

Studi Molekuler Mekanisme Isolat 1,4-bis-(3,4,5-trimetoksi-fenil)-tetrahydro-furo(3,4-c) furan dari Biji Mahoni (*Swietenia macrophylla* King.) sebagai Kandidat Anti-Diabetes Terhadap Model Resistensi Insulin *In Vitro* Pada Cell line C2C12 dan HepG2

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ABSTRAK

Latar Belakang: Resistensi insulin menjadi target terapi yang penting karena mendasari patogenesis diabetes melitus (DM) tipe 2 yang kasusnya mencapai lebih dari 90% diabetes. Tingginya asam lemak bebas merupakan faktor risiko resistensi insulin dan DM tipe 2 karena memicu akumulasi metabolit lipid di otot skelet dan hepar. Hal ini mengakibatkan gangguan sinyal insulin, salah satunya jalur PI3K/Akt yang krusial dalam memediasi transport glukosa dan sintesis glikogen. Studi *in silico* dan *in vivo* membuktikan isolat 1,4-bis-(3,4,5-trimetoksi-fenil)-tetrahydro-furo(3,4-c) furan, golongan saponin dari biji *S. macrophylla* King memiliki aktivitas penurunan glukosa sehingga mendukung potensinya untuk dikembangkan lebih lanjut. Namun, penelusuran jalur molekuler efek isolat tersebut pada jalur sinyal insulin belum pernah dilakukan sebelumnya. Penelitian ini bertujuan memahami mekanisme molekuler efek penurunan kadar glukosa oleh isolat 1,4-bis-(3,4,5-trimetoksi-fenil)-tetrahydro-furo(3,4-c) furan pada jalur sinyal insulin PI3K/Akt menggunakan *cell line* C2C12 *myotube* dan HepG2 model resisten insulin dengan induksi asam palmitat.

Metode: Penelitian pada C2C12 *myotube* dan HepG2 model resisten insulin dengan induksi asam palmitat (PA 0,25 mM), masing-masing 12 kelompok yaitu KS (kontrol sel), PA (kontrol negatif), PA IMK, PA IMS dan PA IMB (PA 0,25 mM dengan isolat 1/8, 1/4, dan 1/2 IC₅₀) serta PA Met (PA 0,25 mM + metformin 42 µg/mL) dengan atau tanpa insulin 100 nM. Penilaian konsumsi glukosa dengan GOD/PAP, ekspresi mRNA GSK-3β, PEPCK dan GLUT4 dengan qRT-PCR, penilaian p-IRS1^{Tyr612} dan p-Akt^{Ser473} dengan imunositokimia. Data dianalisis dengan *Oneway* ANOVA dan *Post Hoc* Tukey atau *Kruskal-Wallis* dan *Mann-Whitney* untuk parameter yang tidak memenuhi kriteria uji parametrik dengan tingkat signifikansi $\alpha = 0,05$.

Hasil: Isolat 1,4-bis-(3,4,5-trimetoksi-fenil)-tetrahydro-furo(3,4-c) furan secara signifikan meningkatkan konsumsi glukosa (menekan kadar glukosa di media), menekan ekspresi mRNA GSK-3β dan meningkatkan mRNA GLUT4 di C2C12 *myotube*, menekan ekspresi mRNA GSK-3β dan PEPCK di HepG2 serta meningkatkan fosforilasi IRS1^{Tyr612} dan Akt^{Ser473} di C2C12 *myotube* dan HepG2. Kemampuan isolat dalam memodulasi parameter di jalur PI3K/Akt tersebut sebanding dengan metformin sebagai kontrol positif. Selain itu, isolat tersebut tidak toksik pada C2C12 *myotube* dan HepG2 dengan IC₅₀ sebesar $223,24 \pm 12,70$ dan $131,90 \pm 12,90$ µg/mL.

Kesimpulan: Isolat 1,4-bis-(3,4,5-trimetoksi-fenil)-tetrahydro-furo(3,4-c) furan, golongan saponin dari biji *S. macrophylla* King mampu menghambat progresivitas resistensi insulin dan meningkatkan konsumsi glukosa pada otot dan hepar. Kemampuan tersebut diperantarai aktivitasnya dalam memodulasi jalur sinyal insulin PI3K/Akt seperti menekan ekspresi mRNA GSK-3β dan PEPCK, meningkatkan mRNA GLUT4, fosforilasi IRS1^{Tyr612} dan Akt^{Ser473} di otot skelet dan hepar.

Kata kunci: resistensi insulin, PI3K/Akt, isolat *S. macrophylla*, C2C12 *myotube*, HepG2

The Molecular Mechanism Study of of 1,4-bis-(3,4,5-Trimethoxy-Phenyl)-Tetrahydro-Furo(3,4-c) Furan Isolate from Mahoni (*Swietenia macrophylla* King.) Seeds as Anti-Diabetic Candidate Towards In Vitro Insulin Resistance Model on C2C12 and HepG2 Cell line

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ABSTRACT

Background: Insulin resistance is an important therapeutic target because it underlies the pathogenesis of type 2 diabetes mellitus (T2DM), which reached for more than 90% of diabetes cases. The increasing of free fatty acids are a risk factor for insulin resistance and T2DM because it triggers the lipid metabolites accumulation in skeletal muscle and liver. This impaired insulin signaling, including the PI3K/Akt pathway which is crucial in mediating glucose transport and glycogen synthesis. In silico and in vivo studies proved that 1,4-bis-(3,4,5-trimethoxy-phenyl)-tetrahydro-furo(3,4-c) furan isolate, saponin group from *S. macrophylla* King seeds had ability in decreasing glucose level thus potential for further development. However, study focusing on the molecular mechanism of its effect on insulin signaling pathways had not been clearly elucidated yet. This study aims to explore the molecular mechanism of glucose lowering effect of 1,4-bis-(3,4,5-trimethoxy-phenyl)-tetrahydro-furo(3,4-c) furan on the PI3K/Akt insulin signaling pathway using cell lines C2C12 myotube and HepG2 insulin resistance model induced by palmitic acid.

Methods: This study was conducted on C2C12 myotube and HepG2 insulin resistance models induced by palmitic acid (PA 0.25 mM). There were 12 groups on each cell line, including KS (cell control), PA (negative control), PA IMK, PA IMS and PA IMB (PA 0, 25 mM + isolates 1/8, 1/4, or 1/2 IC₅₀) and PA Met (0.25 mM PA + metformin 42 g/mL), with or without 100 nM insulin stimulation. Assessment of glucose consumption by GOD/PAP, GSK-3 β , PEPCK and GLUT4 mRNA expression by qRT-PCR, p-IRS1^{Tyr612} and p-Akt^{Ser473} by immunocytochemistry. Data were analyzed by *Oneway ANOVA* and *Post Hoc Tukey* or *Kruskal-Wallis* and *Mann-Whitney* for parameters which did not meet the parametric test criteria with a significance level of $\alpha = 0.05$.

Results: The isolate significantly increased glucose consumption, down regulated GSK-3 β while up regulated GLUT4 mRNA expression in C2C12 myotube, downregulated GSK-3 β and PEPCK mRNA expression in HepG2 and increased phosphorylation of IRS1^{Tyr612} and Akt^{Ser473} in both C2C12 myotube and HepG2. The isolate ability to modulate the parameters in the PI3K/Akt pathway was comparable to metformin as a positive control. In addition, this isolate was not toxic to C2C12 myotube and HepG2 with IC₅₀ of 223.24 ± 12.70 and 131.90 ± 12.90 g/mL, respectively.

Conclusion: The 1,4-bis-(3,4,5-trimethoxy-phenyl)-tetrahydro-furo(3,4-c) furan, a saponin isolate from the seeds of *S. macrophylla* King was able to inhibit insulin resistance and increase glucose consumption in muscles and liver. These abilities were mediated by its activity in modulating the PI3K/Akt insulin signaling pathway, such as suppressing GSK-3 β and PEPCK mRNA expression while increasing GLUT4 mRNA and phosphorylation of IRS1^{Tyr612} and Akt^{Ser473} in skeletal muscle and liver.

Keywords: insulin resistance, PI3K/Akt, *S. macrophylla* isolate, C2C12 myotube, HepG2