Apigenin induce cell death of mice leukemia WEHI-3 cells via cell cycle arrest and induce apoptosis

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ABSTRACT

Apigenin (4,5,7-trihydroxylfavone), found in many plants, is a nature product belonging to the flavone class. It was reported that apigenin could inhibit cancer cell proliferation, especially in leukemia cell line such as human leukemia cancer HL60 and U937 cells via cell cycle arrest or DNA damage. However, it is still unclear whether apigenin induces murine WEHI-3 cell apoptosis *in vitro*. Thus, we at first investigated the effect of apigenin on WEHI-3 cell viability. WEHI-3 cells were treated with apigenin at various concentration, and the viability was measured by using flow cytometry. We found that IC50 is 15 μM for apigenin decreased cell number. Furthermore, we focus on cell cycle in WEHI-3 cells after exposed to apigenin. Apigenin was used at 15μM for treating WEHI-3 cells and cell cycle was arrested at G2/M phase. In addition, DNA damage was determined by DAPI staining and DNA gel electrophoresis and results shown that apigenin induce DNA damage in WEHI-3 cells. Except using flow cytometry, we also use Western blotting to detect association protein from WEHI-3 cells after exposed to apigenin. We found that the vital factors of cell cycle and DNA repair was greatly changed. Based on these observations, apigenin suppresses WEHI-3 cells *in vitro*, we may suggest that apigenin may be used chemotherapeutic candidate for anti-leukemia in the future.

Keyword: WEHI-3 cells, apigenin cell cycle arrest, apoptosis, DNA damage

RESULTS

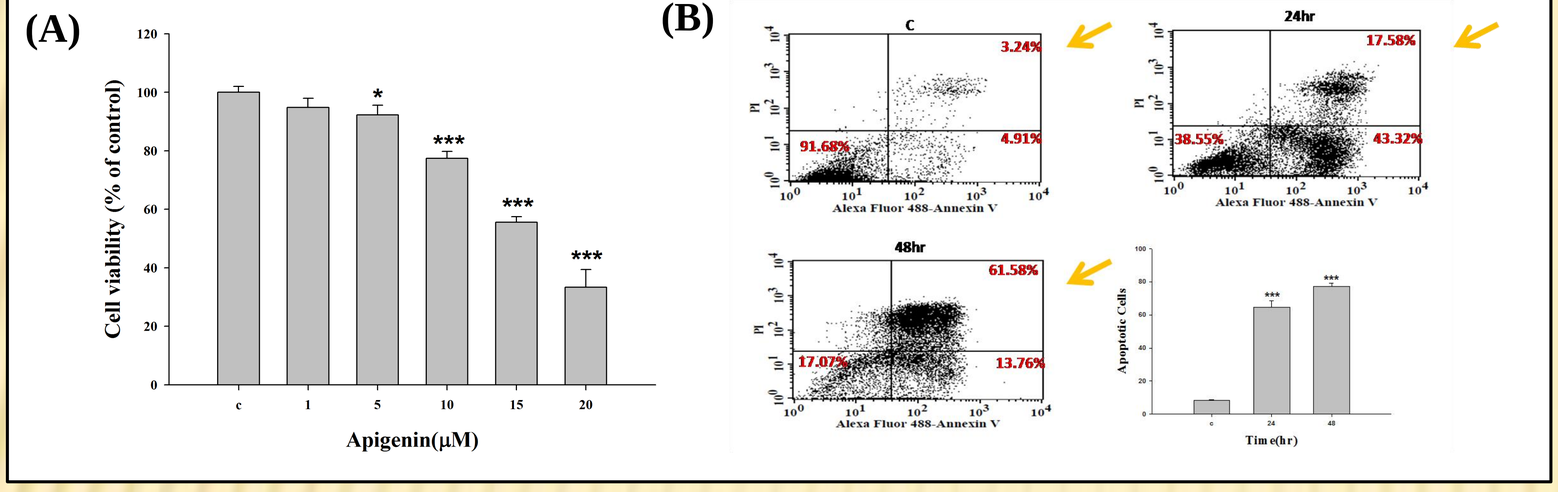

Figure.1 Effect of apigenin on WEHI-3 cell viability. **The WEHI-3 cells (1 × 10^5 cell/well) were treated with several concentration of apigenin for 48h for determining cell viability (A). The cells were harvested individually and then measure apoptosis by annexin V staining (B). *P=0.05,***P=0.001, shows significant difference between control and WEHI-3 - treated groups.**

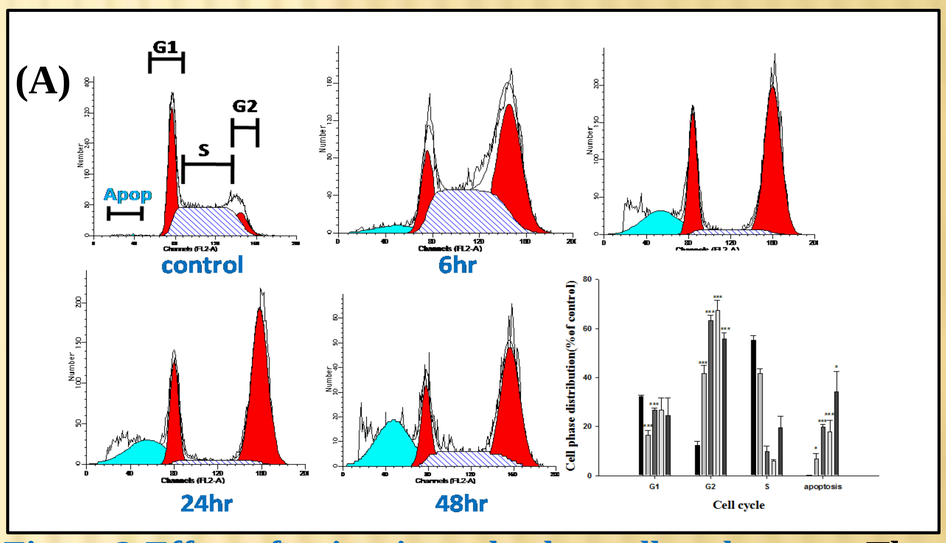

Figure.2 Effect of apigenin on lead to cell cycle arrest **The WEHI-3 cells (1 × 10^5 cell/well) were treated with 15μM of apigenin for several time for determining cell cycle and qualification (A) *P=0.05,***P=0.001, shows significant difference between control and WEHI-3 - treated groups.**

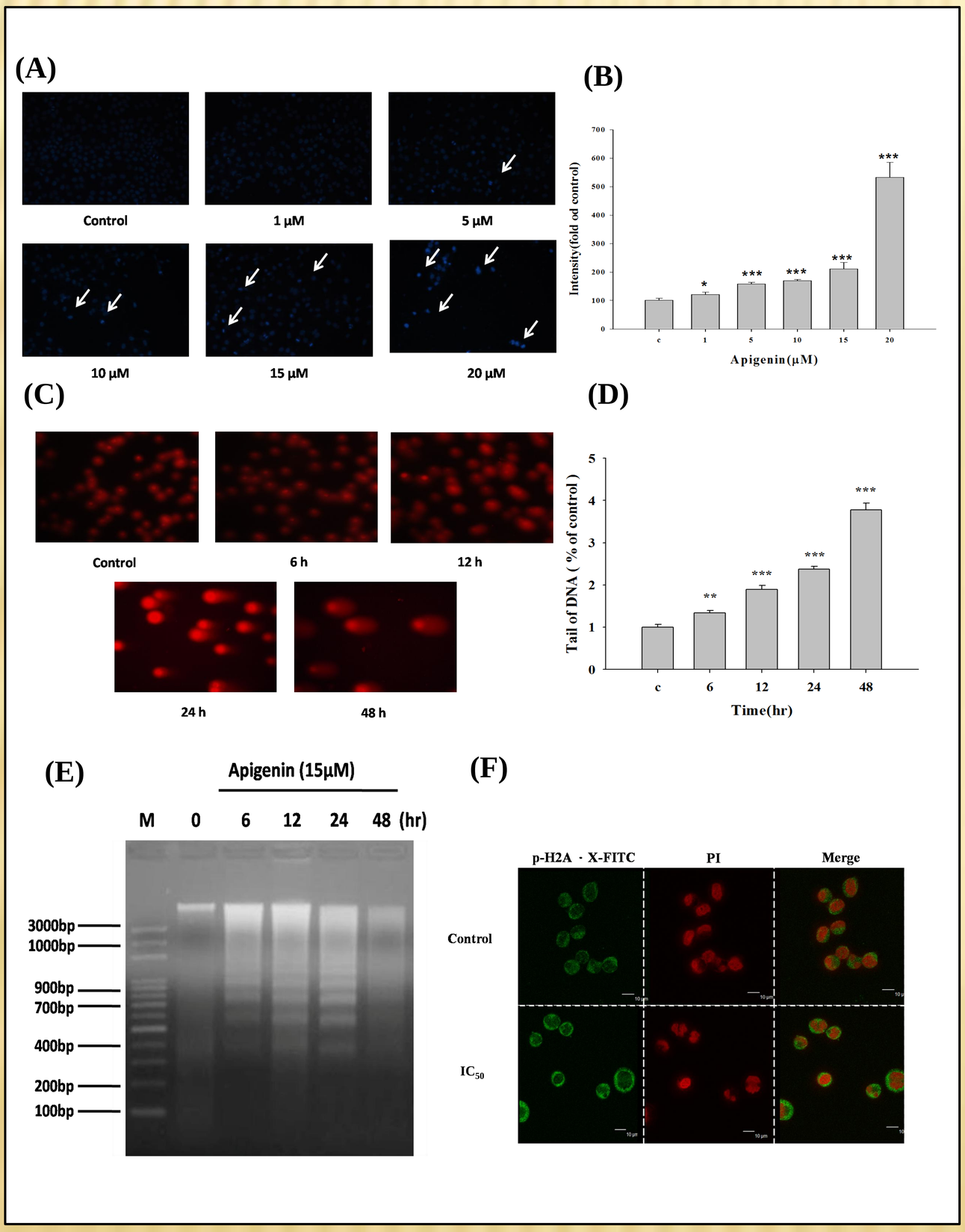

Figure.3 Effect of apigenin on cell cycle arrest and DNA damage of DNA damage. **The WEHI-3 cells (1 × 10^5 cell/well) were treated with 15μM of apigenin for several time for determining DAPI staining (A) and qualification (B), comet assay (C) and qualification (D), DNA fragemntation (E), and DNA repair protein, p-H2A · X, translocation. *P=0.05, **=0.01, ***P=0.001, shows significant difference between control and WEHI-3 - treated groups.**

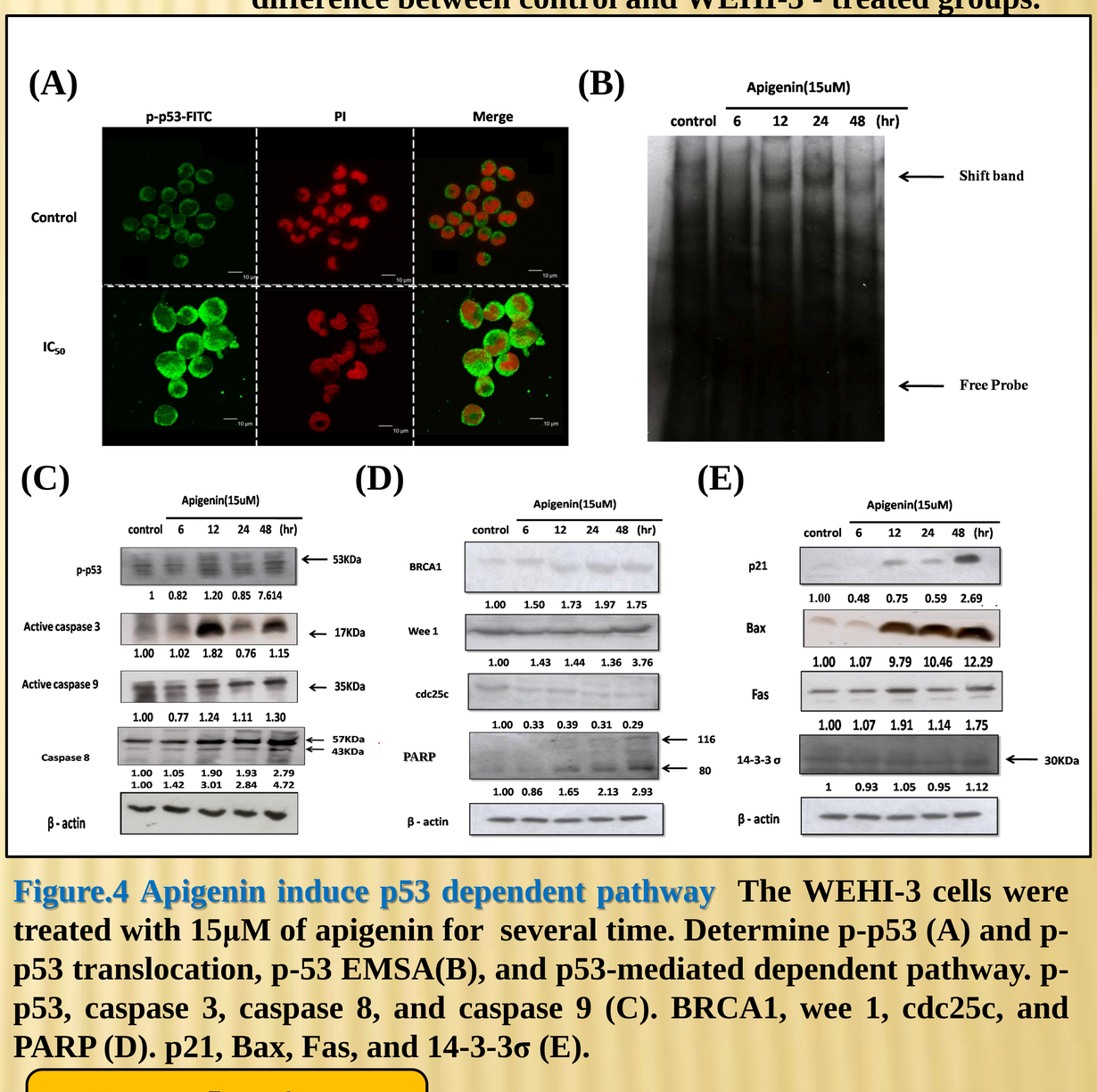

Figure.4 Apigenin induce p53 dependent pathway **The WEHI-3 cells were treated with 15μM of apigenin for several time. Determine p-p53 (A) and p-p53 translocation, p-53 EMSA(B), and p53-mediated dependent pathway. p-p53, caspase 3, caspase 8, and caspase 9 (C). BRCA1, wee 1, cdc25c, and PARP (D). p21, Bax, Fas, and 14-3-3σ (E).**

Conclusion