

## **SITOTOKSISITAS DAN INDUKSI APOPTOSIS *VENOM* KOBRA JAWA *Naja sputatrix* Boie, 1827 TERHADAP SEL KANKER KOLOREKTAL WiDr**

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### **INTISARI**

Kanker kolorektal menempati urutan ke-3 kanker dengan kasus terbanyak dan menjadi kanker penyebab kematian ke-2 di dunia. Komponen protein dalam *venom* ular dilaporkan memiliki aktivitas sitotoksik, sehingga *venom* ular berpotensi dimanfaatkan sebagai sumber senyawa antikanker alami. Penelitian ini bertujuan untuk mempelajari potensi sitotoksik dan induksi apoptosis *venom* kobra jawa *Naja sputatrix* terhadap sel kanker kolorektal WiDr serta menganalisis spesifitasnya menggunakan sel normal Vero. Metode yang digunakan berupa uji sitotoksitas terhadap sel WiDr dan Vero menggunakan *MTT assay* dengan konsentrasi *venom* 1,25; 2,5; 5; 10; 20; 40; 80  $\mu\text{g/mL}$ . Selanjutnya nilai  $\text{IC}_{50}$  ditentukan dengan interpolasi linear. Uji apoptosis dilakukan menggunakan metode *flow cytometry* dengan Annexin V-FITC dan *propidium iodide* (PI) pada konsentrasi  $\frac{1}{2} \text{IC}_{50}$  dan  $\text{IC}_{50}$  *venom* kobra jawa berdasarkan hasil *MTT assay*. Uji sitotoksitas dianalisis dengan *one way-ANOVA* ( $p \leq 0,05$ ) diikuti dengan metode Tukey HSD dan Games-Howell. Sedangkan data apoptosis, dianalisis dengan uji nonparametrik karena data tidak memenuhi asumsi uji parametrik, khususnya distribusi normal dan homogenitas varians, yaitu uji Kruskal–Wallis dan dilanjutkan dengan uji Mann–Whitney U untuk mengetahui perbedaan antar kelompok. Hasil uji sitotoksitas menunjukkan bahwa *venom* kobra jawa menurunkan viabilitas sel WiDr dengan  $\text{IC}_{50}$  sebesar 56  $\mu\text{g/mL}$  dan sel Vero sebesar 22,9  $\mu\text{g/mL}$ . Indeks selektivitas (IS) sel WiDr terhadap sel Vero sebesar 0,41. Hasil uji apoptosis menunjukkan bahwa *venom* kobra jawa dominan menginduksi nekrosis daripada apoptosis awal maupun akhir. *Venom* kobra jawa pada konsentrasi 28  $\mu\text{g/mL}$  ( $\frac{1}{2} \text{IC}_{50}$ ) menyebabkan kematian sel yang didominasi oleh nekrosis ( $52,1 \pm 3,11\%$ ), dengan apoptosis awal dan akhir yang jauh lebih rendah sebesar  $0,6 \pm 0,12\%$  dan  $2,3 \pm 0,32\%$ . Pola serupa juga diamati pada konsentrasi  $\text{IC}_{50}$ , di mana nekrosis tetap mendominasi ( $46,2 \pm 3,59\%$ ), sedangkan apoptosis awal dan akhir yaitu masing-masing sebesar  $0,1 \pm 0,00\%$  dan  $0,9 \pm 0,15\%$ . *Venom* kobra jawa menunjukkan aktivitas sitotoksik kategori sedang terhadap sel kanker kolorektal WiDr, namun memiliki spesifitas yang rendah serta tidak menyebabkan kematian sel melalui mekanisme nekrosis.

Kata kunci: apoptosis, kanker kolorektal, kobra jawa, sitotoksitas, spesifitas

**CYTOTOXICITY AND APOPTOSIS INDUCTION OF JAVANESE  
COBRA *Naja sputatrix* Boie, 1827 VENOM ON COLORECTAL CANCER  
CELL WiDr**

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**ABSTRACT**

Colorectal cancer ranks 3<sup>rd</sup> in cancer with the most cases and is the 2<sup>nd</sup> cause of cancer death in the world. Protein components in snake venom are reported to have cytotoxic activity, so snake venom has the potential to be used as a source of natural anticancer compounds. This study aims to study the cytotoxic potential and apoptosis induction of Javan cobra venom *Naja sputatrix* against WiDr colorectal cancer cells and analyze its specificity using normal Vero cells. The method used is a cytotoxicity test on WiDr and Vero cells using MTT assay with venom concentrations of 1.25; 2.5; 5; 10; 20; 40; 80 µg/mL. Furthermore, the IC<sub>50</sub> value is determined by linear interpolation. The apoptosis test was carried out using the flow cytometry method with Annexin V-FITC and propidium iodide (PI) at a concentration of ½ IC<sub>50</sub> and IC<sub>50</sub> of Javan cobra venom based on the results of the MTT assay. The cytotoxicity test was analyzed by one-way ANOVA ( $p \leq 0.05$ ) followed by the Tukey HSD and Games-Howell methods. Meanwhile, apoptosis data were analyzed by nonparametric tests because the data did not meet the assumptions of parametric tests, especially normal distribution and homogeneity of variance, namely the Kruskal–Wallis test and continued with the Mann–Whitney U test to determine differences between groups. The results of the cytotoxicity test showed that Javan cobra venom reduced the viability of WiDr cells with an IC<sub>50</sub> of 56 µg/mL and Vero cells by 22.9 µg/mL. The selectivity index (IS) of WiDr cells against Vero cells was 0.41. The results of the apoptosis test showed that Javan cobra venom predominantly induced necrosis rather than early or late apoptosis. Javan cobra venom at a concentration of 28 µg/mL (½ IC<sub>50</sub>) caused cell death dominated by necrosis (52.1 ± 3.11%), with much lower early and late apoptosis of 0.6 ± 0.12% and 2.3 ± 0.32%, respectively. A similar pattern was also observed at the IC<sub>50</sub> concentration, where necrosis remained dominant (46.2 ± 3.59%), while early and late apoptosis were 0.1 ± 0.00% and 0.9 ± 0.15%, respectively. Javan cobra venom showed moderate cytotoxic activity against WiDr colorectal cancer cells, but had low specificity and did not cause cell death through necrosis.

*Keywords:* apoptosis, colorectal cancer, Javan cobra, cytotoxicity, specificity