Taiwanin C down-regulates COX2-EGFR and up-regulates P27 pathways to suppress Arecoline-induced oral cancer cell proliferation via ERK1/2 inactivation.

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Introduction: Oral cancer is one of the most common cancers reported in Taiwan and other Southeast Asian countries, caused due to the betel nut chewing habit that is prevalent in their population. Arecoline, the most abundant alkaloid in betel nut is known to induce abnormal proliferation of epithelial cells by facilitating the activation of epidermal growth factor receptor (EGFR) and its downstream mechanisms and there by promotes the expression of a crucial downstream protein cyclooxygenase-2 (COX2) in oral epithelial cells. EGFR is a vital growth factor receptor, which can functionally activate cell differentiation and proliferation. Taiwanin C, a naturally occurring lignan extracted from *Taiwania cryptomerioides*, has been reported to potentially inhibit the expression of COX2. However, the potential of Taiwanin C to inhibit the oral cancer cell proliferation and the related upstream mechanism responsible for COX2 inhibition are not clear yet.

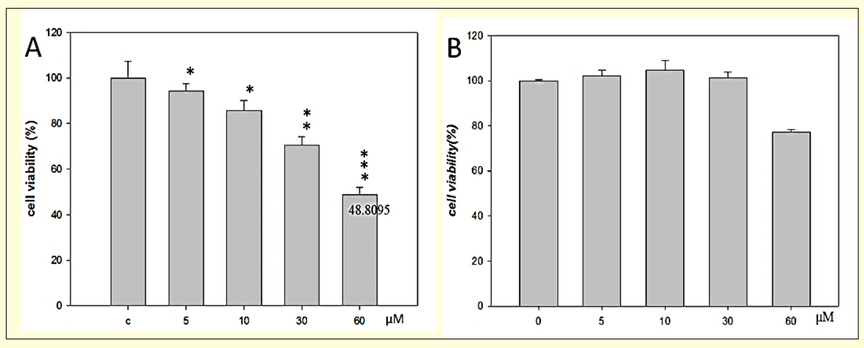


Fig. 1. Influence of Taiwanin C on normal (N28) and oral cancer (T28) cells *in vitro*. Treatment with Taiwanin C reduced the cell viability of the T28 oral cancer cells (A) but 5-30 μM of Taiwanin did not show any cytotoxicity in N28 normal cells (B). The cell viability was measured by MTT assay after treating with different concentrations of TaiwaninC (0, 5, 10, 30, 60 μM).

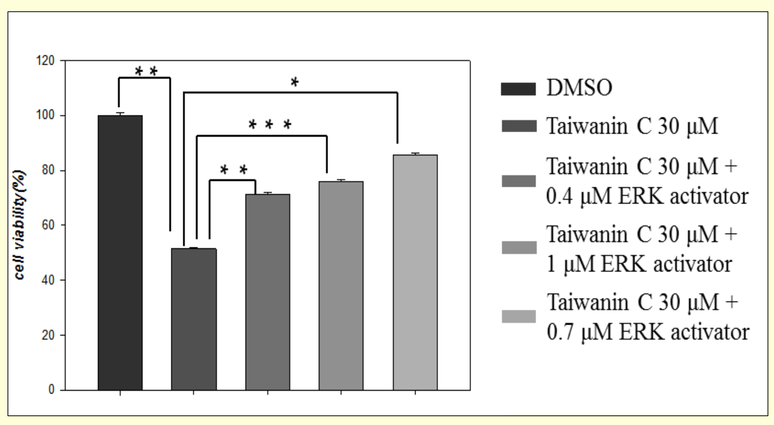


Fig. 2. Influence of ERK activator on the inhibition effect of TaiwaninC on T28 cell proliferation. T28 cells were treated with Cell viability was measured by MTT assay after treatment with 30μM TaiwaninC and co-treatement with different concentrations of ERK activator (0.4, 0.7, 1 μM).

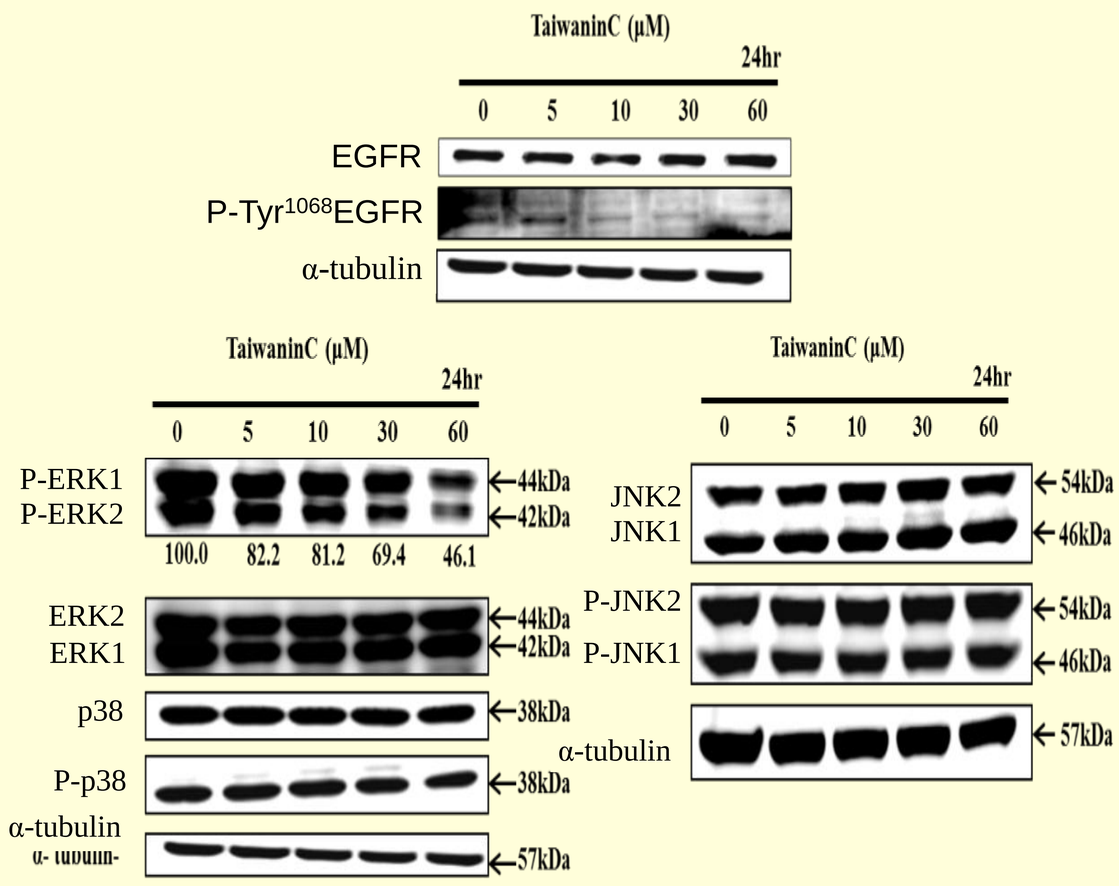


Fig. 3. TaiwaninC Modulate EGFR signal pathway and MAPK pathway proteins in oral cancer cells. After treatment with Taiwanin C T28 cells the levels of EGFR, P-Tyr[1068] EGFR and MAPK proteins were analyzed by Western blot using specific antibodies. The blots were probed with α-tubulin antibody for comparison of protein loading.

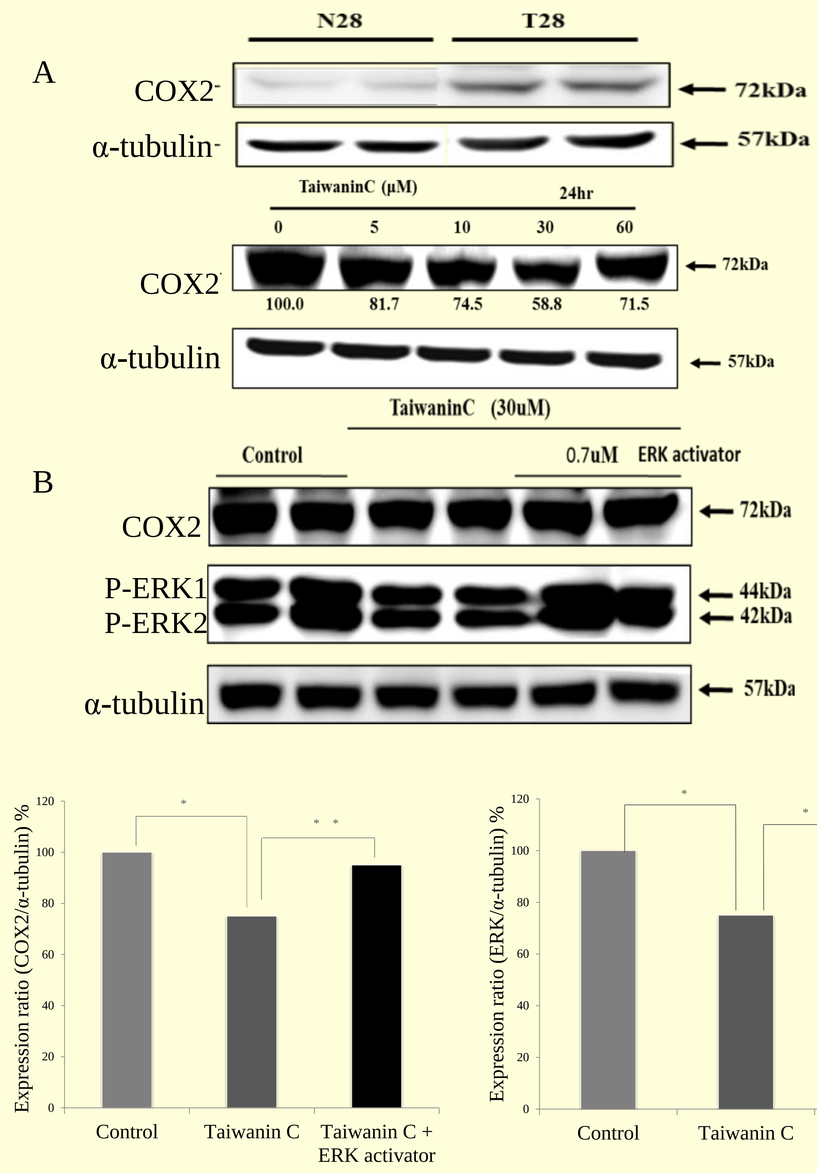


Fig. 4. Western blot analysis to determine the influence of Taiwanin C on COX2 expression in T28 oral cancer cells, in N28 normal oral cells (A) and to determine the influence of ERK activator- Taiwanin C co-treatment on COX2 and P-ERK1/2 expression in the T28 oral cancer cells (B). (** p <0.01 * p <0.05)

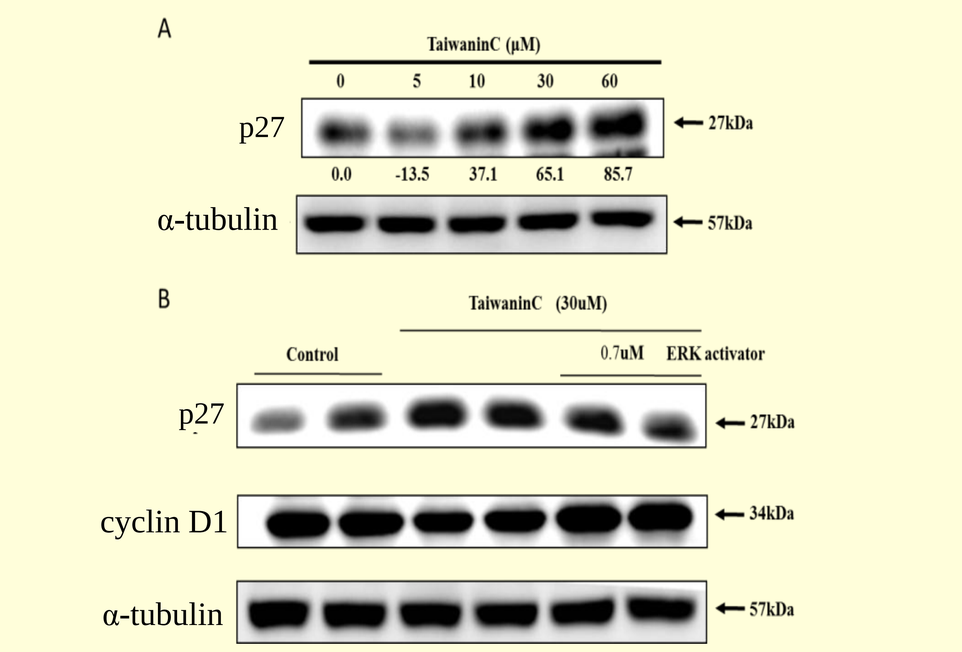
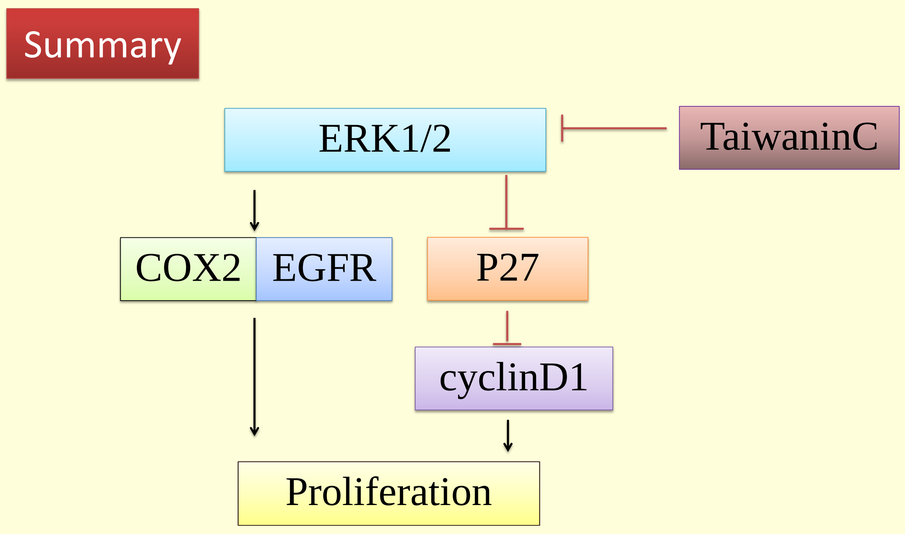


Fig. 5. Western blot analysis to determine the influence of Taiwanin C on p27 protein levels and the influence of ERK activator co-treatment on the Taiwanin C induced effect on p27 (A) and corresponding cytidin D1 expression leves (B). * p < 0.05 represent significant differences when compared with the control group.



Conclusion:

In conclusion our results indicate that Taiwanin C down-regulates COX2/EGFR and up-regulates P27 pathways to suppress Arecoline-induced oral cancer cell proliferation via ERK1/2 inactivation.