Cytotoxicity effects and apoptosis induction by cycleanine and tetrandrine

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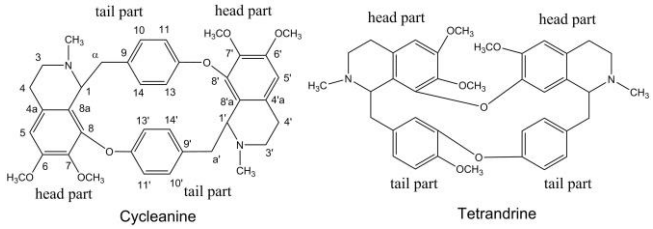
Ovarian cancer remains one of the main causes of death in all gynecologic malignancies.[1] Natural products continue to be important sources of clinically approved anti-cancer drugs.[2-3] *Triclisia subcordata* Oliv (Menispermeaceae) is a medicinal plant traditionally used for the treatment of various diseases,[4] including cancer, in West Africa. This study aims to evaluate the *in vitro* anti-ovarian cancer activities of the crude extracts and the isolated components in *T. subcordata*. The ethanol extract of *T. subcordata* and its fractions (crude alkaloids) were screened for *in vitro* anti-ovarian cancer activities on Ovcar-8, Ovcar-4, A2780, and Igrov-1 ovarian cancer cell lines using the Sulforhodamine B assay method to measure cell growth. Bioassay-guided fractionation using silica gel column chromatography and HPLC were used to isolate the bioactive compound, whose identity and structure was identified by NMR and LC-MS techniques. Caspase and PARP cleavage assays were used to detect apoptotic activities. The effect of isolated pure compounds on cell cycle and apoptosis was analyzed by flow cytometry. *Results*: The crude alkaloids showed the strongest activity in cell growth assays on A2780 and Ovcar-8 cell lines (IC_{50} < 2.4 µg/mL). A bisbenzylisoquinoline alkaloid-cycleanine was isolated using HPLC and identified by MS and NMR analyses. The IC_{50} values of cycleanine and tetrandrine (an alkaloid previously reported from this plant) ranged from 7 to 14 µM on A2780, Ovcar-8, Ovcar-4 and Igrov-1 ovarian cancer cell lines. The IC_{50} of cycleanine on human normal ovarian surface epithelial cells was 35 ± 1 µM hinting at modest selectivity towards cancer cells. Both cycleanine and tetrandrine caused apoptosis as shown by activation of caspases 3/7 (increased levels of caspases 3/7) and cleavage of poly (ADP) ribose polymerase (PARP) to form PARP-I. The percentage of Ovcar-8 cells in $subG_1$ phase increased after exposure of cycleanine and tetrandrine to cells for 48h compared to control. In conclusion, cycleanine, like its isomer – tetrandrine isolated from *Triclisia subcordata*, could be a potential new anti-ovarian cancer agent acting through the apoptosis pathway.



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Keywords: *Triclisia subcordata*, cycleanine, tetrandrine, anti-proliferation, apoptosis, ovarian cancer

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