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Ekspresi mRNA dan Stabilisasi Protein Hypoxia Inducible Factor-1α (HIF-1α) oleh Mangiferin: Studi in vitro pada Lini Sel HepG2 = MRNA Expression and Protein Stabilization of Hypoxia Inducible Factor-1α (HIF-1α) by Mangiferin: in vitro study in cell line HepG2

Himmi Marsiati, author

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Abstrak

Pendahuluan: Penelitian dilakukan untuk mengetahui peran senyawa flavonoid mangiferin dalam meningkatkan ekspresi mRNA HIF-1α dan sebagai pencekal besi dalam menstabilkan HIF-1α pada lini sel HepG2 dan menganalisis interaksi mangiferin dengan prolil hidroksilase (PHD2) secara simulasi docking.

Metode: Sel HepG2 dikultur hingga >80% konfluen dan selanjutnya diberikan mangiferin konsentrasi 25-200μM. Kuersetin digunakan sebagai pembanding flavonoid mangiferin yang bekerja di dalam inti sel, sedangkan DFO dan CuCl2 digunakan sebagai pembanding daya ikat terhadap besi. Ekspresi mRNA HIF-1α ditentukan dengan real time RT- PCR/q-PCR, dan stabilisasi protein HIF-1α ditentukan mengunakan teknik ELISA. Simulasi docking dilakukan terhadap protein PHD2 dengan mangiferin, CuCl2, deferoksamin (DFO), dan campuran mangiferin+ kuersetin.

Hasil: Uji viabilitas sel menggunakan metode MTS dengan pemberian mangiferin, kuersetin, campuran mangiferin-kuersetin, DFO dan CuCl2 (25-200μM) memperlihatkan hasil diatas 85%. Ekspresi mRNA HIF-1α dengan mangiferin, kuersetin, mangiferin+kuersetin, dan DFO menunjukkan hasil sedikit lebih tinggi dibanding kontrol. Konsentrasi protein HIF-1α pada pemberian mangiferin, kuersetin, mangiferin-kuersetin, DFO dan CuCl2 lebih tinggi dibanding kontrol. Simulasi docking mangiferin terhadap PHD2 memperlihatkan ΔG= -16,22, dan DFO menunjukkan ΔG= -17,15. Terdapat interaksi antara mangiferin, dan DFO dengan besi dan asam amino pada situs katalitik domain PHD2, sedangkan CuCl2 tidak berinteraksi dengan residu asam amino pada domain PHD2, tetapi langsung menggantikan Fe. Efek penghambatan terhadap PHD2 oleh mangiferin dan kuersetin disebabkan oleh delokalisasi elektron melalui kompleks transfer elektron.

Kesimpulan: Mangiferin dapat meningkatkan ekspresi mRNA HIF-1α dan meningkatkan protein HIF-1α, menurun protein PHD2 dan menurunkan protein HO-HIF-1α pada lini sel HepG2 secara in vitro. Analisis docking terdapat interaksi antara mangiferin, dan DFO dengan besi dan asam amino PHD2. Mangiferin memiliki stabilitas pengkikatan dengan besi yang berdekatan dengan DFO.
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Introduction: This research was conducted to determine the role of flavanoid mangiferin to increase expression HIF-1α mRNA, and as an iron chelator to stabilize protein HIF-1α in cell line HepG2 and analyzes the interaction of mangiferin with prolil hidroksilase (PHD2) by docking simulation.

Methods: HepG2 cells were cultured and treated by mangiferin with concentration between 25-200μM. Quercetin is used as a comparison mangiferin flavonoid that works in the nucleus and DFO, CuCl2 is used as a comparison to iron-binding. HIF- 1α mRNA expression was determined by real time RT-PCR/q-PCR, and the stability HIF-1α protein were measured by the increase in HIF-1α protein, decreased PHD2 protein and decreased HO-HIF-1α using ELISA. Docking simulation was conducted between PHD2 protein and mangiferin, CuCl2, desferoxamine (DFO), and quercetin.

Results: Cell viability with MTS assay showed that cell exposure with 25μM-200μM concentrations of mangiferin, quercetin, mangiferin+quercetin mixture, DFO, and CuCl2 is above 85%. HIF-1α mRNA expression was slightly higher than in controls with mangiferin, quercetin, mangiferin quercetin mixture and DFO. HIF-1α protein concentration and ratios vs untreated controls were above 1 with mangiferin, quercetin, mangiferin quercetin mixture, DFO, and CuCl2. Docking simulation mangiferin with PHD2 showed ΔG= -16,22. Docking simulation with DFO showed ΔG= -17,15, and interact mangiferin, and DFO with iron in the catalytic site of PHD2 and with amino acid residues, whereas CuCl2 does not react with amino acid residues in the PHD2 domain, but directly replaces Fe. The inhibitory effect to PHD2 by mangiferin and quercetin is considered by electron delocalisation through an electron transfer complex.

Conclusion: Mangiferin can increase HIF-1α mRNA expression and HIF-1α protein levels in HepG2 cell line by in vitro. Binding interaction with iron and PHD2 amino acids occurs by mangiferin and DFO. Mangiferin has stability iron binding a similar with DFO.