# Berberine induces apoptosis *via* increased ROS level and induced DNA damage in B16F10 melanoma cell line

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## Abstract

*Coptis rhizome* is a traditional medicine in China and berberine is one of the main active components of coptis rhizome. Berberine has the effects of antibacterial, lower blood pressure control and anti-cancer. Numerous studies indicated that berberine could inhibit cancer cell proliferation, such as human ovarian cancer, human osteosarcoma, and human breast cancer cells. Melanoma is the most lethal and malignant in human skin cancer. It was recently shown that berberine could inhibit cancer cell proliferation, especially in PANC-1 and MIA-PaCa2 pancreatic cell lines *via* ROS generation. However, it is still unclear whether or not berberine induces melanoma B16F10 cells apoptosis. In the present study, B16F10 cells were treated with berberine for various concentration. Cell viability was measured by using flow cytometry, and we found the $IC_{50}$ was 25 μM at 48 hour treatment. Reactive oxygen species level was measured by utilizing flow cytometry via $H_2$DCFDA staining and results indicated that ROS level was increased obviously. Furthermore, We also use DAPI staining to investigate berberine induce DNA damage and results shown berberine induce DNA damage. We predict that DNA damage cause down-regulated of DNA repair gene which *via* ATM pathway and we will focus on this area. Based on these observations; we may suggest that berberine may be used as chemotherapeutic candidate for anti-melanoma in the future.
Keyword: Berberine, B16F10 cell, apoptosis, reactive oxygen species

## Result

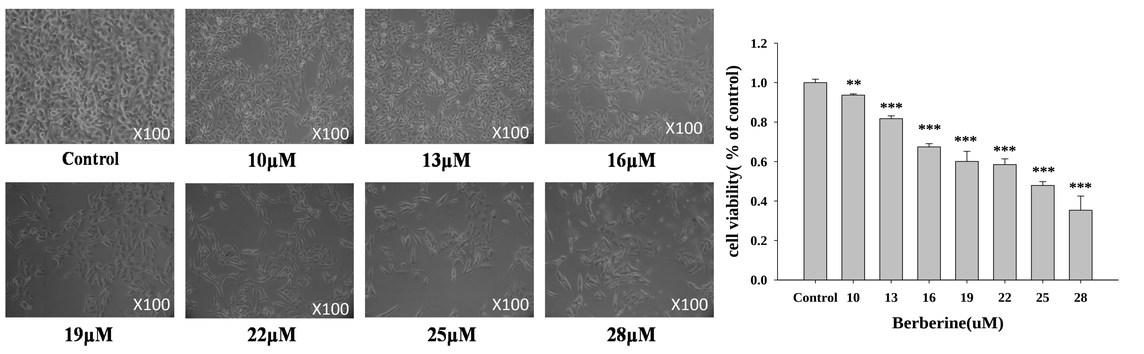



**Figure 1. Effects of berberine on cell viability in B16-F10 melanoma cells.** Viability of B16F10 cells (1X10$^5$cells/well) were treated with 0, 10, 13, 16, 19, 22, 25 and 28μM berberine for 48 hours by flowcytometry and qualification. After 48 hour, over 50% B16F10 melanoma cells were suppressed on 25μM berberine-treated groups. Our data suggest that the 50% inhibition concentration was 25μM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus control.

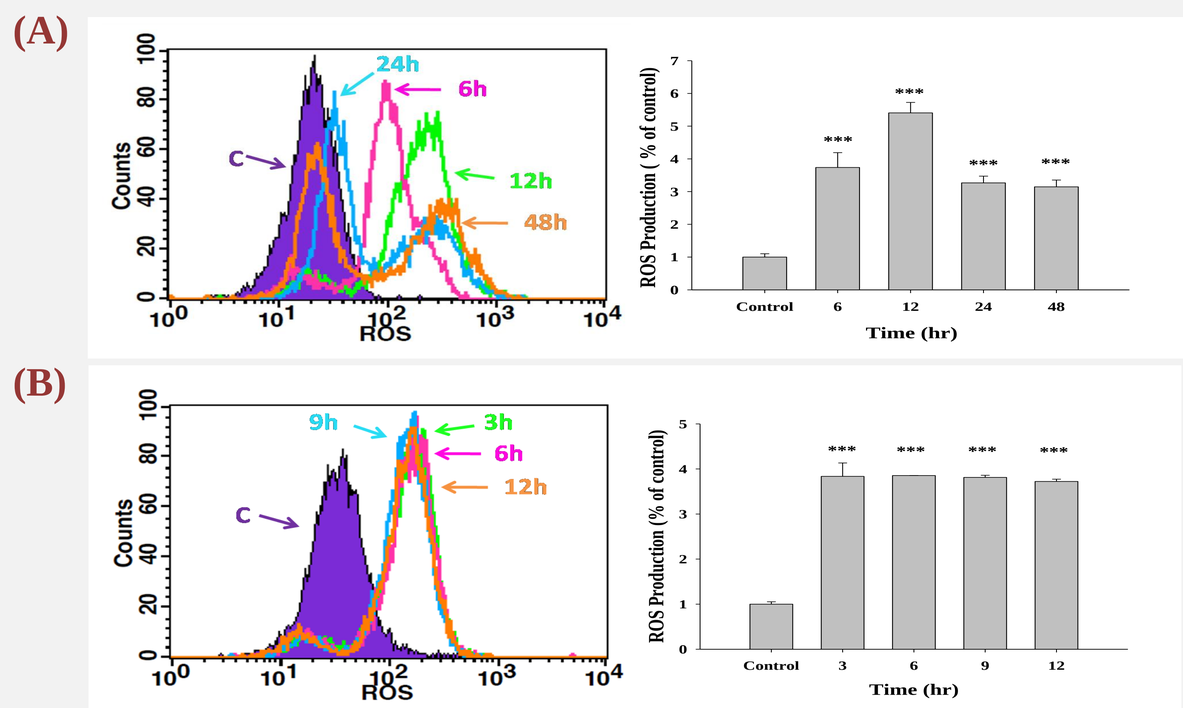



**Figure 2. Intracellular reactive oxygen species (ROS) levels after berberine treatment.** ROS levels of B16F10 melanoma cells treat with 0 and 25μM berberine for 6, 12, 24 and 48 hours by flowcytometry and qualification, (A). ROS levels of B16F10 cells (1X10$^5$cell/well) were treat 0 and 25μM berberine for 3, 6, 9 and 12 hours by flowcytometry and qualification, (B). Our data suggest that ROS levels of B16F10 melanoma cells significantly increased within 12 hours. *** $p < 0.001$ versus control.

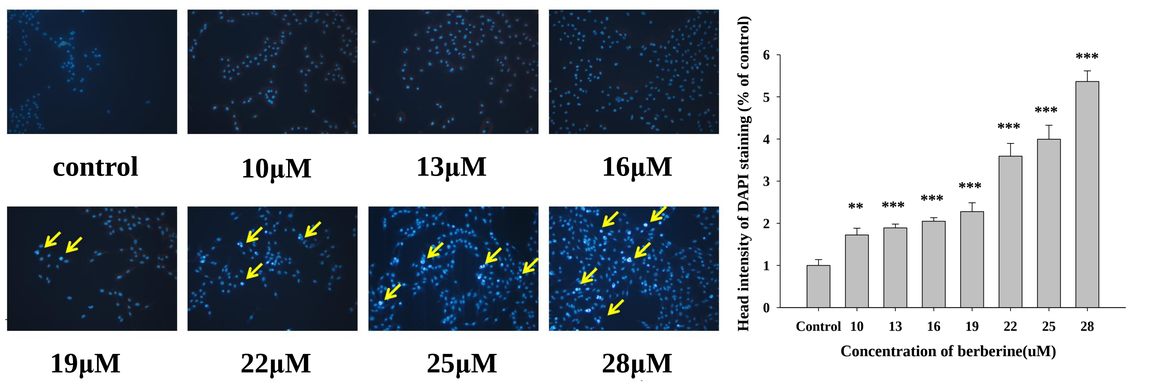



**Figure 3. Berberine induce B16F10 melanoma cells DNA condensation.** The B16F10 cells (2X10$^5$ cells/well) were treated with 0 ,10, 13, 16, 19, 22, 25 and 28μM berberine for 48 hour for determining DAPI staining and qualification. Our data suggest that significant difference between control and berberine–treated groups. * $p < 0.05$ versus control.

## Result

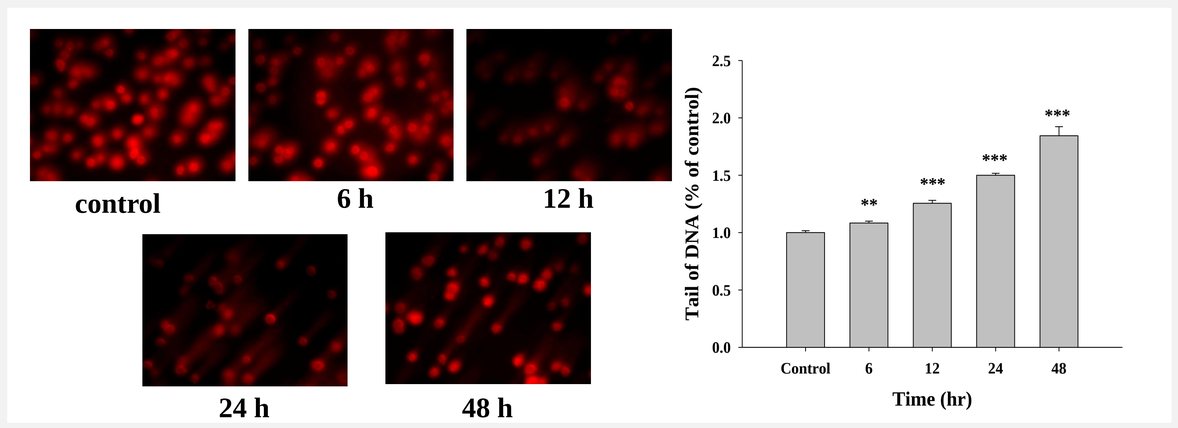



**Figure 4. DNA damage of B16F10 melanoma cells after berberine treatment .** The B16F10 cells (2X10$^5$ cells/well) were treated with 0 and 25μM berberine for 6, 12, 24 and 48 hours for determining comet assay and qualification. Our data suggest that DNA damage significant increase between control and berberine–treated groups. ** $p < 0.01$, *** $p < 0.001$ versus control.

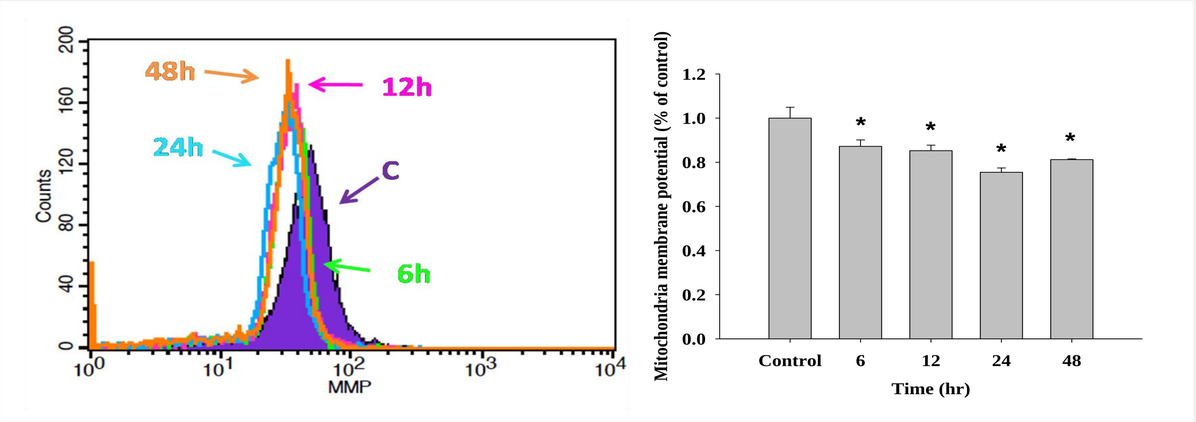



**Figure 5. Mitochondria membrane potential (MMP) levels of B16F10 melanoma cells after berberine treatment .** MMP levels of B16F10 melanoma cells (1X10$^5$ cells/well) treat with 0 and 25μM berberine for 6, 12, 24 and 48 hours by flow cytometry and qualification. Our data suggest that MMP levels of B16F10 melanoma cells decreased between control and berberine–treated groups. * $p < 0.05$ versus control.

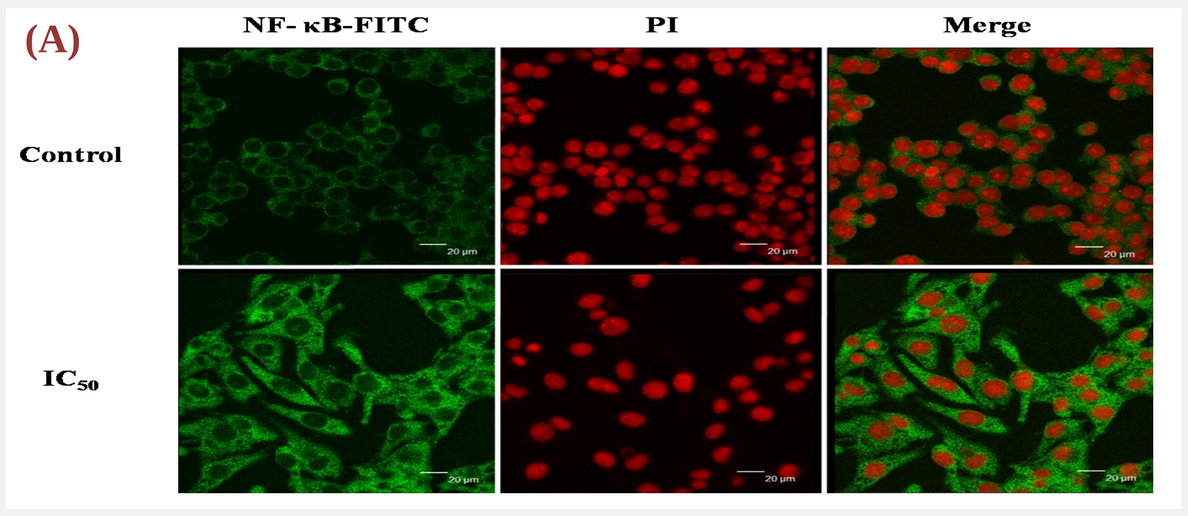
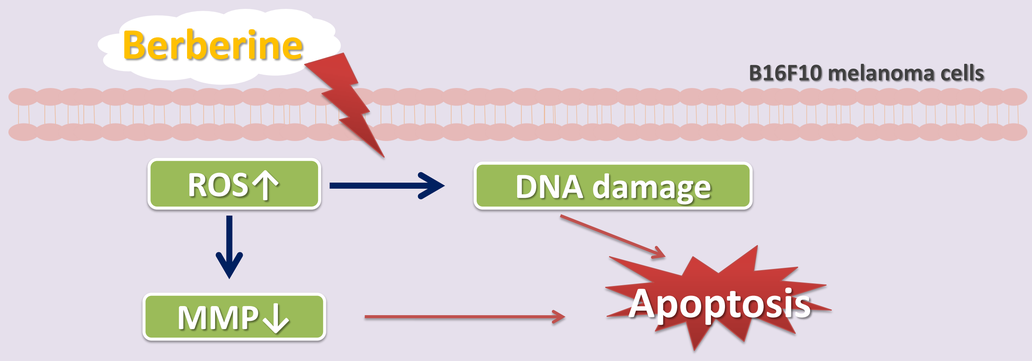



**Figure 6. NF-κB (p65) and p-p38 translocation.** B16F10 melanoma cells (1X10$^5$ cells/well) treat with 0 and 25μM berberine for 48 hour by confocal laser microscopy, NF-κB (p65) (A), and p-p38 (B). Our data suggest that NF-κB (p65) and p-p38 significant translocation.

## Conclusion




1. **Berberine induce B16F10 cell death *via* apoptosis.**
2. **Berberine induce B16F10 cell apoptosis *via* ROS dependent pathway and mitochondrial control of apoptosis.**
3. **Berberine increase expression of NF-κB (p65) and p-p38 and downregulate apoptosis.**