

EFFECTS OF *Andrographis paniculata* Burm.F Ness AND *Centella asiatica* L. Urban PURIFIED EXTRACT COMBINATION ON PPAR γ AND GLUT-4 EXPRESSION OF 3T3-L1 ADIPOCYTE CELLS

ABSTRACT

The use of the sambiloto herb (*Andrographis paniculata* (Burm.F) .Ness) and *Centella asiatica* L. Urban herb are reported can improve the insulin resistance and type-2 diabetes mellitus. However, the molecular mechanisms of those combined plants in lowering blood glucose are unknown. The object of this study was to examine the toxicities and effects of sambiloto herb purified extracts (SHPE) and pegagan herb purified extracts (PHPE) combined on glucose uptake, PPAR γ , and GLUT-4 expression at mRNA level of insulin resistance 3T3-L1 adipocyte cells.

Levels of andrographolid and asiaticoside in SHPE and PHPE were measured with densitometric Thin Layer Chromatography-Densitometry. The toxicity of both extracts was tested on 3T3-L1 cells. LC₅₀ was determined by probit analysis. The adipocyte cells were differentiated by using insulin, dexamethasone and IBMX from 3T3-L1 cells. Induction of insulin resistance in adipocyte cells by using glucose 55 mM and insulin 100 nM for 10 days. Pioglitazone, ETHS, ETHP and the extracts combination were tested on glucose uptake activity and PPAR γ and GLUT-4 expression. The obtained data were analyzed statistically by using unpaired t-test or ANOVA oneway or Mann-Whitney test.

The andrographolid levels in SHPE were 17.41 \pm 0.42% and asiaticosid levels in PHPE were 11.34 \pm 0.69%. The LC₅₀ of SHPE and PHPE on 3T3-L1 cells were 72.71 μ g/ml and 46.29 μ g/ml. The purified extracts combination (30:10) μ g/ml did not show cell toxicity. By the 10 days, the adipocyte cells had larger in form, size, lipid droplet, and increased lipid accumulation was 4.8-fold greater than preadipocytes with significantly different ($p < 0.05$), and PPAR γ expressions were significantly different to preadipocyte cells. Results of insulin resistance test with GLUT-4 expression analysis had significant differences ($p < 0.05$) compared to normal adipocyte cells. The glucose uptake of the extract combination (30:10) μ g/ml was significantly different to negative control (DMSO) at 180 minutes. The combination of the purified extracts (30:10) μ g/ml showed an increasing in regulation of PPAR γ and GLUT-4 expression at mRNA levels significantly relative to negative control, and has not significant difference ($p > 0, 05$) compared to pioglitazone 0.02 μ M and each purified single extract.

Keyword : sambiloto, pegagan, purified extract, 3T3-L1 cells, PPAR γ , GLUT-4

INTISARI

Penggunaan herba sambiloto (*Andrographis paniculata* (Burm.F).Ness) dan herba pegagan (*Centella asiatica* L. Urban) dilaporkan dapat memperbaiki kondisi resistensi insulin pada diabetes melitus. Namun, mekanisme aksi pada tingkat molekuler dalam menurunkan kadar glukosa darah belum diketahui apabila kedua tanaman tersebut dikombinasikan. Penelitian ini bertujuan untuk menguji potensi dan efek kombinasi ekstrak terpurifikasi herba sambiloto (ETHS) dan pegagan (ETHP) pada sel 3T3-L1 adiposit resistensi insulin terhadap *uptake* glukosa dan ekspresi PPAR γ dan GLUT-4 pada tingkat mRNA dibandingkan dengan obat pioglitazon dari golongan tiazolidinedion maupun masing-masing ekstrak.

ETHS dan EHP ditentukan kadarnya dengan kromatografi lapis tipis-densitometri. Toksisitas kedua ekstrak diujikan terhadap sel 3T3-L1. LC₅₀ ditentukan dengan analisis probit. Differensiasi sel adiposit menggunakan insulin, dexametason dan IBMX dari sel 3T3-L1. Induksi resistensi insulin pada sel adiposit menggunakan glukosa 55 mM dan insulin 100 nM selama 10 hari. Aktivitas *uptake* glukosa dan ekspresi PPAR γ dan GLUT-4 diujikan terhadap kelompok pioglitazon, ETHS, EHP dan kombinasi ekstrak konvensional. Data yang diperoleh dianalisis secara statistik menggunakan *unpaired t-test* atau uji ANOVA *oneway* atau Man-Whitney.

Kadar andrografolid dan asiatikosid dalam ETHS dan EHP masing-masing sebesar 17,41 \pm 0,42% dan 11,34 \pm 0,69%. Nilai LC₅₀ ETHS dan EHP terhadap sel 3T3-L1 masing-masing sebesar 72,71 μ g/ml dan 46,29 μ g/ml. Pengujian kombinasi ekstrak terpurifikasi (30:10) μ g/ml terhadap sel tidak menunjukkan toksisitas sel. Differensiasi sel adiposit pada hari 10 menunjukkan bentuk, ukuran dan droplet lipid yang lebih besar, akumulasi lipid meningkat 4,8 kali atau berbeda signifikan dengan preadiposit ($p < 0,05$), dan regulasi ekspresi PPAR γ berbeda signifikan dibanding sel preadiposit ($p < 0,05$). Hasil uji resistensi insulin dengan analisis ekspresi GLUT-4 menunjukkan perbedaan signifikan dibandingkan sel adiposit normal ($p < 0,05$). Analisis ANOVA *oneway*, aktivitas *uptake* glukosa kelompok kombinasi ekstrak terpurifikasi konsentrasi (30:10) μ g/ml berbeda signifikan dengan kontrol negatif (DMSO). Kombinasi ekstrak terpurifikasi konsentrasi (30:10) μ g/ml menunjukkan peningkatan regulasi terhadap ekspresi PPAR γ dan GLUT-4 pada tingkat mRNA yang signifikan dibandingkan kontrol negatif dan tidak berbeda signifikan ($p > 0,05$) ketika dibandingkan dengan pioglitazon 0,02 μ M dan masing-masing ekstrak terpurifikasi.

Kata kunci : sambiloto, pegagan, ekstrak terpurifikasi, sel 3T3-L1, PPAR γ , GLUT-4