ditingkatkan dengan suntikan 5 % etanol. Ini menunjukkan 5%

ethanol boleh menyebabkan kesan negative atas sel otak. Namun demikian, apabila 5 % etanol disuntik bersama-sama dengan vanilin, penzahiran gen kembali ke paras normal. Vanilin dijangka mempunyai kesan perlindungan sel neuro. Untuk kesan pencegahan, tikus yang disuntik dengan AOM dan dirawat dengan vanilin mempunyai kiraan ACF dan keserbaragaman ACF yang lebih tinggi ($p<0.05$) berbanding dengan kumpulan kawalan. Analisis penzahiran gen menunjukkan bahawa vanilin boleh meningkatkan mekanime pembaikian DNA rekombinasi, pembaikian DNA mismatch repair (MMR), menahan kitaran sel, meningkatkan penzahiran gen penindas kanser, biomarker kanser kolon dan proto-onkogen. Walau bagaimanapun, vanilla tidak mengaruh apoptosis atau keradangan dalam kolon yang mengandungi ACF. Kesimpulannya, vanilin boleh mengarah kesan sitotoksik ke atas sel kanser kolon dan tidak memberi kesan negatif apabila diberi pada kepekatan yang tinggi secara oral dan suntikan intra-peritoneal. Walau bagaimanapun, vanilin merupakan co-mutagen dan bukan agen perlindungan kimia dalam tikus yang disuntik dengan AOM. Namun demikian, hanya vanillin yang diberi dengan cara suntikan akan menjadi co-mutagen. Vanillin yang diberikan secara oral bukan co-mutagen.

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I certify that a Thesis Examination Committee has met on 18th August, 2011 to conduct the final examination of Ho Ket Li on his thesis entitled "Properties of Vanillin on Colorectal Cancer *in vitro* and *in vivo*" 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 Doctor of Philosophy.
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This thesis submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the supervisory Committee were as followed:

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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 other institutions.

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HO KET LI

Date: 18th August 2011

TABLE OF CONTENT