Intisari

Latar belakang: *Diabetic peripheral neuropathy* (DPN) mempengaruhi kualitas hidup pasien dengan terapi definitif yang belum ditemukan. Kondisi hiperglikemik merusak neuron dan menurunkan kapasitas regenerasi perifer. Schwann *cell* (SC), yang berperan krusial dalam regenerasi perifer, mengalami kerusakan dan penurunan regulasi ekspresi gen terkait mielinasi yang berakibat menurunkan kapasitas regenerasi. Oleh karena itu, terapi DPN dapat dilakukan tertarget SC untuk meningkatkan regenerasi perifer. DLBS1033N merupakan fraksi protein *Lumbricus rubellus* terhidrolisis yang terkonfirmasi meningkatkan pertumbuhan dan *survival* Schwann *cell line* RSC96 dengan menginduksi ekspresi NGF melalui aktivasi jalur PI3K.

Tujuan: Penelitian ini bertujuan mengevaluasi aktivitas DLBS1033N terkait regenerasi perifer dalam model *in vitro* diabetik neuropati dalam aspek viabilitas, migrasi, dan regulasi gen-gen terkait mielinasi.

Metode: DLBS1033N berbentuk serbuk kering dikarakterisasi meliputi kuantitas total protein menggunakan Bradford *assay* dan visualisasi profil protein menggunakan SDS-PAGE. Model *in vitro* DPN dioptimasi dengan pemberian variasi konsentrasi glukosa (50, 100, dan 150 mM) terhadap RSC96 selama 24, 48, dan 72 jam. Evaluasi aktivitas DLBS1033N konsentrasi 25, 50, dan 100 µg/mL terhadap viabilitas RSC96 dalam kondisi hiperglikemik dilakukan dengan metode MTS *assay*. Potensi DLBS1033N dalam menginduksi migrasi RSC96 dianalisis menggunakan metode *scratch assay*. Pengaruh DLBS1033N terhadap regulasi gen terkait mielinasi meliputi Egr-2/Krox-20 dan MBP dievaluasi dengan metode RT-PCR.

Hasil: Total protein DLBS1033N sebesar 11,45 mg/g dan memiliki protein baru berukuran < 20 kDa. Glukosa 50 mM merupakan model *in vitro* DPN pada penelitian ini. DLBS1033N meningkatkan viabilitas RSC96 secara *dose-dependent* pada inkubasi 48 jam sebesar 24%, 49% ($p < 0,05$), dan 71% ($p < 0,05$) oleh variasi dosis 25, 50, dan 100 µg/mL secara berurutan. DLBS1033N 100 µg/mL signifikan meningkatkan migrasi sel sebesar 16% ($p < 0,05$). Variasi dosis DLBS1033N tidak signifikan meningkatkan ekspresi *Egr-2* dan *Mbp* ($p > 0,05$) pada pengamatan 48 dan 72 jam.

Kesimpulan: Aktivitas DLBS1033N meningkatan regenerasi perifer tertarget Schwann sel hanya dapat terkonfirmasi pada aspek viabilitas dan migrasi sel.

Kata kunci: Diabetik neuropati, *Lumbricus rubellus*, sel Schwann, regenerasi sistem saraf perifer

Abstract

Background: Diabetic Peripheral Neuropathy (DPN) significantly affects the quality of life with no definitive therapy currently. Hyperglycemic condition implicates neuronal loss and peripheral regeneration capacity reduction. Schwann cells that play pivotal roles in peripheral regeneration manifest cell injury and down-regulation of myelin-related gene expression in diabetic and consecutively decreased regeneration capacity. Therefore, DPN treatment with SC-targeted to enhance peripheral regeneration is potential therapy strategy. DLBS1033N is hydrolyzed *Lumbricus rubellus* protein fraction which confirmed promotes Schwann cell line RSC96 growth and survival by induction of NGF expression via PI3K pathway.

Objective: This study aims to evaluate DLBS1033N activity related to peripheral regeneration in *in vitro* diabetic model in the aspect of cell viability, migration, and myelin-related gene regulation.

Method: Dried powder DLBS1033N was characterized including total protein quantity using Bradford assay and protein profile visualization using SDS-PAGE. DPN model in RSC96 was optimized using high-glucose levels (50, 100, and 150 mM) in 24, 48, 72 h incubation. DLBS1033N effect against cell viability was evaluated in variation doses of 25, 50, and 100 µg/mL using MTS assay. Migration activity induction of DLBS1033N was determined at 100 µg/mL using scratch assay. The effect of DLBS1033N in myelin-related gene regulation including *Egr-2*/*Krox-20* and *Mbp* expression was confirmed using RT-PCR method.

Result: DLBS1033N contains of 11,45 mg/g of total protein and has new protein profile under 20 kDa compare to unhydrolyzed. Glucose 50mM was used to establish DPN *in vitro* model. DLBS1033N improves cell viability of 24%, 49% ($p < 0,05$), and 71% ($p < 0,05$) at 25, 50, and 100 µg/mL, respectively. DLBS1033N significantly stimulates cell migration at 16% ($p < 0,05$) in 48 h incubation. Meanwhile, *Egr-2* and *Mbp* expression had not significantly improved by DLSB1033N in 48 and 72 h incubation.

Conclusion: DLBS1033N activity in Schwann cell-targeted peripheral regeneration was performed in the aspect of viability and migration cells.

Key words: diabetic neuropathy, *Lumbricus rubellus*, Schwann cell, peripheral regeneration