

INTISARI

Latar Belakang: Tingkat kematian yang tinggi pada penderita kanker serviks uteri terutama di negara berkembang, belum maksimalnya terapi, masih banyaknya efek samping serta harga obat yang mahal mendorong para peneliti untuk mencari obat kanker yang efektif dengan target molekuler yang tepat.

Tujuan: Menguji efek antikanker dan ko-kemoterapi 1,2-Epoksi-3(3-(3,4-dimetoksifenil)-4H-1-benzopiran-4on)propana (EPI) serta mekanismenya.

Metode: Merupakan penelitian eksperimental *in vitro* pada kultur kanker serviks uteri. Uji sitotoksik dilakukan untuk menentukan IC_{50} , dan uji kombinasi untuk Indek Kombinasi (IK). Sel HeLa, sebanyak 32 sumuran dibagi menjadi 8 kelompok yaitu kontrol negatif, yang diberi senyawa EPI $\frac{1}{2}IC_{50}$, EPI IC_{50} , EPI $2IC_{50}$, doksorubisin (DOX) IC_{50} , kombinasi EPI+DOX, cisplatin (CIS), kombinasi EPI+CIS. Setelah diberi perlakuan selama 24 jam, diukur ekspresi miR-21, miR-214, dan miR-34a menggunakan qRT-PCR, sedangkan protein p53, TIMP-3, dan Bcl-2 dengan metode ELISA. Kecocokan target diuji dengan metode *in silico*. Uji *one-way* ANOVA dan *post hoc* Tukey digunakan untuk menguji beda rerata seluruh variabel antar kelompok penelitian, sedangkan untuk mengetahui hubungan ekspresi miRNA dengan konsentrasi protein dilakukan dengan uji korelasi Pearson dan regresi linier sederhana. Sekuensing dilakukan untuk melihat mutasi pada miR-21, dan analisis target menggunakan *BLAST*.

Hasil: IC_{50} EPI adalah $33,24 (\pm 3,01) \mu\text{g/ml}$, IK $\frac{1}{2}EPI+\frac{1}{2}DOX < 0,1$, dan IK $\frac{1}{2}EPI+\frac{1}{2}CIS < 0,9$. Ekspresi miR-21, -214, dan -34a lebih tinggi pada kelompok perlakuan dibandingkan dengan kelompok kontrol ($p < 0,05$), Ekspresi p53 pada kelompok 6 ($1,67 \pm 0,31 \mu\text{g/ml}$) dan 8 ($1,18 \pm 0,18 \mu\text{g/ml}$), ekspresi TIMP-3 kelompok 6 ($3,81 \pm 0,49 \mu\text{g/ml}$) dan 8 ($2,93 \pm 0,42 \mu\text{g/ml}$) lebih tinggi dari kelompok kontrol ($p < 0,05$), ekspresi Bcl-2 lebih rendah pada semua kelompok perlakuan ($p < 0,05$), koefisien korelasi antara miR-214 dan Bcl-2 adalah $-0,908$. Analisis *in silico* dengan metode *docking* menunjukkan energi ikatan reseptor p53 dan Bcl-2 dengan EPI lebih tinggi dari energi ikatan dengan ligan nativ. Hasil sekuensing menunjukkan terjadi mutasi pada miR-21, dan uji *in silico* dengan *BLAST* menunjukkan terjadi perubahan target pada miR-21 mutan.

Simpulan: Efek antikanker EPI bersifat sedang, bersinergi sangat kuat dengan DOX dan bersinergi lemah dengan CIS. Kombinasi EPI+DOX, dan EPI+CIS dapat meningkatkan MiR-214, MiR-34a, p53, dan TIMP-3 dan menurunkan Bcl-2, terdapat korelasi negatif sangat kuat antara ekspresi miR-214 dengan Bcl-2. Berdasarkan metode *docking* protein p53 dan Bcl-2 bukan target yang cocok dari senyawa EPI. Terjadi mutasi pada miR-21 dan perubahan target miR-21 mutan.

Kata kunci: Kanker serviks, Epoksi, miRNA, Index Kombinasi, miR-21 mutan

ABSTRACT

Background: *The insufficiency of treatment for cervical cancer, numerous side effects, and costly drugs encourage researchers to look for effective cancer drugs with accurate molecular target.*

Aim: *This study aims to examine the anticancer and co-chemotherapy effects of 1,2-Epoxy-3(3-(3,4-dimetoxyphenyl)-4H-1-benzopiran-4on) propane (EPI) compounds and their mechanism of EPI compounds on cultures of cervical cancer.*

Method: *This study was an experimental study in vitro on cervical cancer uteri culture. Cytotoxic test was performed to determine IC_{50} , while the combination test was conducted to determine the Combination Index (CI). HeLa cells in 32 wells were divided into eight groups as negative control, which were given $EPI/1/2IC_{50}$, $EPI IC_{50}$, $EPI 2IC_{50}$, doxorubicin IC_{50} , combination of $EPI+DOX$, cisplatin (CIS), and the combination of $EPI+CIS$ respectively. After 24 hours of treatment, HeLa cells expression of miR-21, miR-214, and miR-34a were measured using qRTPCR. Expression of p53, TIMP-3, and Bcl-2 proteins were measured by ELISA method. Suitability of protein target were measured by docking method. One-way ANOVA and post hoc Tukey test was performed to test the mean difference of all variables between the study groups, whereas Pearson's correlation test and simple linear regression were conducted to see the relationship of miRNA expression and its protein concentration. Sequencing is done to see the mutation in miR-21, and miR-21 target were analysed using BLAST.*

Results: *IC_{50} for EPI was $33.24 (\pm 3.01) \mu\text{g/ml}$, $1/2EPI+1/2DOX$ CI was <0.1 , and $1/2EPI+1/2CIS$ CI <0.9 . The expression of miR-21, -214, and -34a were higher in the treatment group compared to the control group ($p < 0.05$). Expression of p53 in group 6 ($1,67 \pm 0.31 \mu\text{g/ml}$) and 8 ($1.18 \pm 0.18 \mu\text{g/ml}$), TIMP-3 6 ($3,81 \pm 0,49 \mu\text{g/ml}$) and 8 ($2,93 \pm 0,42 \mu\text{g/ml}$) were significantly higher compared to the control group ($p < 0.05$), and Bcl-2 expression were significantly lower in all treatment groups ($p < 0.05$). The correlation coefficient between miR-214 and Bcl-2 is -0.908 . In silico analysis by docking method showed that EPI bond energy with p53 and Bcl-2 receptors was higher than native ligand binding energy with p53 and Bcl-2 receptors. The sequencing results showed mutations in miR-21, and test with BLAST showed a target change in the miR-21 mutant.*

Conclusion: *EPI compounds have a moderate anticancer effect, strong synergy with DOX and weak with CIS. The combination of $EPI+DOX$ and $EPI+CIS$ can increase miR-214, miR-34a, p53 and TIMP-3 expression and decrease Bcl-2 expression. There is a robust negative correlation between the expression of miR-214 and Bcl-2. Based on the docking method, proteins p53 and Bcl-2 are unsuitable targets of EPI compounds. There was a mutation in miR-21 and a change in mutant miR-21 target.*

Keywords: *Cervical cancer, Epoxy, miRNA, Combination Index, MiR-21mutan.*