RESEARCH ARTICLE

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Synergistic Action of 1,2-Epoxy-3 (3- (3,4-dimethoxyphenyl)-4H-1-benzopiyran-4-on) Propane with Doxorubicin and Cisplatin through Increasing of p53, TIMP-3, and MicroRNA-34a in Cervical Cancer Cell Line (HeLa)

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Abstract

Objective: Cervical cancer is the second most common cancer among women worldwide, with a high mortality rate especially in developing countries. Insufficient treatment for cervical cancer, multiple side effects, and high drug prices encourage researchers to look for effective and selective cancer drugs with appropriate molecular targets. This study explored the cytotoxicity of (1,2-epoxy-3(3-(3,4-dimethoxyphenyl)-4H-1-benzopyran-4-on) propane (EPI) synthesized from clove leaves oil on HeLa cells, its combination with doxorubicin (DOX) and cisplatin (CIS), and also their influence on p53, TIMP-3, and miR-34a as therapeutic targets. **Materials and Methods:** This research was an experimental in vitro study on cervical cancer uteri culture. The cytotoxicity was analyzed by MTT assay. The drug combination synergisms were indicated by the combination index (CI) (using CompuSyn 1.4). HeLa cells in 32 wells were divided into eight groups as negative control, which were given EPI ½IC_{50}, EPI IC_{50}, EPI 2IC50, DOX IC_{50}, combination of EPI+DOX, CIS, and the combination of EPI+CIS. The p53 and TIMP-3 concentrations were measured using ELISA, and expressions of miR-34a with qRT-PCR. One-way ANOVA and post hoc Tukey tests were performed to determine the mean difference of all variables between the study groups. **Results:** IC_{50} for EPI was 33.24 (±3.01) µg/ml, while DOX and CIS were 4.8 µg/ml (±0.1), and 23.34 µg/ml (±3.01), respectively, while CI values for EPI-DOX were <0.1 and for EPI-CIS <0.9. Expression of p53 in group 6 (1.67±0.31) µg/ml and 8 (1.18±0.18) µg/ml, TIMP-3 6 (3.81±0.49) µg/ml and 8 (2.93±0.42) µg/ml were significantly higher compared to the control group ($p<0.05$). All treatment groups showed significantly increased miR-34a expressions compared to the control group ($p<0.05$). **Conclusion:** The combinations showed a very strong synergism and a moderate slight synergism for EPI-DOX and EPI-CIS. Both combinations were able to increase the expressions of p53, TIMP-3 proteins, and MiR-34a in the HeLa cells.

Keywords: Cervical cancer- Epoxy- MicroRNA-34a- p53- TIMP-3

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Introduction

Cervical cancer has a high incidence and mortality rate and is the second most common cancer among women worldwide. About 200,000 women in developing countries died of this disease in 2010, and 46,000 of them were aged 15-49 years (Forouzanfar et al., 2014; Nindrea et al., 2018).

Cervical cancer is caused by a long-term infection of Human Papillomavirus (HPV). DNA viruses merge with human DNA resulting in an increased expression of E6 and E7 proteins, and subsequently interact with the oncogenic proteins and interfere with the cell cycle (Steben and Duarte-Franco, 2007). Protein E6 inhibits the tumor suppressor gene of p53 by increasing its proteasomal degradation. p53 is a tumor suppressor gene that acts as a transcription factor in regulating the transcription process of hundreds of genes encoding proteins for protection and maintaining genome integrity. It induces cell cycle arrest and DNA repair in the event of DNA damage, and triggers apoptosis when DNA repair is unsuccessful (Kumari et al., 2014).

Inactivation of p53 will cause down-regulation of miR-34a expression, since miR-34a is a direct target of transcriptional p53 transcription factor. Transactivation of expression of miR-34a is triggered by the p53 bond at

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