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Triclosan-induced breast cancer growth was antagonized by kaempferol, a phytoestrogen, via regulating cell cycle, migration and apoptosis related genes in MCF-7 breast cancer cells

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ABSTRACT

Triclosan (TCS) is one of endocrine disrupting chemicals (EDCs) derived from toothpastes, deodorant and cleaning supplies. As a phytoestrogen, kaempferol (Kaem) is found at variety of vegetables. In this study, we examined anti-proliferative effects of Kaem in TCS-induced cell growth in MCF-7 breast cancer cells. In MTT assay, TCS (10^{-6} M) increased the cell viability of MCF-7 cells, while Kaem (50 mM) significantly reduced the cell viability compared to a control (0.1% DMSO). Kaem reversed TCS-induced MCF-7 cell growth at 50 mM. To confirm that Kaem inhibited TCS-induced cell growth, we examined the transactional levels of cell growth and apoptosis-related markers using reverse transcription (RT)-PCR. The expression levels of cyclin D, cyclin E and were increased, while that of p21 and bax mRNAs was decreased by TCS in MCF-7 cells. In addition, Kaem treatment significantly reversed TCS-induced gene expressions. In parallel with its mRNA level, the protein level of cyclin E, cyclin D, cathepsin D, p-IRS1, p-AKT, p-ERK and p-MEK1/2 were induced by TCS while it was reversed by Kaem. The expression levels of p21 and bax genes was altered by TCS and reversed by Kaem treatment. For *in vivo* assay, a xenografted mouse model was generated following injection with MCF-7 breast cancer cells. In parallel with *in vitro* results, tumor volumes following treatment with E2 and TCS were continually increased compared to a vehicle (corn oil). It was of interest that treatment of the mice with combination of E2 plus Kaem or TCS plus Kaem showed less tumor formation rather than that of single treated mice with E2 or TCS. Taken together, these results indicate that Kaem may inhibit the growth MCF-7 cells via regulating the expression of cell cycle, migration and apoptosis-related genes, suggesting that TCS-induced progression of breast cancer may be suppressed by a phytoestrogen.

Results

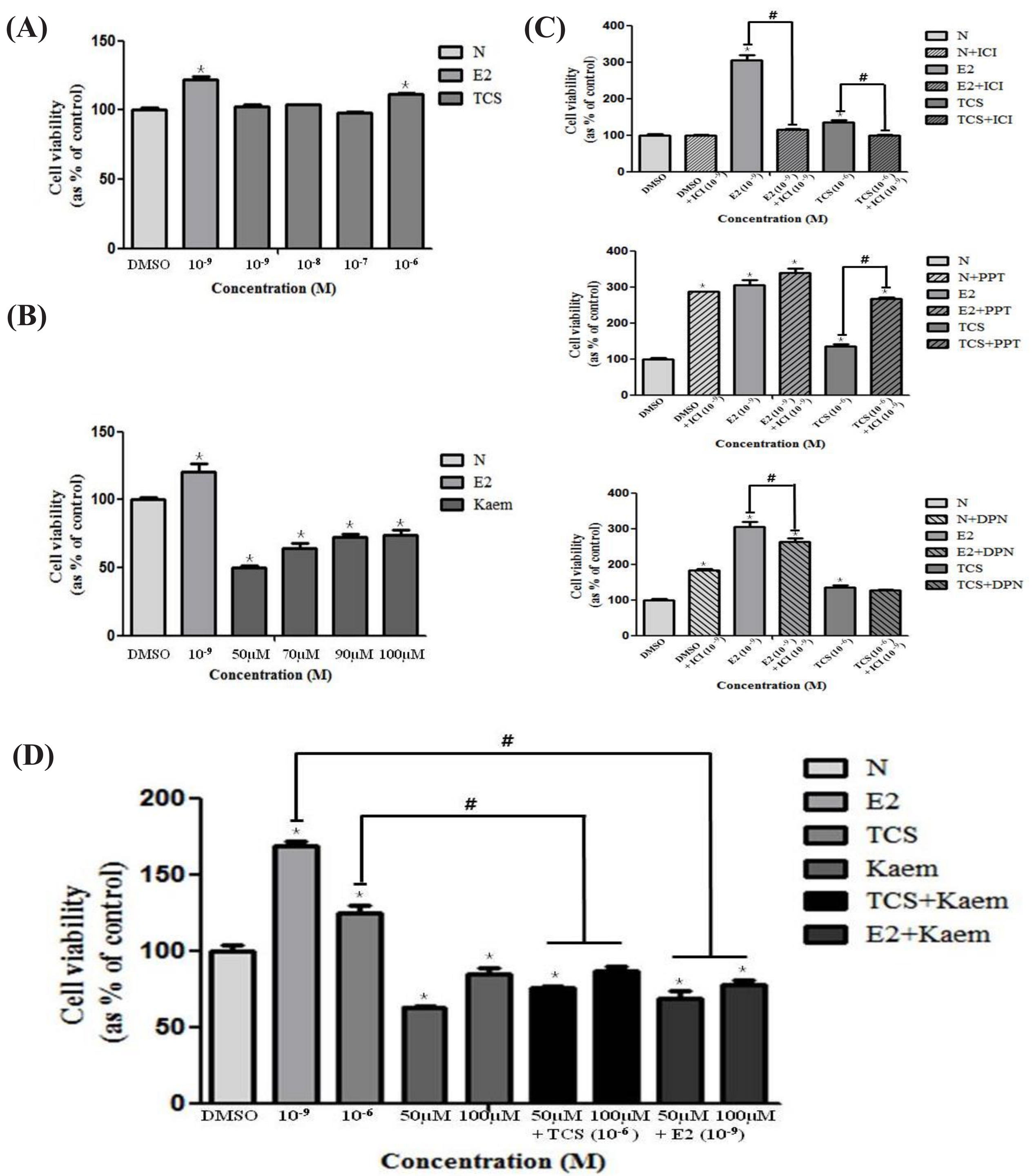
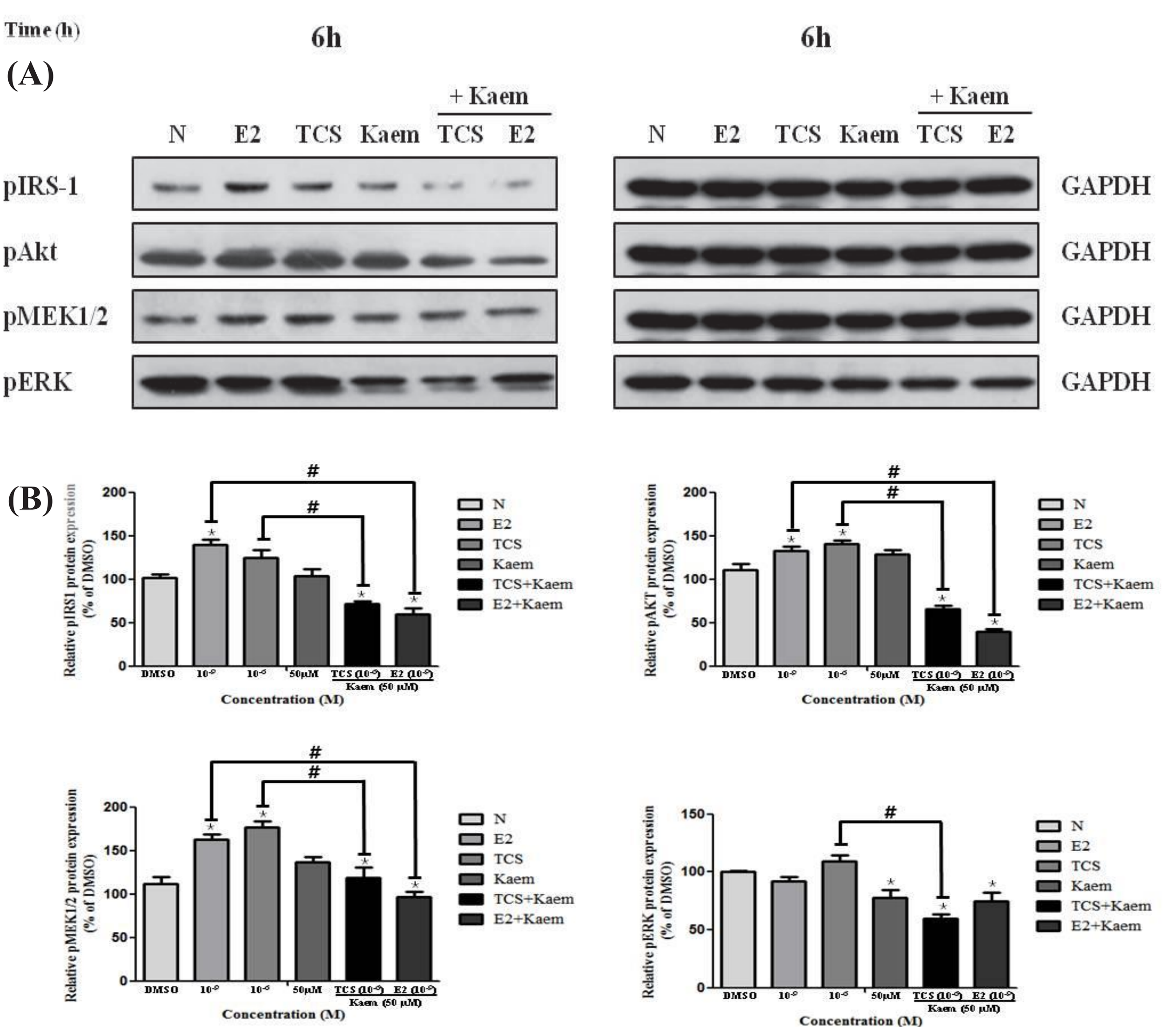


Figure 1. Viability of MCF-7 breast cancer cells following treatment with chemicals. Cells were treated with DMSO (0.1%) as a control, E2, or TCS (A) or Kaempferol (B) for 4 days. E2 and TCS were co-treatment with ER modulator, ICI 182,780, PPT, DPN (C) E2 or TCS were treated in the presence of absence of Kaem. Cell viability was then measured using an MTT assay. Data represent the mean ± SD of triplicated experiments. *Significant difference compared to the control cells ($p<0.05$ according to Dunnett's multiple comparison test)



RESULTS

Figure 2. Altered protein expression levels of IGF related genes following treatments with chemicals. DMSO (vehicle), E2 (10^{-9}M), TCS (10^{-6}M), Kaem (50μM) were treated. Total proteins were extracted after the treatment period (6h) (A) Protein bands of pIRS-1, pAkt, pMek1/2, pERK and GAPDH genes were detected by using Western blot analysis. Quantification of proteins of pIRS-1 ,pAkt, pMek1/2, pERK and GAPDH genes were conducted by scanning the densities of bands on a transfermembrane using Gel Doc 2000. Data represent the means ± S.D. of triplicate experiments. *: a significant elevation or reduction comparing with control treated with DMSO (p b 0.05 in Dunnett's multiple comparison test). #: a significant elevation or reduction expression by treatment of E2 + Kaem or TCS + Kaem comparing with treatment of E2 or TCS, respectively (p b 0.05 in Student's t-test).

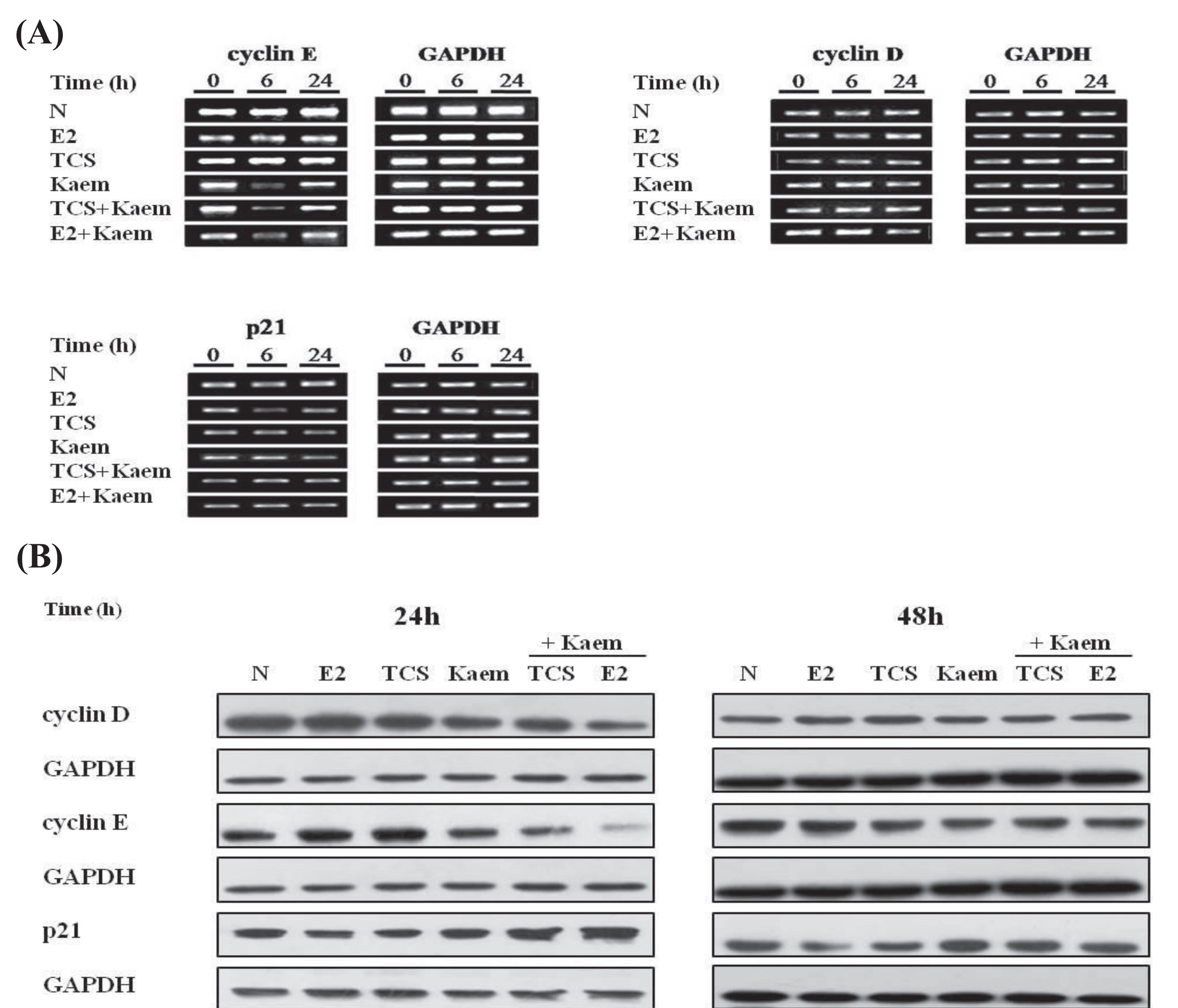


Figure 3. Altered expression levels of cell cycle related genes following treatments with chemicals. DMSO (vehicle), E2 (10^{-9}M), TCS (10^{-6}M), Kaem (50μM) were treated. Total RNA and proteins were extracted after the treatment period (A) DNA bands of cyclin D,cyclin E, p21 and GAPDH genes were detected by using Reverse transcription PCR (A).Western blot analysis were used to measure protein levels of cyclin D, cyclin E, p21 and GAPDH. Data represent the means ± S.D. of triplicate experiments.

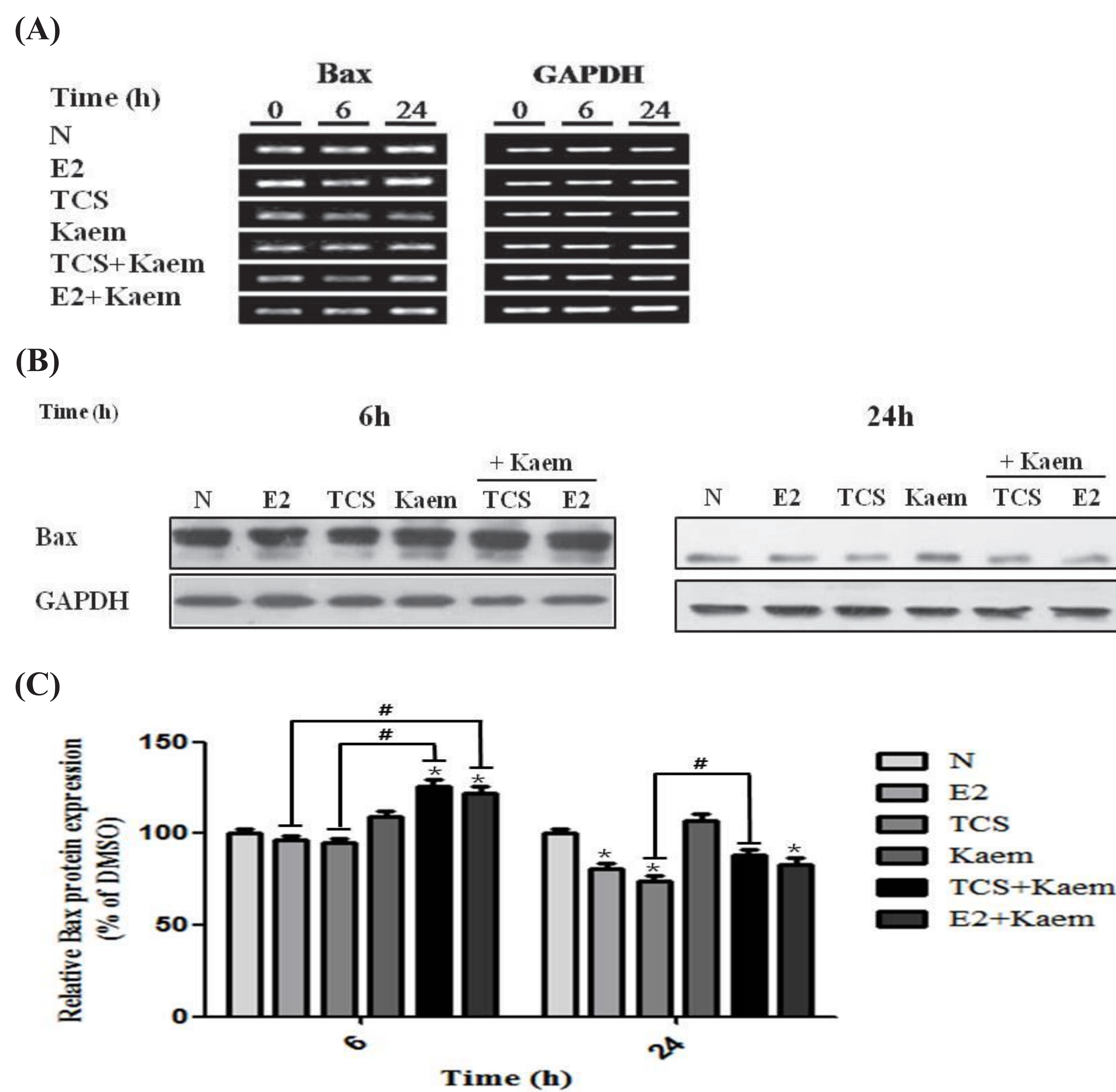
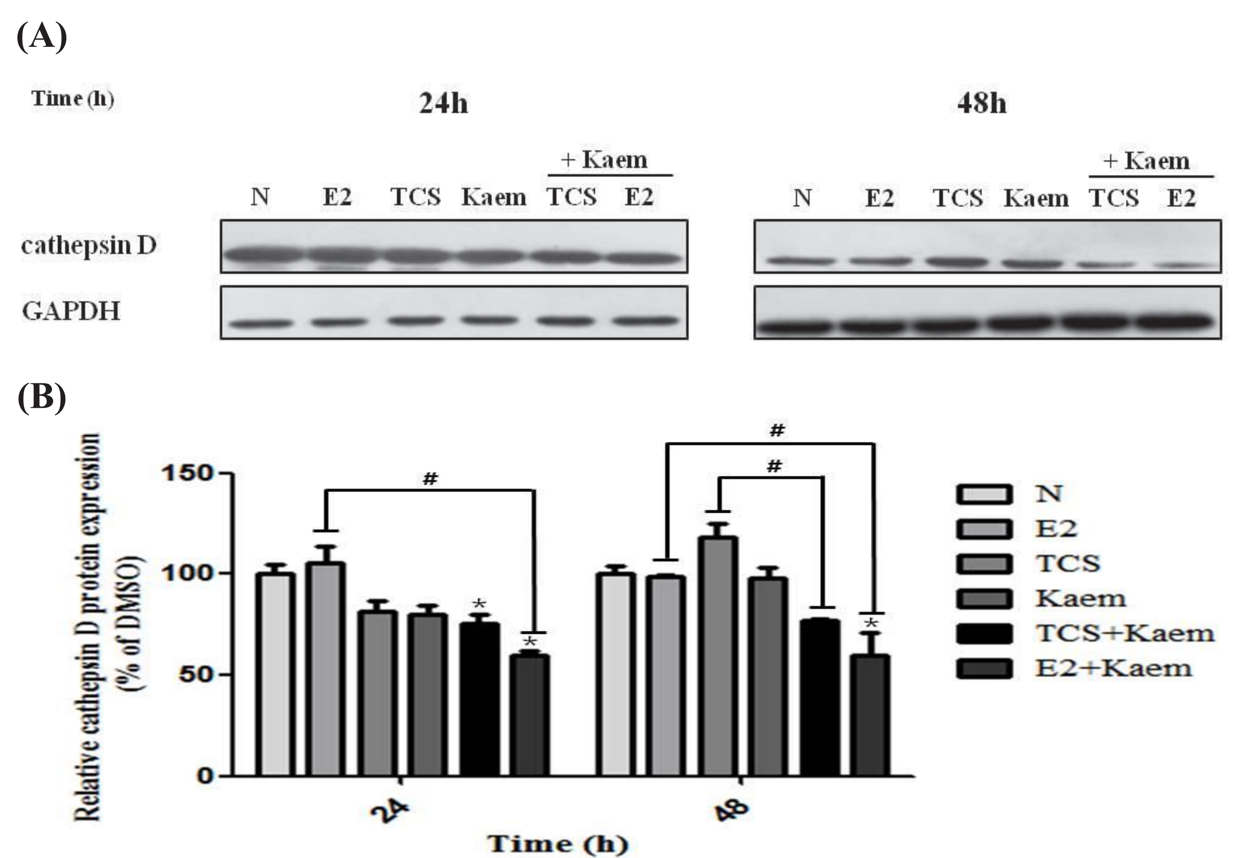


Figure 4. Altered expression levels of apoptosis related gene, Bax following treatments with chemicals. DMSO (vehicle), E2 (10^{-9}M), TCS (10^{-6}M), Kaem (50μM) were treated. Total RNA and proteins were extracted after the treatment period (A) DNA bands of Bax and GAPDH genes were detected by using Reverse transcription PCR (A).Western blot analysis were used to measure protein levels of Bax and GAPDH. Data represent themeans ± S.D. of triplicate experiments. Quantification of proteins of Bax and GAPDH genes were conducted by scanning the densities of bands on a transfermembrane using Gel Doc 2000. Data represent the means ± S.D. of triplicate experiments. *: a significant elevation or reduction comparing with control treated with DMSO (p b 0.05 in Dunnett's multiple comparison test). #: a significant elevation or reduction expression by treatment of E2 + Kaem or TCS + Kaem comparing with treatment of E2 or TCS, respectively (p b 0.05 in Student's t-test).

Figure 5. Altered expression levels of metastasis related gene, cathepsin D following treatments with chemicals. DMSO (vehicle), E2 (10^{-9}M), TCS (10^{-6}M), Kaem (50μM) were treated. Total proteins were extracted after the treatment period (A) Western blot analysis were used to measure protein levels of cathepsin D and GAPDH. Data represent the means ± S.D. of triplicate experiments. Quantification of proteins of Bax and GAPDH genes were conducted by scanning the densities of bands on a transfermembrane using Gel Doc 2000. Data represent the means ± S.D. of triplicate experiments. *: a significant elevation or reduction comparing with control treated with DMSO (p b 0.05 in Dunnett's multiple comparison test). #: a significant elevation or reduction expression by treatment of E2 + Kaem or TCS + Kaem comparing with treatment of E2 or TCS, respectively (p b 0.05 in Student's t-test).



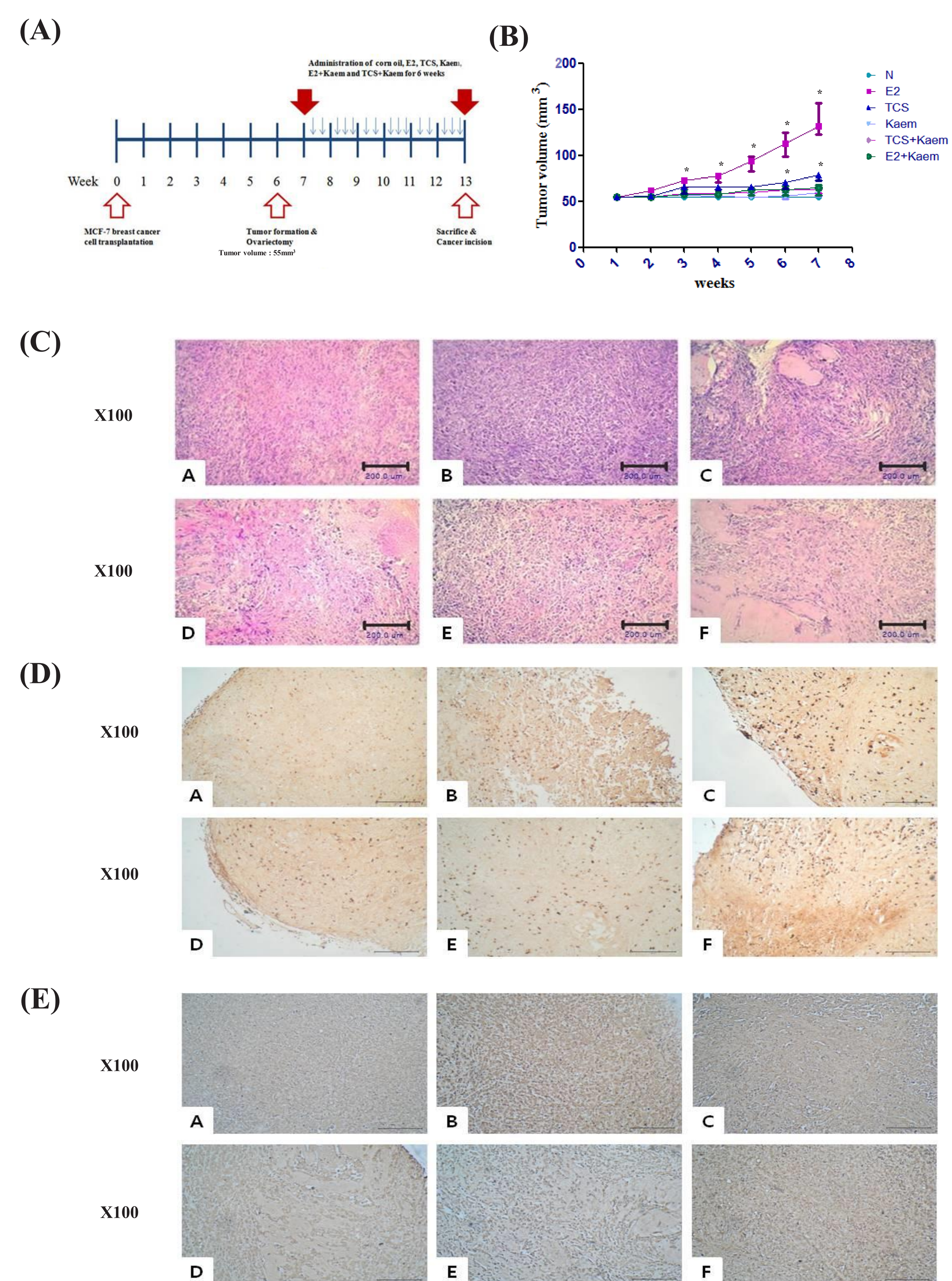


Figure 6. Effect of E2, TCS, and Kaem on cancer proliferation in animal models. (A) The schedule of the animal experiments. MCF-7 cells were transplanted to 6 week old female nude mice to manufacture breast cancer xenograft models. After the tumor formation (55 mm3 in tumor volume) the mice were ovariectomized and then administered with corn oil (vehicle), E2 (20 μg/kg b.w.), TCS (100 mg/kg b.w.), and treated in the presence of absence of Kaem (100 mg/kg b.w.) for 6 weeks.*: a significant elevation or reduction in tumor volume of BPA + GEN group comparing with BPA group (p b 0.05 in Student's t-test). (B) Tumor volume for 6 weeks. (C) Histological observation of tumor tissues by hematoxylin and eosin staining. (magnification ×100). (D) Identification of cell proliferation in tumor tissues via BrdU incorporation assay. At the end of the animal experiments, mice were injected with BrdU solution. After 2 h, the mice from each treatment group (E)The immunohistopathological images of PCNA proteins in tumor sections. At the end of the animal experiments, tumor tissues were excised from each treatment group xenografted mice after sacrifice and then embedded in paraffin. Paraffin blocks were cut into 3 μm thick sections and each section was treated for measuring the immunohistological images of PCNA proteins using each antibody and IHC staining protocol described in Materials and methods. IHC image of each protein was observed under a light microscopy (magnification ×100)

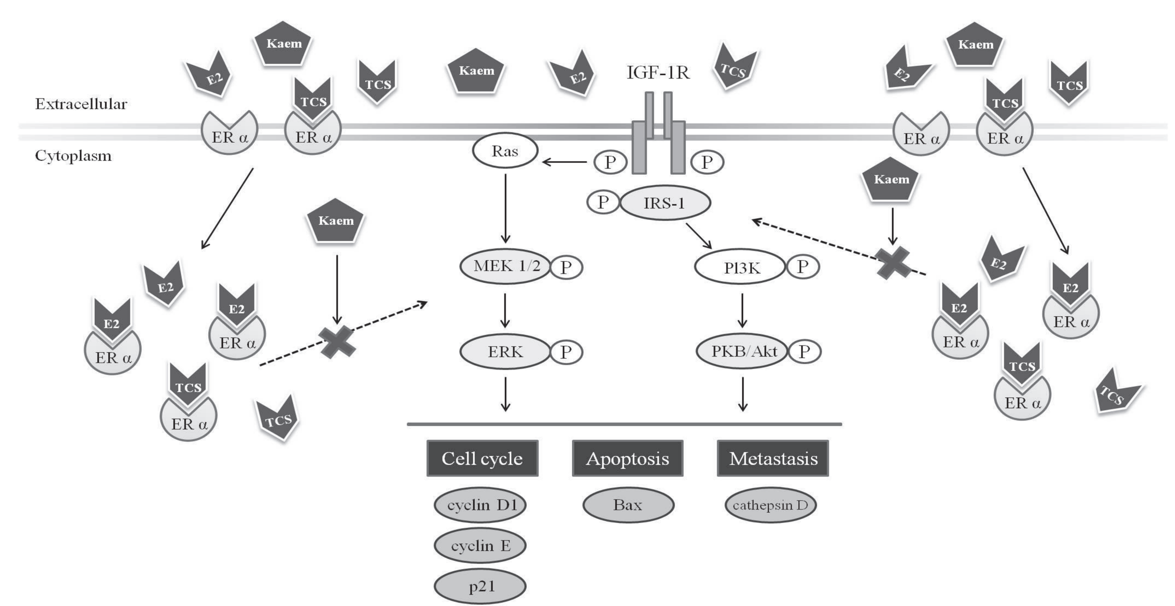


Figure 7. The anti-proliferative activity of Kaem in estrogen-dependent MCF-7 breast cancer treated with E2 or TCS. E2 or TCS has a distinct estrogenicity by upregulating the crosstalk between ERα and IGF-1R signaling pathways and MCF-7 breast cancer proliferation. On the contrary, Kaem as a phytoestrogen effectively reversed the increased cancer progression by modulating cell cycle, apoptosis and metastasis that induced by E2 or TCS.

Conclusion

In conclusion, our findings provide a more specific mechanism that TCS and E2 induce MCF-7 breast cancer proliferation through ER or IGF signaling pathways. And promote cancer progression by modulating the activities of cell cycle, apoptosis and metastasis related genes. In addition, we demonstrated that as a novel phytoestrogen, Kaem effectively act as a chemopreventive agent by suppressing carcinogenesis risks induced by TCS and E2.

Acknowledgments

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