KESAN ANTI PROLIFERASI γ-TOKOTRIENOL PADA SEL HEPG2 MELALUI PENGEKSPRESAN GEN

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TESIS YANG DIKEMUKAKAN UNTUK MEMPEROLEH
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PENGAKUAN

Saya akui karya ini adalah hasil kerja saya sendiri kecuali nukilan dan ringkasan yang tiap-tiap satunya telah saya jelaskan sumbernya.

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ABSTRAK

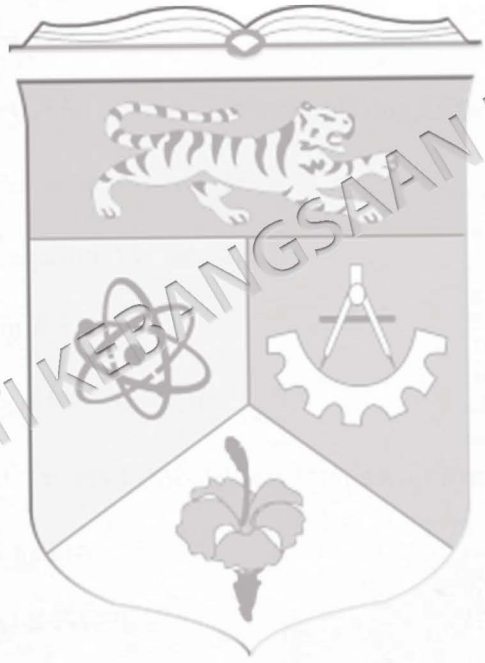
Tokotrienol terutamanya γ-tokotrienol (GTT) daripada minyak kelapa sawit mempunyai kesan yang lebih signifikan sebagai antikanser berbanding tokoferol. GTT kini dianggap sebagai ejen antikanser yang berpotensi disebabkan kesan yang efektif terhadap pelbagai kanser. Namun mekanisme tindakan yang khusus masih tidak jelas. Kajian ini dijalankan untuk menentukan kesan GTT pada sel selanjar HepG2 melalui pengekspresan gen global. Sel dirawat dengan media kultur yang mengandungi GTT (0-160 μM) selama 48 jam dan 72 jam, TNF-α (0-1000 $ngmL^{-1}$) dan NF-κB (0-100 $ngmL^{-1}$) selama 48 jam. Viabiliti sel ditentukan dengan menggunakan asai viabiliti sel (MTS). RNA diektrak daripada sel dirawat dengan GTT dan dianalisis menggunakan *Nanodrop* dan *Bioanalyzer*. Proses melabel, menghibrid, membasuh dan mengimbas bagi analisis mikroatur menggunakan *Gene chip Human Gene 1.0 Array Kit* dan *Gene chip Hybridization Wash and Stain Kit* dilakukan. Perisian *GeneSpring GX 10* dan *Pathway Studio 6.0* digunakan bagi menganalisis data mikroatur. Keputusan eksperimen mikroatur disahkan dengan lebih lanjut melalui analisis kuantitatif PCR masa nyata (RT-qPCR) serta analisis pemedapan Western. Asai viabiliti sel menunjukkan viabiliti sel HepG2 dan sel WRL68 menurun secara signifikan ($p<0.01$) apabila kepekatan GTT meningkat dan kesan pada nilai IC_{50} tidak bergantung pada tempoh masa eraman. Nilai IC_{50} untuk GTT bagi sel HepG2 adalah 110 ± 0.91 μM dan 130 ± 3.98 μM bagi sel WRL68. GTT tidak menunjukkan kesan penurunan yang signifikan ($p<0.01$) melalui TNF-α dan NF-κB dalam kedua-dua sel. Analisis statistik bagi data mikroatur menunjukkan perubahan 393 gen ($p<0.01$, $FC\geq1.3$) dengan rawatan GTT dalam sel HepG2 manakala hanya 8 gen berubah ($p<0.01$, $FC\geq1.3$) dalam sel WRL68. Analisis ontologi gen menunjukkan GTT mengaruh perbezaan ekspresi pada kumpulan gen yang berperanan dalam pengawalaturan kitar sel (CCNB1, CCNA2, CDC2, BUB1B, MAD2L1, CDC25A, CDC45L), replikasi DNA (MCM10, RRM2, DNA2), pembaikan DNA (RAD51AP1, CLSPN, RAD54L), proliferasi sel (PTGS2, DKC1, MKI67), apoptosis (BOK, BAX, BCL-2, BIRC5, HMGB1, BCL2L14, SLC25A6), modifikasi kromatin (RNF168, SETD8, E2H2) dan pengawalaturan transkripsi (TCEA3, OLIG1, SIM2). Selain itu, kebanyakan gen yang berkaitan dengan progresi kanser menurun dengan rawatan GTT. GTT tidak menunjukkan perubahan yang signifikan dalam sel WRL68. Hasil RT-qPCR dan pemedapan Western menunjukkan keputusan yang selari dengan analisis data mikroatur. Kesimpulannya, GTT bertindak sebagai agen antikanser dengan menindas proses kitaran sel, replikasi DNA, proliferasi sel, modifikasi kromatin, pembaikan DNA dan pengawalaturan transkripsi serta meningkatkan apoptosis dalam sel HepG2.

ANTIPROLIFERATIVE EFFECT OF γ-TOCOTRIENOL IN HEPG2 CELLS VIA DIFFERENTIAL GENE EXPRESSION

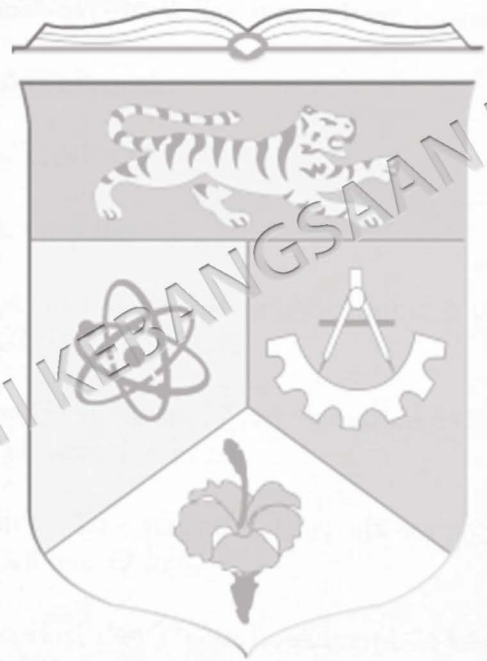
ABSTRACT

Tocotrienols especially γ-tocotrienol (GTT) are primarily derived from palm oil possess powerful anticancer compare to tocopherols. GTT is now considered to be a promising anticancer agent due to its potent effects against a wide range of cancers. But the specific mechanisms of action were still not clear. The objective of this study is to determine the effect of GTT in HepG2 cells via differential global gene expression. The cells were treated with various doses of GTT (0-160 μM) for 48-h and 72-h, TNF-α (0-1000 $ngmL^{-1}$) and NF-κB (0-100 $ngmL^{-1}$) for 48-h. Cell viability was assessed by the cell proliferation assay (MTS). Total RNA was extracted from cells treated with GTT and quantified using the Nanodrop and Bioanalyzer. Labeling, hybridization, washing and scanning for microarray analysis were performed using commercially available GeneChip Human Gene 1.0 Array Kit and Gene chip Hybridization Wash and Stain Kit. The GeneSpring GX 10 and Pathway Studio 6.0 software were used for further microarray data analysis. The microarray results were validated by quantitative real time PCR analysis (RT-qPCR) and Western blot of a few representative genes. Cell viability assay showed that the viability of HepG2 and WRL68 cells were significantly ($p<0.01$) decreased dose-dependently with increasing GTT concentration and the effects on the IC_{50} are not time-dependent over the incubation period tested. The IC_{50} for GTT is 110 ± 0.91 μM for HepG2 and 130 ± 3.98 μM for WRL68. GTT did not show any significant inhibitory effect ($p<0.01$) through TNF-α and NF-κB in both cells. Statistical analysis for microarray data showed that 393 genes were differentially expressed ($p<0.01$, $FC\geq1.3$) with GTT treatment in HepG2 cells whereas only 8 differentially expressed ($p<0.01$, $FC\geq1.3$) genes in WRL68 cells. Gene ontology investigation displayed that GTT-affected clusters of genes involved in cell cycle regulation (CCNB1, CCNA2, CDC2, BUB1B, MAD2L1, CDC25A, CDC45L), DNA replication (MCM10, RRM2, DNA2), DNA repair (RAD51AP1, CLSPN, RAD54L), cell proliferation (PTGS2, DKC1, MKI67), apoptosis (BOK, BAX, BCL-2, BIRC5, HMGB1, BCL2L14, SLC25A6), chromatin modification (RNF168, SETD8, E2H2) and regulation of transcription (TCEA3, OLIG1, SIM2). Moreover, most of the genes associated with cancer progression were down regulated by GTT. GTT did not show any significant changes in the WRL68 cells. RT-qPCR and Western-blot results showed trends that agreed with microarray data analysis. In conclusion, GTT exhibit anticancer effects by suppression of cell cycle progression, DNA replication, cell proliferation, chromatin modification, DNA repair and regulation of transcription and also increased apoptosis in HepG2 cells.

KANDUNGAN



SENARAI JADUAL



SENARAI ILUSTRASI

SENARAI SIMBOL

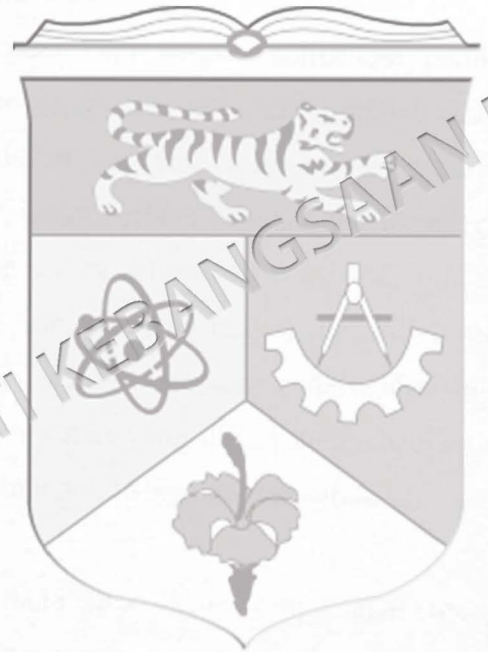
%	Peratus
β	beta
≤	Sama dengan atau kurang daripada
≥	Sama dengan atau lebih daripada
°C	Darjah celsius
cm^2	Sentimeterpadu
g	gram
kDa	Kilodalton
L	Liter
M	Molar
ml	Mililiter
nm	Nanometer
V	Voltan
X	Kali
α	alfa
γ	gamma
δ	delta
μ	Mikro
μg	Mikrogram
μl	Mikroliter
μm	Mikrometer
μM	Mikromolar

SENARAI SINGKATAN

ANOVA	*Analysis of variance*
Apaf-1	*Apoptosis-protease-activating factor 1*
ATF	Alfa Tokoferol
ATT	Alfa Tokotrienol
BAX	*BCL2-associated X protein mRNA, transcript variant alpha*
BCL-2	*B-cell CLL/lymphoma 2*
CDKs	*Cyclin Dependent Kinase*
DMSO	Dimetilsulfoksida
EDTA	Asidetilenediaminetetrasetik
ELISA	*Enzyme linked immunosorbent assay*
EMEM	*Eagle's Minimum Essential Medium*
GTT	Gamma Tokotrienol
HCL	Asidhidroklorik,
HEPES	Asid N-2 hidroksietilpiperazin-N'-2-etansulfonik
HMG-CoA	*5-hydroxy-3-methylglutaryl-coenzyme A*
IC_{50}	50% kepekatanperencatan
IL-6	Interleukin 6
MTS	*3-(4,5-dimetylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)2-(2-sulfophenyl)-2H-tetrazolium*
NEAA	Asid amino takesseintial
NF-κB	*Nuklear factor κappa B*
PBS	LarutanPenimbalfosfat
PMSF	Fenilmetilsulfonil fluoride
PVDF	Polivinildifluorida
RNA	Asidribonukleik
ROS	Spesisreaktifoksigen
Rpm	Kitaran per minit
TNF-α	*Tumor necrosis factor alfa*
TRF	Fraksi kaya tokotrienol
UV	Ultra ungu

SENARAI TATANAMA

HCl	Asid hidroklorik
KCl	Kalium klorida
KH_2PO_4	Kalium dihidrogen fosfat
Na_2HPO_4	Dinatrium hidrogen fosfat
NaCl	Natrium klorida,
NaOH	Natrium hidroksida



BAB I

PENDAHULUAN

1.1 PENGENALAN

Kanser merupakan kumpulan penyakit yang dicirikan oleh pembahagian sel yang tidak terkawal yang membawa kepada pertumbuhan tisu yang tidak normal. Buat masa kini, rawatan utama bagi kanser melibatkan pembedahan, kemoterapi dan radioterapi. Tujuan kemoterapi dan radioterapi adalah untuk membunuh sel kanser dengan menggunakan dadah sitotoksik, hormon atau radiasi dengan dos yang tinggi. Tetapi kesan sampingan lazim berlaku kerana rawatan sebegini tidak dapat disasarkan hanya kepada sel kanser kerana sel normal yang sedang membahagi pada kadar yang tinggi juga boleh termusnah. Rawatan radiasi juga boleh menyebabkan penghapusan sel normal berhampiran sel kanser yang sedang dirawat. Sesetengah sel kanser menjadi rentan kepada rawatan yang diberi menyebabkan sel-sel ini tidak terhapus tetapi terus membahagi mengakibatkan kanser berulang.

Sehingga kini tiada ubat khas yang dapat menyembuhkan kanser dan mengurangkan kesan sampingan yang teruk. Usaha masih berterusan untuk mencari penyembuh kanser yang paling ampuh yang dapat memperlahankan sel kanser dari terus merebak di dalam badan manusia. Salah satu pendekatan untuk menghalang insiden kejadian kanser adalah dengan agen antikanser (Pan et al. 2011; Tsao et al. 2004). Pada masa kini, perhatian telah diberikan kepada agen antikanser semulajadi yang berupaya menghalang, merencat, membalikkan proses pembentukan karsinogenesis. Oleh sebab itu, para saintis terus berusaha untuk mencari sebatian semulajadi yang mampu untuk membunuh sel kanser, menjadikan sel kanser sensitif

terhadap rawatan dadah atau radiasi atau pun sebatian yang boleh menghalang dan mengurangkan pembentukan kanser.

Kanser merupakan satu proses dinamik yang melibatkan banyak peringkat dan faktor kompleks. Disebabkan oleh itu, maka setiap peringkat dan faktor tersebut akan dijadikan sasaran perspektif dalam pembalikan atau penahanan proses tersebut. Oleh itu, kajian dan pembangunan terhadap agen kemopencegah yang spesifik terhadap peringkat sasaran sebagai kaedah rasional terhadap langkah untuk menghalang penularan kanser. Pelbagai kajian epidemiologi dan intervensi (Sen et al. 2007) mencadangkan vitamin E sebagai agen antikanser yang memberikan perlindungan terhadap pembentukan dan merencat kanser menerusi sifat antioksidannya. Namun, kajian terkini mendapati bahawa tindakan vitamin E sebagai antikanser tidak terbatas sebagai bahan antioksidan semata-mata.

Secara umumnya, vitamin E terbahagi kepada dua kumpulan iaitu tokoferol dan tokotrienol di mana setiap kumpulan mempunyai empat komponen iaitu α, β, γ dan δ. Tokotrienol terutamanya γ-tokotrienol (GTT) dilaporkan mempunyai kesan yang lebih signifikan sebagai antikanser berbanding tokoferol. GTT kini dianggap sebagai ejen antikanser yang berpotensi disebabkan kesan yang efektif terhadap pelbagai kanser seperti kanser serviks (Wu & Ng 2010), kanser prostat (Yap et al. 2008) dan kanser kolon (Xu et al. 2009).

Kesan antikanser GTT telah dilaporkan pada sel kanser HepG2 tetapi mekanisme tindakan GTT masih belum diketahui dengan jelas. Penggunaan sel selanjar HepG2 dan WRL68 ini boleh membantu usaha dalam memahami mekanisme tindakan GTT.

1.2 OBJEKTIF

1.2.1 Objektif Umum

Menentukan kesan anti kanser γ-tokotrienol pada sel HepG2 melalui pengekspresan gen global.

1.2.2 Objektif Khusus

1. Menentukan kesan pelbagai dos GTT, TNF-α dan NF-κB terhadap viabiliti sel kanser hepatoma, HepG2 dan sel normal, WRL68.
2. Menentukan perubahan ekspresi gen dalam sel kanser hepatoma, HepG2 dan sel normal, WRL68 yang dirawat dengan GTT.
3. Mengenalpasti mekanisme tindakan GTT yang terlibat dalam menghalang pertumbuhan sel HepG2.

1.3 HIPOTESIS

1. GTT menurunkan viabiliti sel HepG2.
2. GTT akan merangsang apoptosis sel HepG2 dengan mengganggu tapak jalan NF-κB untuk kelansungan hidup.
3. GTT menurunkan ekspresi gen berkaitan kemandirian sel dan meningkatkan ekspresi gen berkaitan apoptosis dalam sel HepG2 tanpa memberi kesan yang ketara pada sel WRL68.

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