Supplementary File

A Cannabidiol-loaded Mg–gallate Metal-Organic Framework-based Potential Therapeutic for Glioblastomas

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KEYWORDS: Metal-organic Framework, Gallic acid, Cannabidiol, Anti-cancer, ROS-mediated Apoptosis, Anti-Inflammatory, Dual delivery

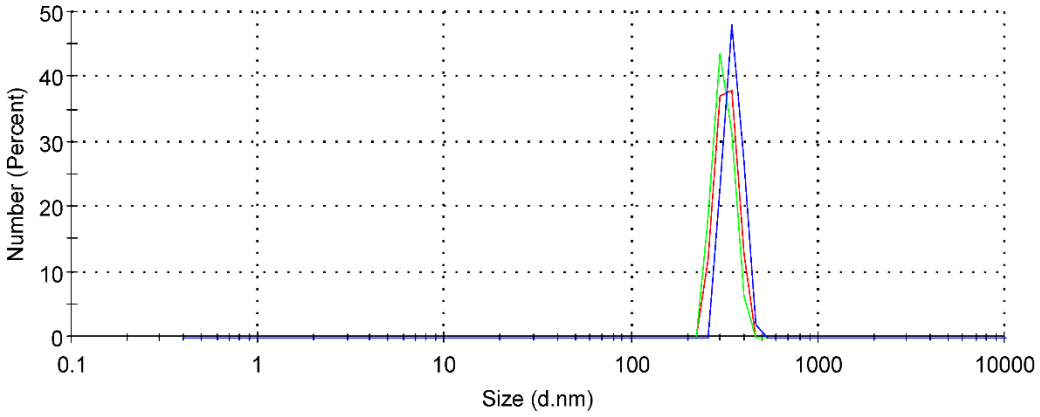


Figure S1. Number-based size distributions from dynamic light scattering (DLS) from three experiments on dilute dispersions of the Mg-GA MOF in ethanol. These measurements yield an overall mean diameter of 321 nm with a standard deviation of 41 nm. Note that the DLS analysis assumes spherical particles, which is not the case here, and these are equivalent diameters based upon diffusion coefficient in the solvent.

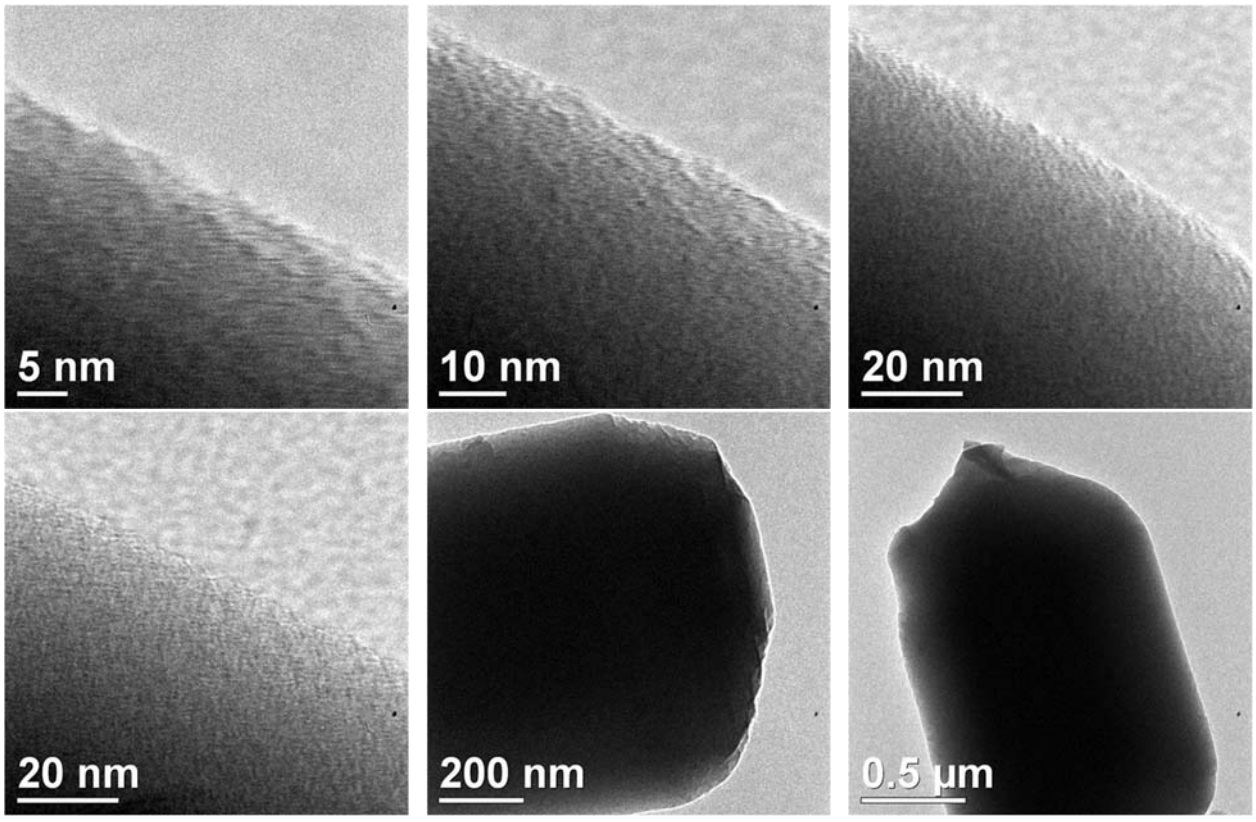


Figure S2. Representative TEM images at various magnifications illustrating the porous surface of the Mg-GA MOF visible at higher magnifications, in contrast to the smooth surface appearance at lower magnification.

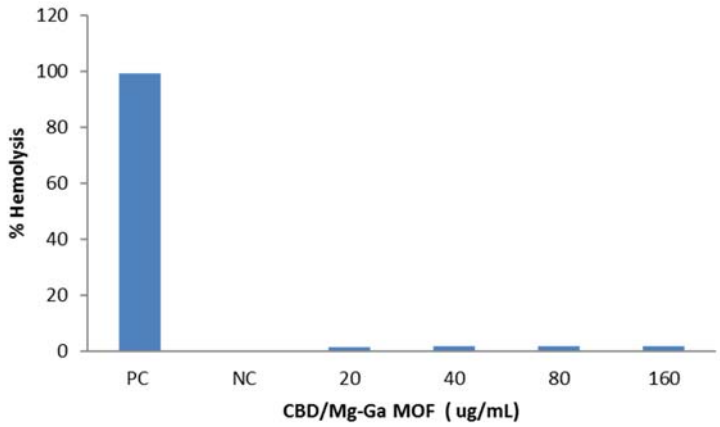


Figure S3. Hemocompatibility testing results for concentrations of CBD/Mg-GA MOF as indicated. PC is positive control (Triton-X) and NC is negative control (PBS buffer).

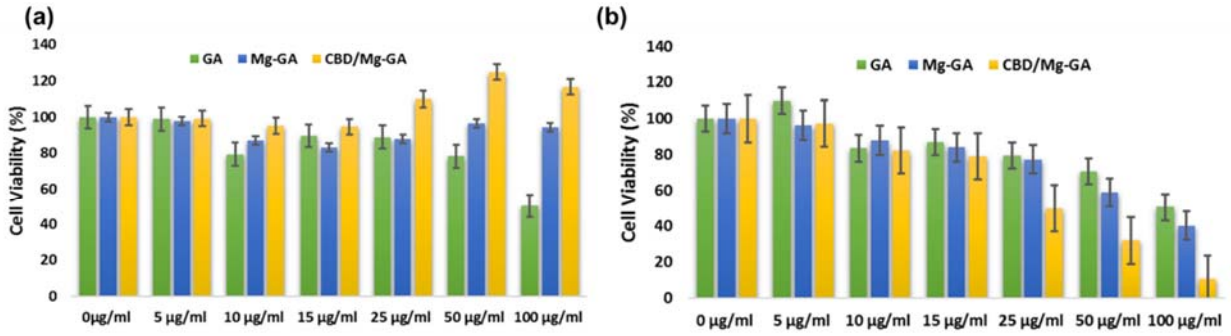


Figure S4. MTT assay showing the time and concentration-dependent cytotoxicity of GA, Mg-GA and CBD/Mg-GA MOF against Rat glioma C6 cells after (a) 24h and (b) 48 h.

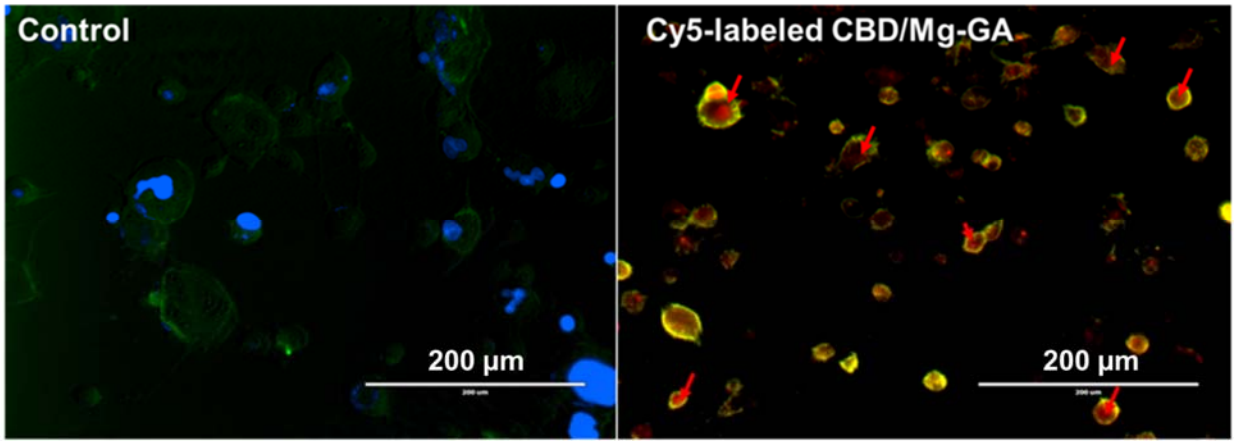


Figure S5. Cellular uptake of Cy5-labelled CBD/Mg-GA MOF. The control cell image shows cells treated with the CBD/Mg-GA MOF without the labeling, but stained with the membrane stain WGA (green) and nuclear staining with DAPI (blue). The Cy5-labelled CBD/Mg-GA MOF treated cells shows significant uptake. Red arrows indicate the uptake of red fluorescent Cy5-labelled CBD/Mg-GA particles and greenish yellow is the WGA membrane stain.

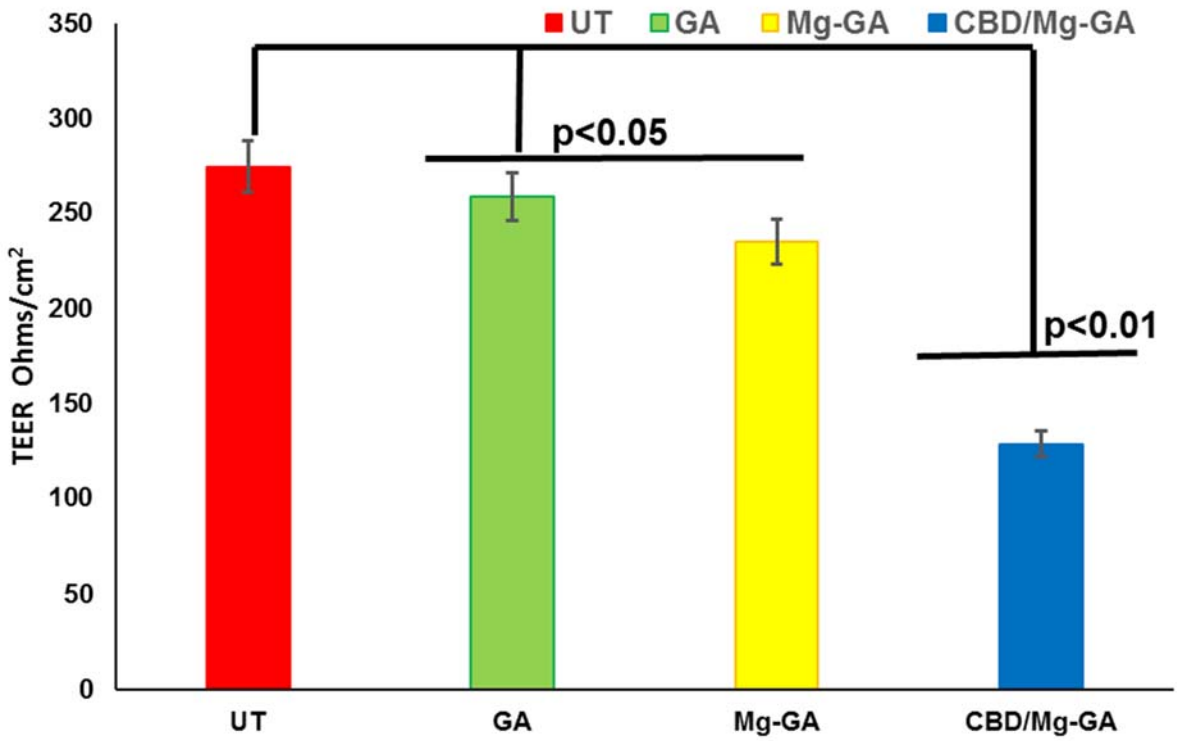
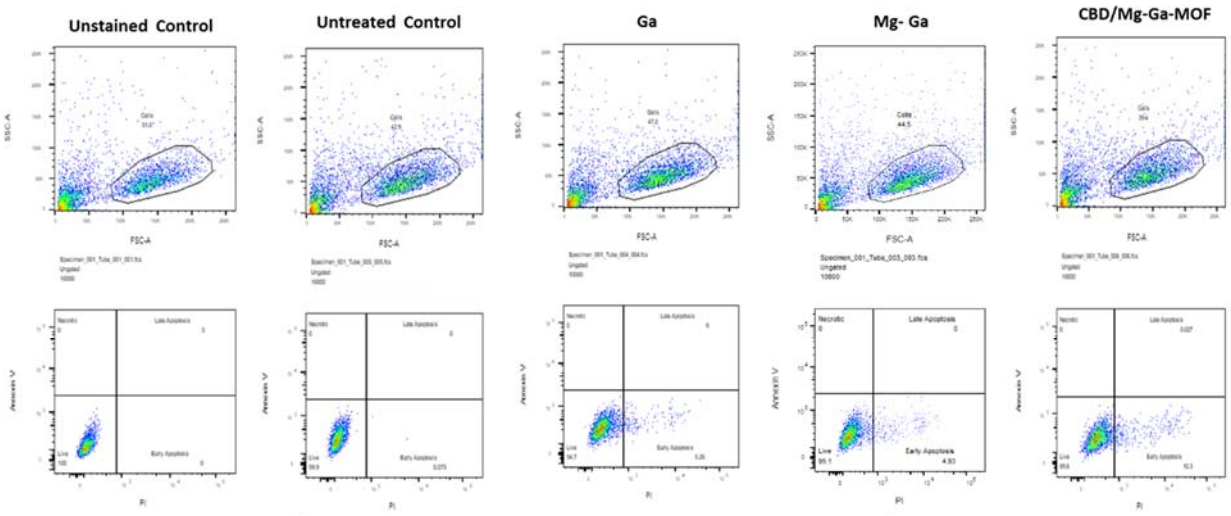


Figure S6. Effect of CBD/Mg-GA MOF on BBB permeability by measuring the TEER across the *in-vitro* BBB model.



Annexin- V staining	% Cells	% Cells	% Cells	% Cells
Sample:	Early Apoptosis	Late Apoptosis	Live	Necrotic
Unstained Control	0	0	100	0
Untreated Control	0.075	0	99.9	0
Ga	4.93	0	95.1	0
Mg-Ga	5.26	0	94.7	0
CBD/Mg-Ga- MOF	10.3	0.027	89.6	0

Figure S7. Flow Cytometric analysis showing effect of CBD/Mg-GA MOF does induce early state apoptosis and no cells were undergoing necrosis. Statistical analysis was done using data from *n*=3 independent experiments. A p-value of 0.05 or less between groups was considered significant.

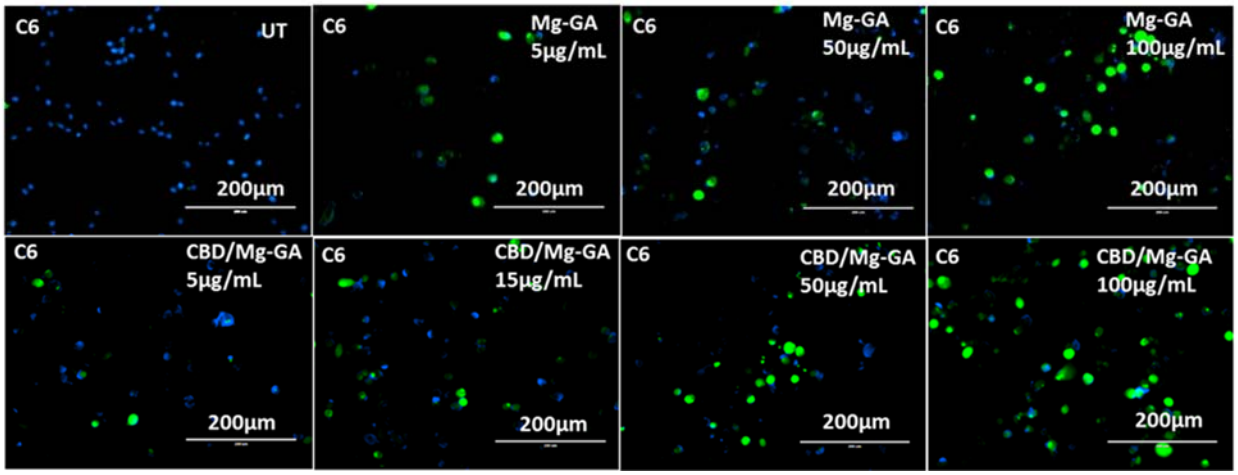


Figure S8. Determination of cellular ROS generation by fluorescence microscopic imaging of treated cells and spectrophotometric fluorescence intensity measurement which indicates the enhancement of ROS in cells treated with different concentrations of Mg-GA and CBD/Mg-GA MOF (0, 5, 15, 50, & 100 µg/mL as labelled). Blue fluorescence is form DAPI staining of the cell nuclei; green fluorescence is from ROS-sensitive dye carboxy-H2DCFDA.

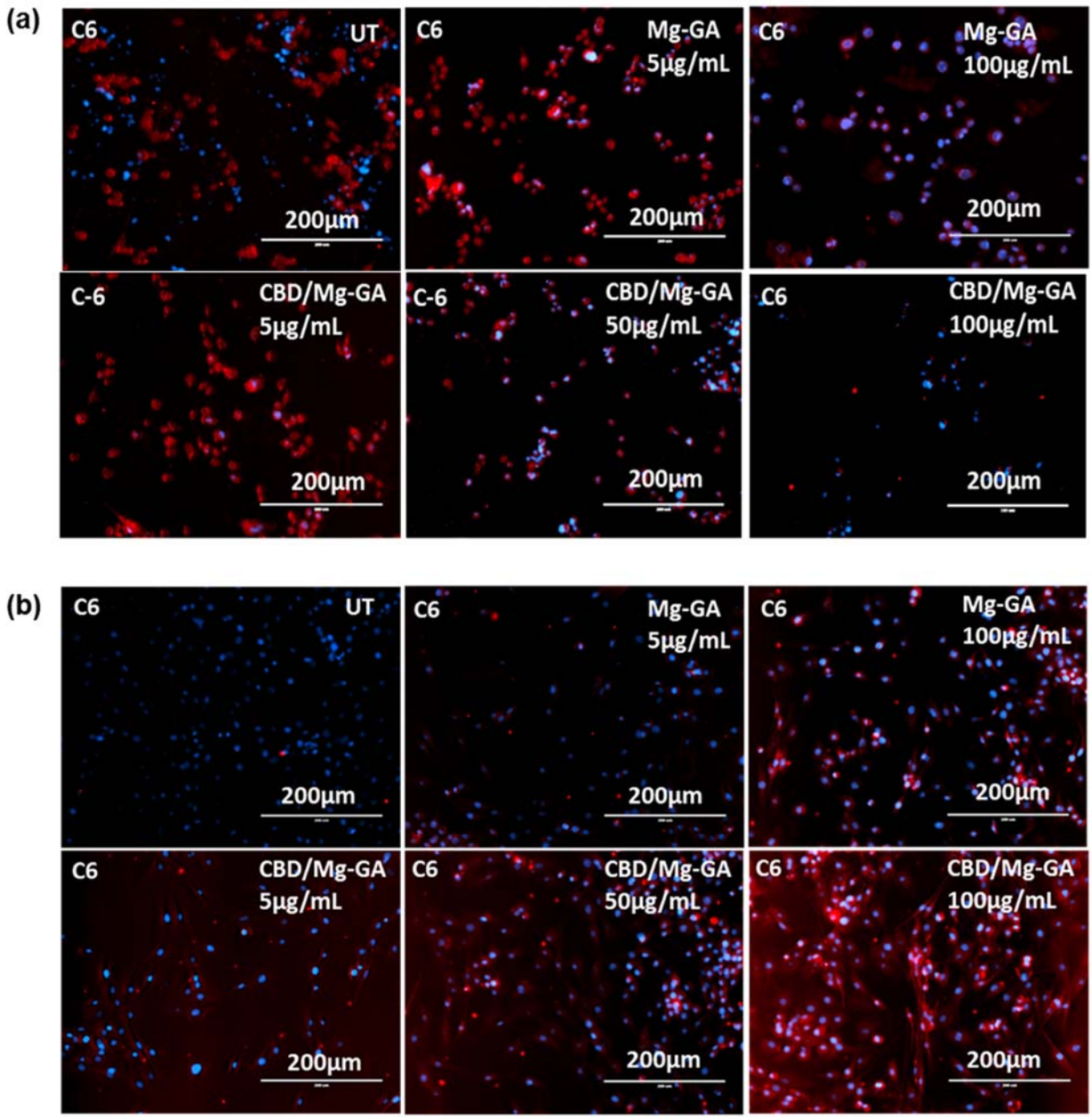


Figure S9. Determination of (a) TNF-α, (b) NF-κB expressions by fluorescence microscopic imaging of treated cells and spectrophotometric fluorescence intensity measurement which indicates the decrement of TNF-α and enhancement of NF-κB expressions in cells treated with different concentrations of Mg-GA and CBD/Mg-GA MOF (0, 5, 50, & 100 µg/mL as labelled).

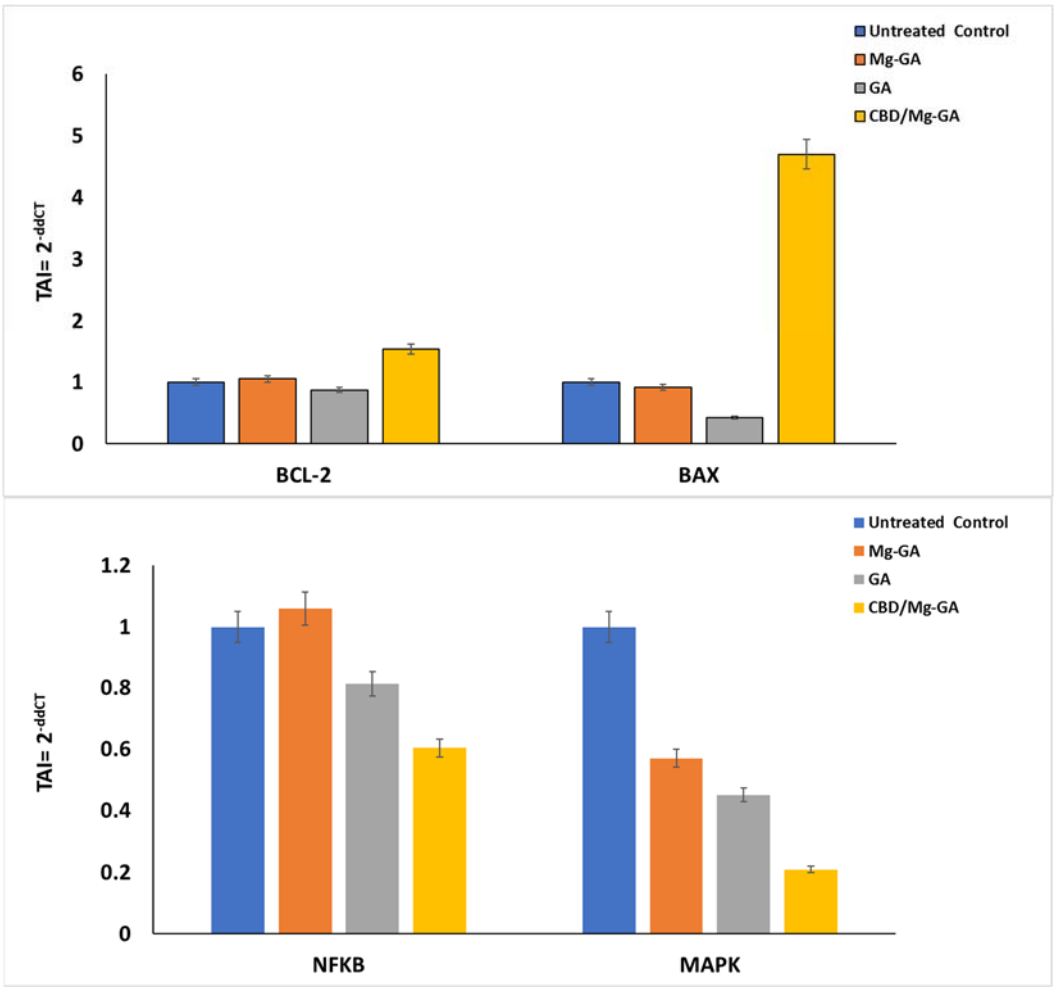


Figure S10. Effect of the CBD/Mg-GA MOF on CBD/Mg-GA MOF nanoformulation significantly increases both BCL-2 and BAX gene expression and decreasing NF-kB and MAPK expressions, indicating that CBD/Mg-GA MOF has potential anti-cancer effects by increasing cell death. Results are expressed as the mean ± SD of 3 separate experiments. A p-value of 0.05 or less between groups was considered significant.