

## CURATIVE EFFECTS OF ETHANOL EXTRACT OF BUTTERFLY PEA FLOWER (*Clitoria ternatea L.*) ON CYTOTOXICITY, SENESCENCE, APOPTOSIS, AND CELL MIGRATION IN NIH3T3 FIBROBLASTS EXPOSED TO HYDROGEN PEROXIDE

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### ABSTRACT

Degenerative diseases are characterized by a progressive decline in organ function, often linked to oxidative stress that triggers the overproduction of reactive oxygen species such as hydrogen peroxide ( $H_2O_2$ ), leading to cellular damage. *Clitoria ternatea L.* (butterfly pea) is known to contain flavonoids and anthocyanins, particularly ternatin, which exhibit strong antioxidant properties and may protect fibroblasts from oxidative stress. This study aimed to evaluate the effects of ethanolic extract of *Clitoria ternatea* (EBT) on the viability and collagen deposition of NIH3T3 fibroblasts under normal conditions and following  $H_2O_2$  induction. Cell viability was measured using the MTT assay, while collagen deposition was quantified by Sirius Red staining. NIH3T3 fibroblasts were treated with EBT at concentrations ranging from 7.8125 to 2000  $\mu\text{g/mL}$ , with or without  $H_2O_2$  150  $\mu\text{M}$ . Data were analyzed using one-way ANOVA followed by Tukey's HSD test ( $p \leq 0.05$ ). EBT demonstrated an  $IC_{50}$  value of 713.34  $\mu\text{g/mL}$ , indicating non-cytotoxicity. At low to moderate concentrations (7.812–500  $\mu\text{g/mL}$ ), EBT enhanced fibroblast viability, whereas at 2000  $\mu\text{g/mL}$  viability decreased significantly. Collagen deposition remained unaffected at most concentrations, with a significant increase observed at 500  $\mu\text{g/mL}$ . At higher concentrations (1000–2000  $\mu\text{g/mL}$ ), collagen levels were relatively stable, but reduced viability and morphological changes, including collagen clumping, suggested impaired fibril organization. In fibroblasts induced with  $H_2O_2$  150  $\mu\text{M}$ , EBT preserved collagen deposition across most doses, although protective effects were less evident at higher concentrations. In conclusion, EBT shows potential as a natural agent capable of stimulating fibroblast viability and collagen deposition under normal and mild oxidative stress conditions. Nevertheless, high concentrations exhibit toxic effects and compromise collagen organization, underscoring the need for further studies to elucidate molecular mechanisms and determine the optimal therapeutic dose.

Keywords: Butterfly Pea, *Clitoria ternatea L.*, Degenerative Diseases, NIH3T3 Fibroblast cell, Oxidative stress

## EFEK KURATIF EKSTRAK ETANOLIK BUNGA TELANG (*Clitoria ternatea L.*) TERHADAP SITOTOKSIK DAN DEPOSISI KOLAGEN PADA FIBROBLAS NIH3T3 YANG TERPAPAR HIDROGEN PEROKSIDA

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### INTISARI

Penyakit degeneratif ditandai oleh penurunan fungsi organ yang sering berkaitan dengan peningkatan stres oksidatif. Kondisi ini memicu akumulasi radikal bebas, termasuk hidrogen peroksida ( $H_2O_2$ ), yang dapat merusak struktur sel. Bunga telang (*Clitoria ternatea L.*) diketahui mengandung flavonoid dan antosianin, terutama ternatin, dengan aktivitas antioksidan kuat sehingga berpotensi melindungi fibroblas dari stres oksidatif. Penelitian ini bertujuan untuk mengevaluasi pengaruh ekstrak etanolik bunga telang (EBT) terhadap viabilitas dan deposisi kolagen fibroblas NIH3T3, baik pada kondisi normal maupun setelah induksi  $H_2O_2$ . Viabilitas sel diuji menggunakan metode MTT, sedangkan deposisi kolagen dianalisis dengan pewarnaan Sirius Red. Perlakuan diberikan pada rentang konsentrasi EBT 7,8125–2000  $\mu\text{g/mL}$ , dengan atau tanpa induksi  $H_2O_2$  150  $\mu\text{M}$ . Data dianalisis menggunakan ANOVA satu arah dilanjutkan uji Tukey HSD ( $p \leq 0,05$ ). Hasil penelitian menunjukkan bahwa EBT memiliki nilai  $IC_{50}$  sebesar 713,34  $\mu\text{g/mL}$  sehingga termasuk kategori tidak sitotoksik. Pada konsentrasi rendah hingga menengah (7,812–500  $\mu\text{g/mL}$ ), EBT menstimulasi viabilitas fibroblas NIH3T3, sedangkan pada konsentrasi tinggi (2000  $\mu\text{g/mL}$ ) viabilitas menurun signifikan. Uji deposisi kolagen memperlihatkan bahwa EBT tidak mengganggu deposisi kolagen pada sebagian besar konsentrasi, dengan peningkatan nyata pada 500  $\mu\text{g/mL}$ . Pada dosis tinggi (1000–2000  $\mu\text{g/mL}$ ), deposisi kolagen tetap relatif normal, tetapi viabilitas menurun dan morfologi menunjukkan kolagen menggumpal, menandakan gangguan kualitas fibril. Pada fibroblas yang diinduksi  $H_2O_2$  150  $\mu\text{M}$ , EBT mempertahankan deposisi kolagen pada sebagian besar konsentrasi meskipun efek protektifnya melemah pada dosis tinggi. Disimpulkan bahwa EBT berpotensi sebagai agen alami yang mampu menstimulasi viabilitas dan deposisi kolagen fibroblas NIH3T3 dalam kondisi normal maupun stres oksidatif ringan, meskipun pada dosis tinggi bersifat toksik dan menurunkan kualitas organisasi matriks.

Kata kunci: Bunga Telang, *Clitoria ternatea L.*, Penyakit Degeneratif, Sel fibroblas NIH3T3, Stres Oksidatif