Supplementary file 1

Enhanced efficacy of breast cancer treatment with etoposide-graphene oxide nanogels: A novel nanomedicine approach

Abbas Asoudeh-Fard[1,2#], Milad Mohkam[3#], Asghar Parsaei[4], Shadi Asghari[5], Antonio Lauto[6,7], Fatemeh Khoshnoudi[8], Mustafa Mhmood Salman Al-Mamoori[9], Mohadeseh Asoudeh-Fard[10], Hossine Ghasemi Sadabadi[11,12], Ahmad Gholami[13,14*]

[1] Institute Galilée-University Sorbonne, University Sorbonne Paris North, Paris, France

[2] Research Center for Pharmaceutical Nanotechnology, Tabriz University of Medical Sciences, Tabriz, Iran

[3] Allergy Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

[4] Niko Gene Saba Company, Rayan Novin Pajoohan Pars, Biotechnology Company, Biotechnology Incubator, Shiraz University of Medicine Sciences, Shiraz, Iran

[5] Department of Microbiology, Shiraz Branch Islamic Azad University, Shiraz, Iran

[6] School of Science, University of Western Sydney, Campbelltown, NSW, 2560, Australia

[7] School of Medicine, University of Western Sydney, Campbelltown, NSW, 2560, Australia

[8] Department of Cellular and Molecular, Zarghan Branch Islamic Azad University, Zarghan, Iran

[9] Department of Cellular and Molecular, Mashhad Branch Islamic Azad University, Mashhad, Iran

[10] Department of General Medicine, Azad Zahedan Medicine University, Zahedan, Iran

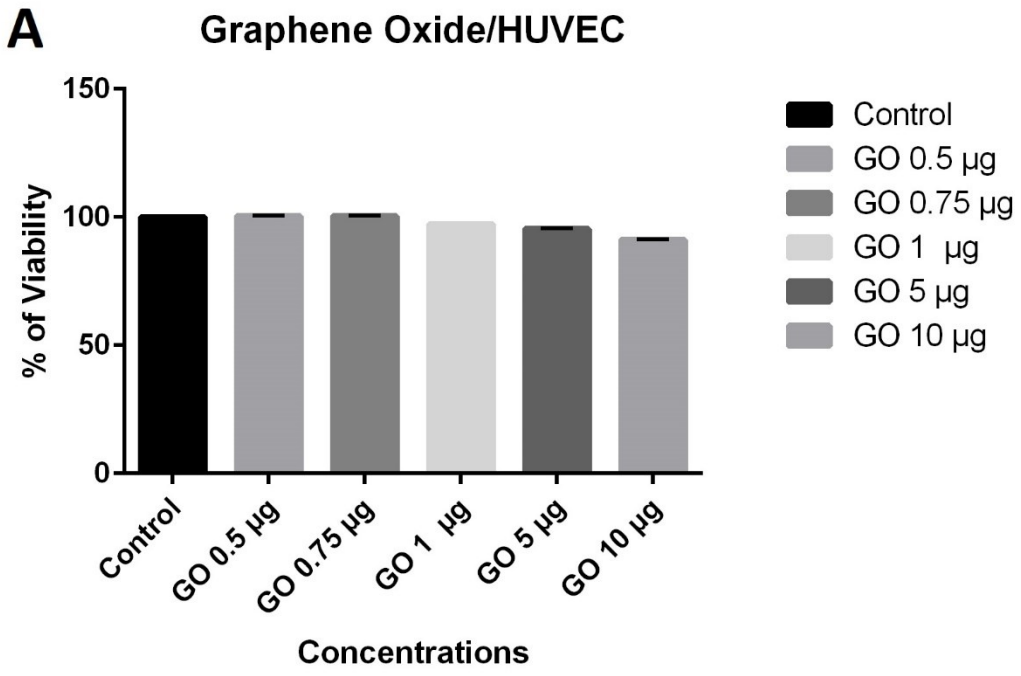
[11] Hematology and Oncology Research Center, Tabriz University of Medical Sciences Tabriz, Iran

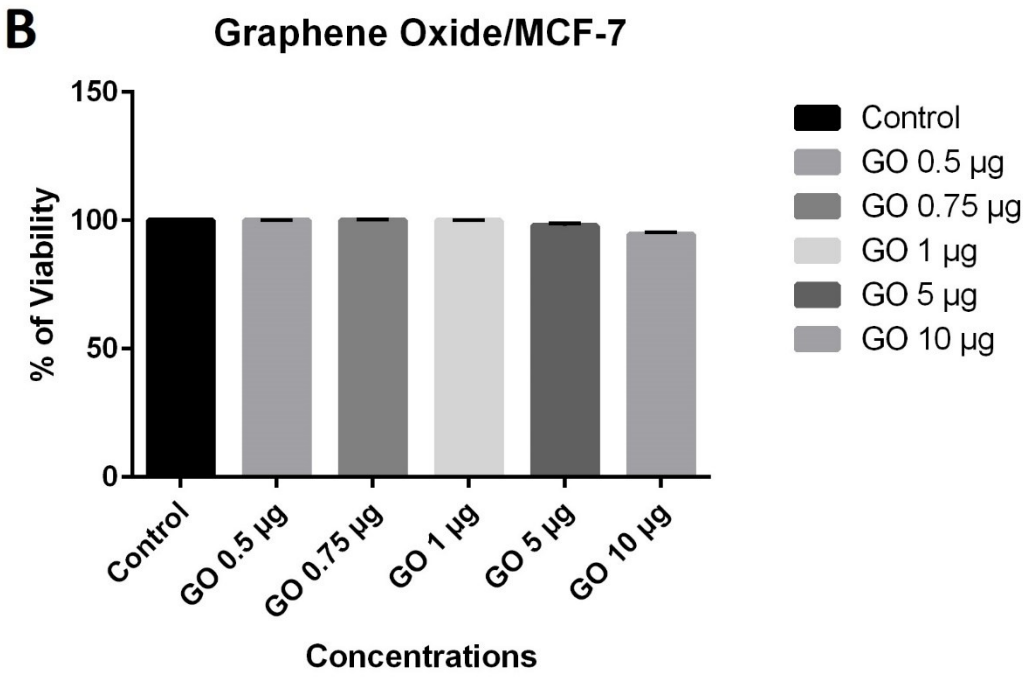
[12] Faculty of Medicine Tabriz, University of Medical Sciences Tabriz, Iran

[13] Biotechnology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

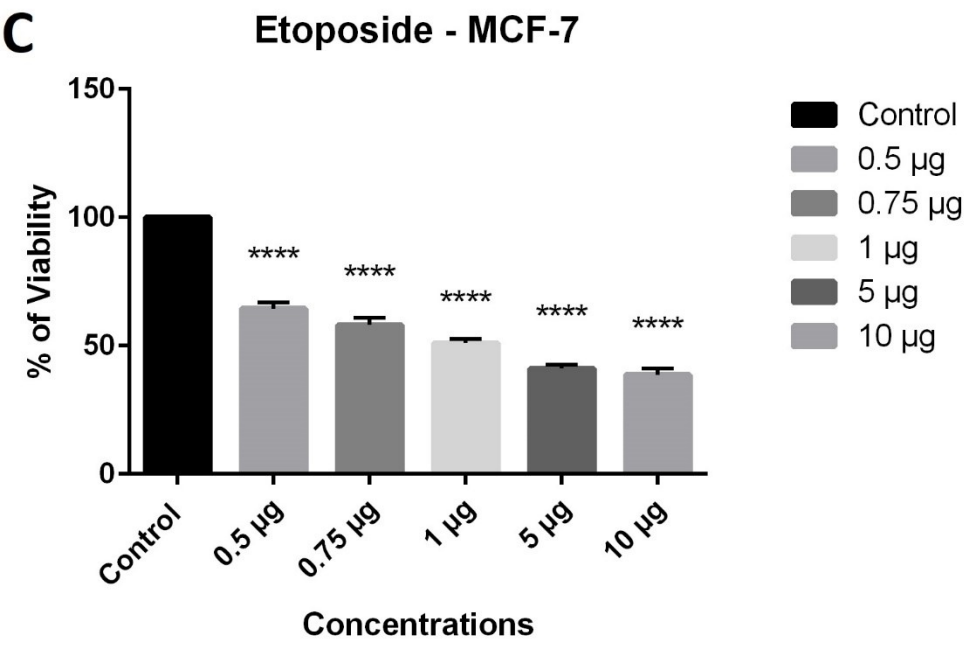
[14] Department of Pharmaceutical Biotechnology, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran

[#]These authors have contributed equally to this work.

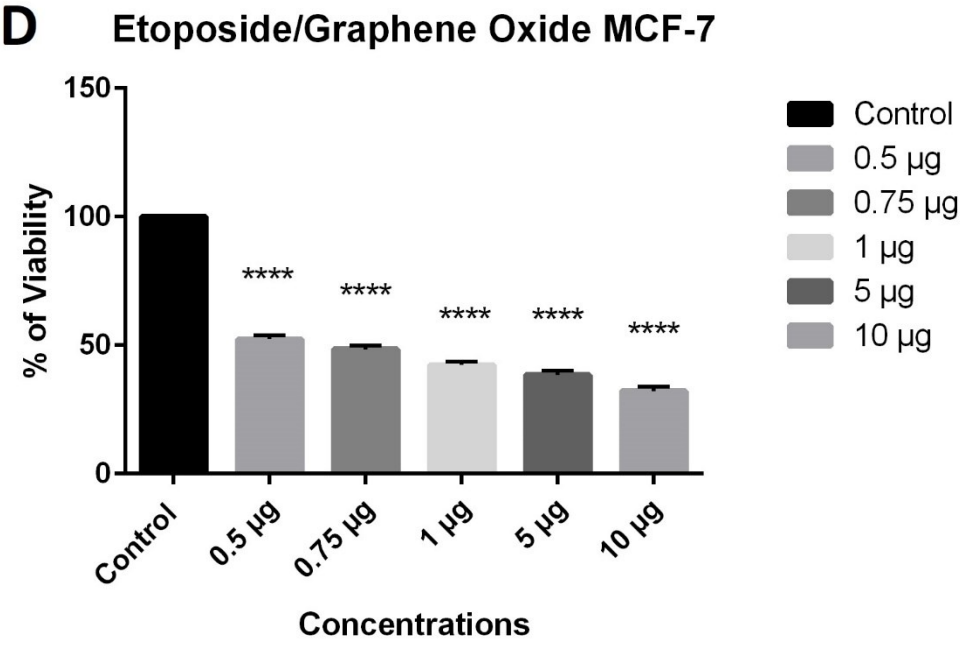

A
Graphene Oxide/HUVEC
150
100
50
0
% of Viability
Control
GO 0.5 µg
GO 0.75 µg
GO 1 µg
GO 5 µg
GO 10 µg
Concentrations


B
Graphene Oxide/MCF-7
150
100
50
0
% of Viability
Control
GO 0.5 µg
GO 0.75 µg
GO 1 µg
GO 5 µg
GO 10 µg
Concentrations

C


Etoposide - MCF-7
150
100
50
0
% of Viability
****
****
****
****
****
Control
0.5 µg
0.75 µg
1 µg
5 µg
10 µg
Control
0.5 µg
0.75 µg
1 µg
5 µg
10 µg
Concentrations

D


Etoposide/Graphene Oxide MCF-7
150
100
50
0
% of Viability
****
****
****
****
****
Control
0.5 µg
0.75 µg
1 µg
5 µg
10 µg
Control
0.5 µg
0.75 µg
1 µg
5 µg
10 µg
Concentrations

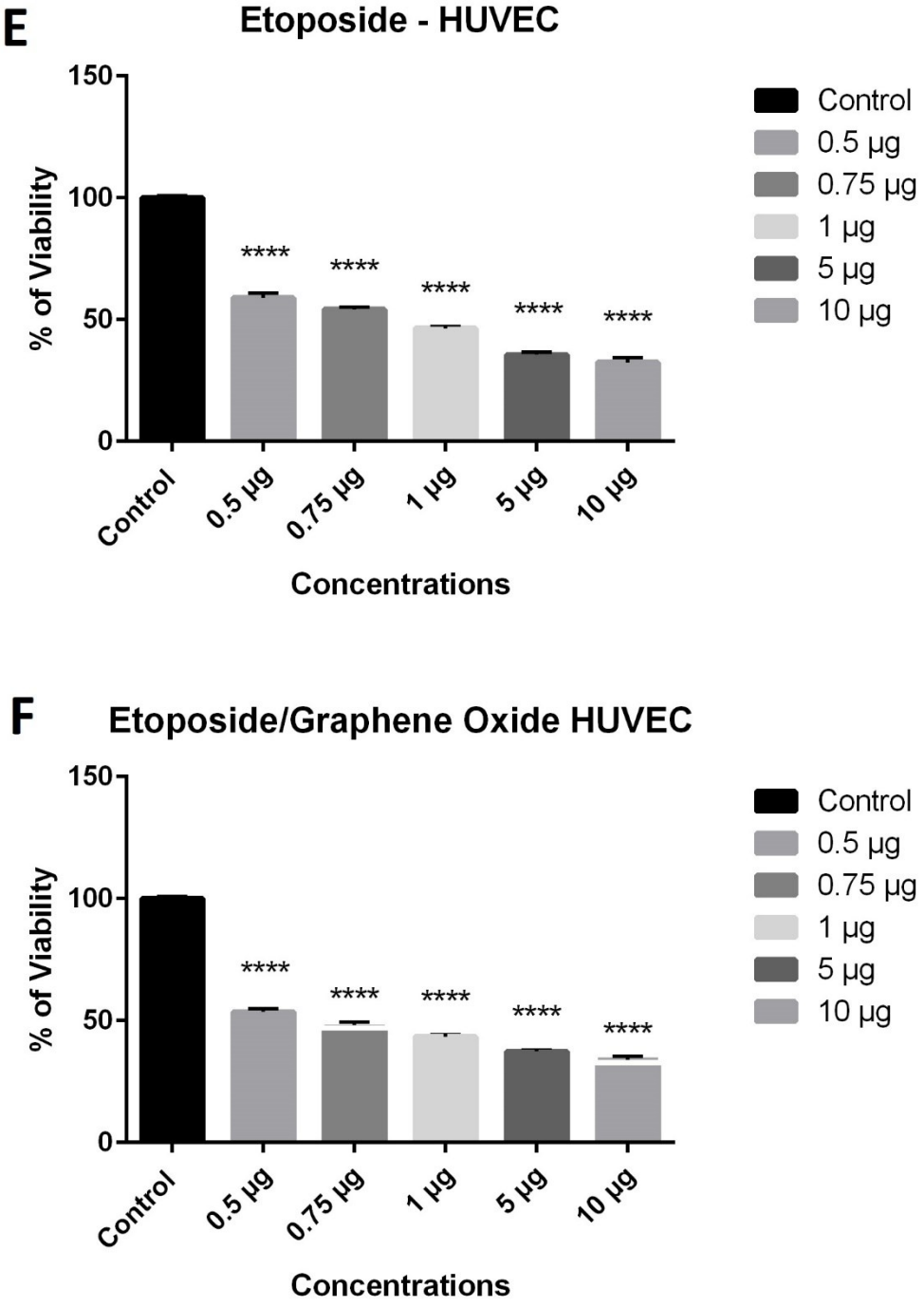


Fig. S1. MTT Assay for Cytotoxicity of Etoposide-Graphene Oxide (GO) Complex Nanogels. **A**: shows the dose-dependent cytotoxicity of ETO on HUVEC cells with viabilities at 32.50%, 35.53%, and 46.46% for 10 µg/mL, 5 µg/mL, and 1 µg/mL, respectively; **B**: illustrates similar effects for the ETO/GO complex with viabilities at 33.70% (10 µg/mL) and 37.29% (5 µg/mL); **C:** indicates minimal cytotoxicity of GO alone on HUVEC cells, maintaining viability above 90% at all concentrations. For MCF-7 cells, **D:** shows ETO's cytotoxicity with viabilities at 38.63%, 41.02%, and 51.09% at the same concentrations; **E:** reflects the impact of the ETO/GO

complex with viabilities at 32.24% (10 µg/mL) and 38.46% (5 µg/mL); **F:** demonstrates negligible effects of GO alone with cell viability exceeding 90% at all concentrations. The IC50 for ETO was approximately 2.5 µg/mL in HUVEC and 1.0 µg/mL in MCF-7 cells, suggesting higher cytotoxicity in cancerous cells, with statistical significance confirmed across treatments ($p < 0.0001$).

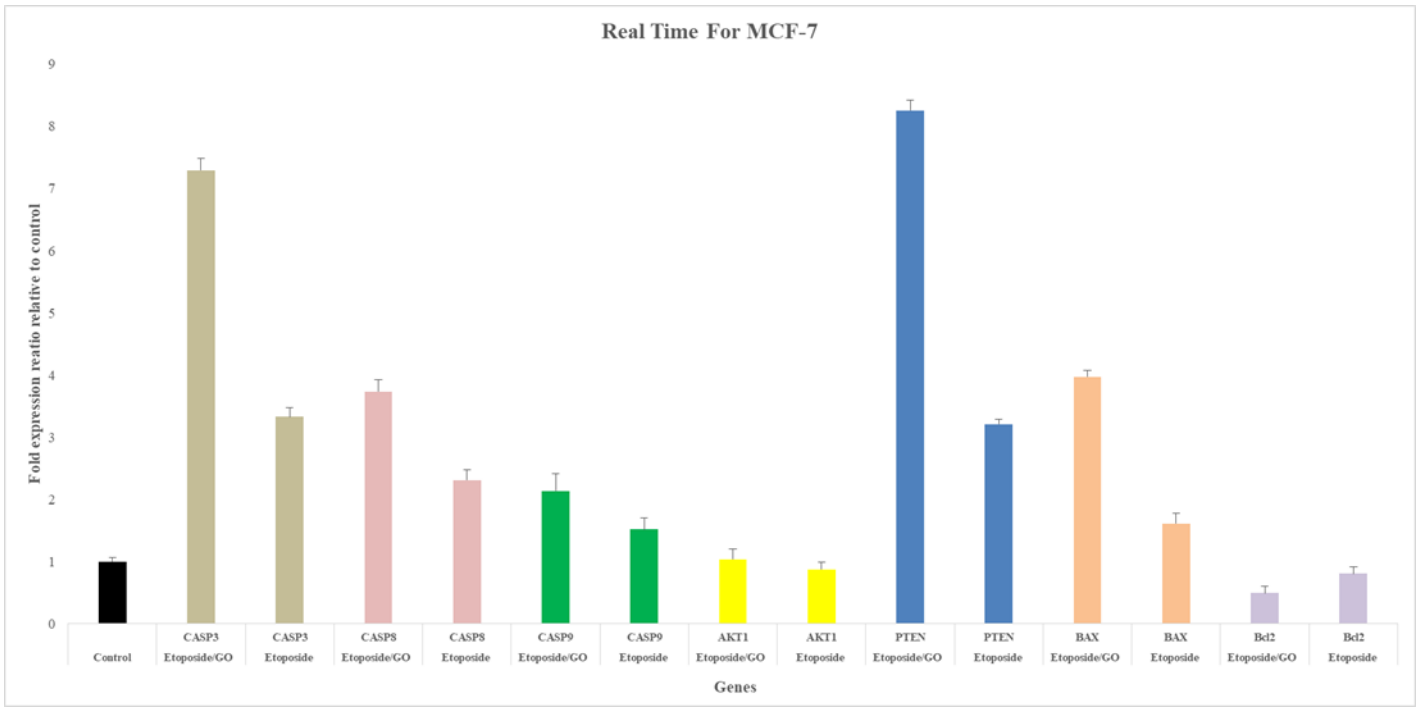


Fig. S2: Q-PCR analysis of gene expression in MCF-7 cells treated with etoposide-graphene oxide (GO) nanogels.