ABSTRAK

Terapi untuk mengatasi *painful diabetic neuropathy (*PDN) cukup menantang salah satunya terkait dengan tingginya efek samping. Salah satu obat yang digunakan untuk PDN adalah capsaicin, suatu agonis *transient receptor protein vanilloid-1* (TRPV1). 6-shogaol merupakan komponen aktif dari jahe yang mempunyai kemiripan struktur dengan capsaicin. Tujuan dari penelitian ini adalah memprediksi energi ikatan 6-shogaol dan derivatnya terhadap TRPV1 menggunakan model *in silico* dan membuktikan mekanismenya menggunakan model *in vivo* pada PDN.

Uji *in silico* dilakukan menggunakan Autodock Vina dengan membandingkan nilai energi ikatan antara 6-shogaol, 8-shogaol, 10-shogaol, 6-gingerol, 8-gingerol dan 10-gingerol terhadap TRPV1 (ID. 3J9J). Model *in vivo* menggunakan streptozotocin 110 mg/kgBB (i.p) untuk menginduksi diabetes. Pada hari ke-28, mencit dengan kadar glukosa darah > 200 mg/dL dirandomisasi menjadi 9 kelompok (@8 ekor) yaitu normal, PDN, perlakuan 6-shogaol 5 mg/kgBB, 6-shogaol 10 mg/kgBB, 6-shogaol 15 mg/kgBB, ekstrak etanol jahe 100 mg/kgBB, ekstrak etanol jahe 200 mg/kgBB, ekstrak etanol jahe 400 mg/kgBB dan gabapentin 100 mg/kgBB. Terapi 6-shogaol, ekstrak etanol jahe dan gabapentin diberikan sekali sehari selama 21 hari setelah hari ke-28. Kadar glukosa darah diamati pada hari ke-0,7,28,35,42 dan 49. Hiperalgesia diukur dengan *hot plate test, tail flick test* dan *Randall-Selitto,* sedangkan alodinia diukur dengan filamen *von Frey* diamati pada hari ke-0,7,14,21,28,35,42 dan 49. Pada hari ke-49, mencit dinekropsi, jaringan nervus ischiadicus, pancreas dan medulla spinalis diisolasi. Volume insula pancreatica diestimasi menggunakan metode hitung titik. Analisis deskriptif digunakan untuk menggambarkan ekspresi insulin pada pancreas dan morfologi akson pada nervus ischiadicus. Ekspresi mRNA dari TRPV1 dan reseptor NMDA subunit NR2B dikuantifikasi menggunakan real time PCR dengan GAPDH sebagai gen pembanding.

Berdasarkan analisis *in silico*, 6-shogaol mempunyai energi ikatan paling kecil terhadap TRPV1 (-7,10 kkal/mol). Pada mencit diabetes, 6-shogaol 15 mg/kgBB dan ekstrak etanol jahe 400 mg/kgBB memperbaiki perilaku nyeri dan morfologi akson nervus ischiadicus. Meskipun demikian pemberian terapi tidak mempunyai efek pada volume insula pancreatica dan ekspresi insulin serta tidak menurunkan kadar glukosa darah ke rentang normal. Pada medulla spinalis mencit yang menerima 6-shogaol 15 mg/kgBB dan ekstrak etanol jahe 400 mg/kgBB, ekspresi mRNA dari reseptor TRPV1 (0,52±0,21 and 0,47±0,08) dan reseptor NMDA subunit NR2B (0,85±0,04 and 0,57±0,10) lebih rendah dibandingkan kelompok PDN ($p < 0,05$)

Kesimpulan dari penelitian ini adalah senyawa 6-shogaol 15 mg/kgBB dan ekstrak etanol jahe 400 mg/kgBB memperbaiki perilaku nyeri dan morfologi akson nervus ischiadicus. Mekanisme senyawa tersebut bukan melalui peningkatan sekresi insulin pada insula pancreatica namun melalui penurunan ekspresi TRPV1 dan reseptor NMDA subunit NR2B pada medulla spinalis.

Kata kunci: PDN, 6-shogaol, jahe, TRPV1, reseptor NMDA subunit NR2B

ABSTRACT

Treatment of painful diabetic neuropathy (PDN) is challenging due to several factors including their many side effects. One of the drug used for PDN is capsaicin, an agonist of transient receptor protein vanilloid-1 (TRPV1). 6-shogaol is an active compound of ginger that has structural similarity to capsaicin. The aims of this study are predicting the binding energy of 6-shogaol and its derivates to TRPV1 using in silico model and investigating its mechanism of action in mice model of PDN.

In silico test was performed using Autodock Vina comparing the binding of 6-shogaol, 8-shogaol, 10-shogaol, 6-gingerol, 8-gingerol, 10-gingerol to TRPV1 (ID. 3J9J). In vivo model used streptozotocin-induced (110 mg/kgBB i.p) diabetic mice. At day-28, mice with blood glucose levels > 200 mg/dL were randomly divided into 9 groups (@8 mice) i.e. normal, PDN, 6-shogaol 5 mg/kgBW, 6-shogaol 10 mg/kgBW, 6-shogaol 15 mg/kgBW, ginger extract 100 mg/kgBW, ginger extract 200 mg/kgBW, ginger extract 400 mg/kgBW and gabapentin 100 mg/kgBW. Treatment of 6-shogaol, ginger extract and gabapentin were given once daily for 21 days after day-28. Blood glucose levels were determined at day-0, 7, 28, 35, 42 and 49. Pain behaviors were determined at day-0, 7, 14, 21, 28, 35, 42 and 49. Hyperalgesia were examined using hot plate, tail flick and Randall-Selitto tests. Allodynia was xamined using von Frey filament. At day-49, the mice were sacrificed; pancreas, sciatic nerve and spinal cord were dissected out. Volume of pancreatic islets were estimated in histological sections using point counting method. Descriptive analysis was performed on the expression of insulin in pancreatic sections and axon morphology in sciatic nerve sections. The mRNA expression of TRPV1 and N-methyl-D-aspartate (NMDA) receptor subunit NR2B in spinal cord were quantified using real time PCR method with GAPDH gene as reference gene.

In the in silico analysis, 6-shogaol had the smallest binding energy to TRPV1 (-7.10 kcal/mol). In the diabetic mice, 6-shogaol 15 mg/kgBW and ginger extract 400 mg/kgBW improved pain behavior and sciatic nerve's axonal morphology. However, the treatments had no effect in pancreatic islet's volume and insulin expression, neither reducing blood glucose level into normal range. In the spinal cord of mice received 6-shogaol 15 mg/kgBW and ginger extract 400 mg/kgBW, the mRNA expression of TRPV1 (0.52±0.21 and 0.47±0.08) and NMDA receptor subunit NR2B (0.85±0.04 and 0.57±0.10) were significantly lower compared to PDN group ($p < 0.05$).

In conclusion, 6-shogaol 15 mg/kgBW and ginger extract 400 mg/kgBW ameliorated pain behavior and sciatic nerve morphology in mice model of PDN. The mechanisms implicated in the drug's effect were not improving structure and function of pancreatic islet but reducing the expression of TRPV1 and NMDA receptor subunit NR2B in the spinal cord.

Key word: PDN, 6-shogaol, ginger, TRPV1, NMDA receptor subunit NR2B