

## Supplementary Information

Fabrication of Methotrexate Conjugated Multi-Walled Carbon Nanotubes for the Evaluation of Cytotoxic Potential at Biochemical and Molecular Level Modulating *BAX*, *BCL-2* and Telomerase Expression

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**Table S1: Quantification of vascular parameters of CAM assay**

Concentration	Time	Vessel length (mm)	Branch Points	Junctions	Vessel Thickness (mm)
Vehicle Control	0 hour	42.3135±0.4055	68±0.5	40±1	1.809±0.575
	48 hours	77.6675±2.3225	114±5.5	71±3	2.737±0.196
10 µg/mL	0 hour	48.2105±2.8325	60±4	26.5±1.5	1.843±0.077
	48 hours	16.9245±0.7115	27±2	18±1	0.61±0.12

**Table S2: Tabular representation of sequences of gene-specific forward and reverse primers.**

Genes	Primer Sequence
<i>18S</i>	FP: GTAACCCGTTGAACCCATT
	RP: CCATCCAATCGGTAGTAGCG
<i>BAX</i>	FP: AGTAACATGGAGCTGCAGAG
	RP: AGTAGAAAAGGGCGACAACC
<i>BCL-2</i>	FP: GGGTATGAAGGACCTGTATTGG
	RP: CATGCTGATGTCTCTGGAATCT

## **Figure Legends**

Figure S1- Dispersion and solubility analysis of r-MWCNTs, p-MWCNTs and c MWCNTs in aqueous medium to validate the carboxylation.

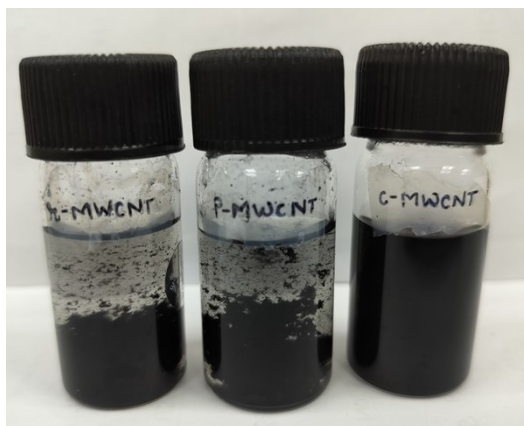
Figure S2- TEM images representing 2D morphology of p-MWCNT and MTX-MWCNT.

Figure S3- Cell viability (MTT) assay to determine the cytotoxicity of synthesized nanoconjugate in non-cancerous (HEK 293T) cells.

Figure S4- Standard curves for TAS and sandwich ELISA-based quantification of protein levels.

**Figure S1**

**A**



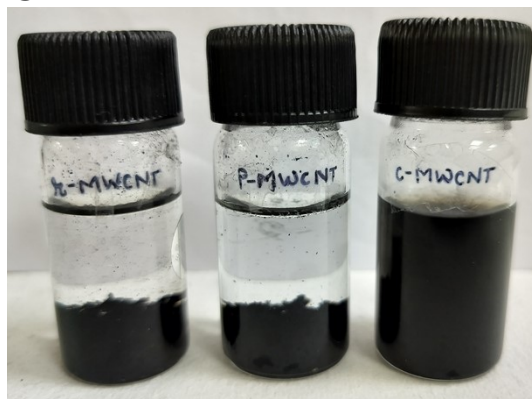
**0 hour**

**B**



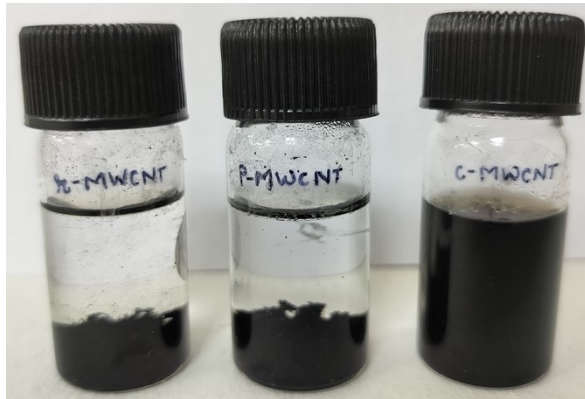
**1 day**

**C**



**7 days**

**D**

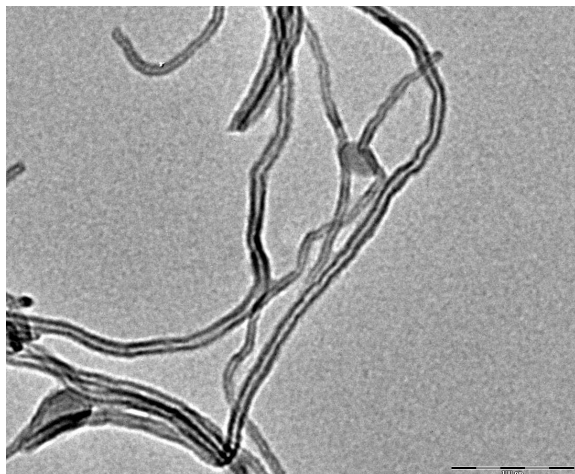


**30 days**

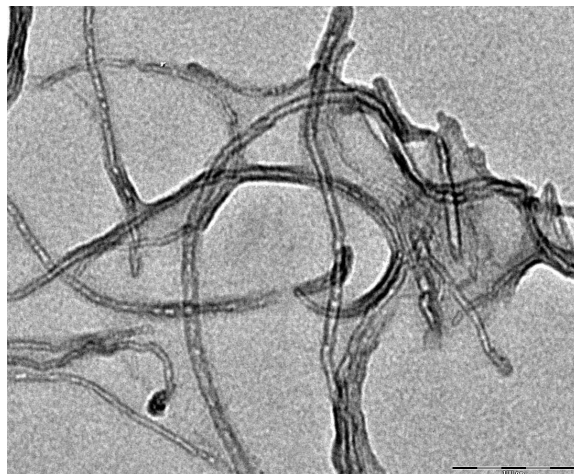
**Figure S1:** Dispersion and solubility analysis of r-MWCNTs, p-MWCNTs and c-MWCNTs in aqueous medium to validate the carboxylation. Visual observations were captured at **(A)** 0 hour, **(B)** 1 day, **(C)** 7 days and **(D)** 30 days to assess dispersion stability over time. The enhanced dispersion stability of c-MWCNTs compared to r-MWCNTs and p-MWCNTs confirms successful surface oxidation and introduction of carboxyl groups required for further conjugation.

**Figure S2**

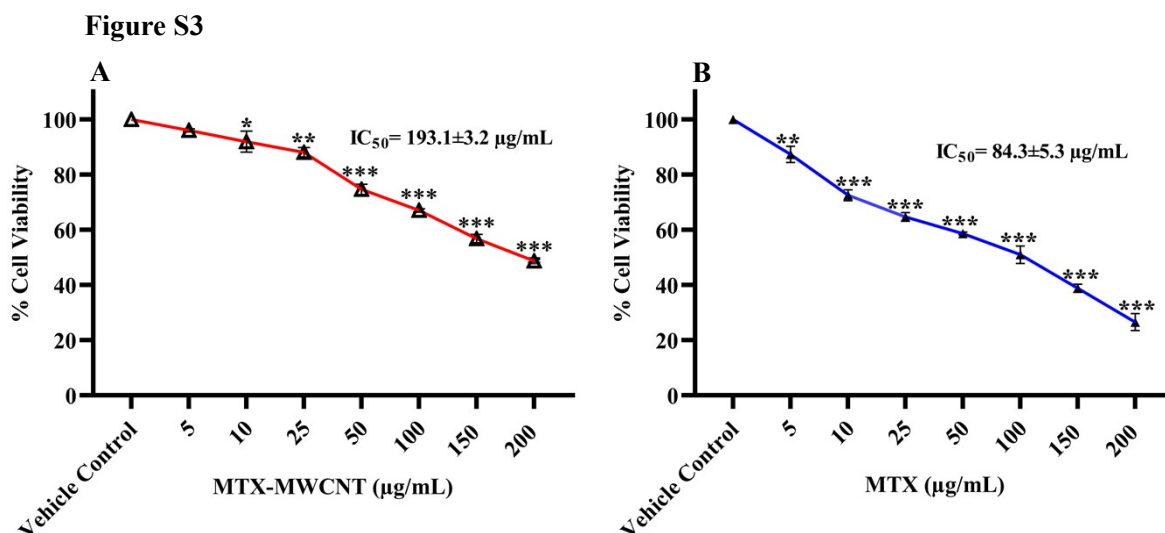
**A**



**B**

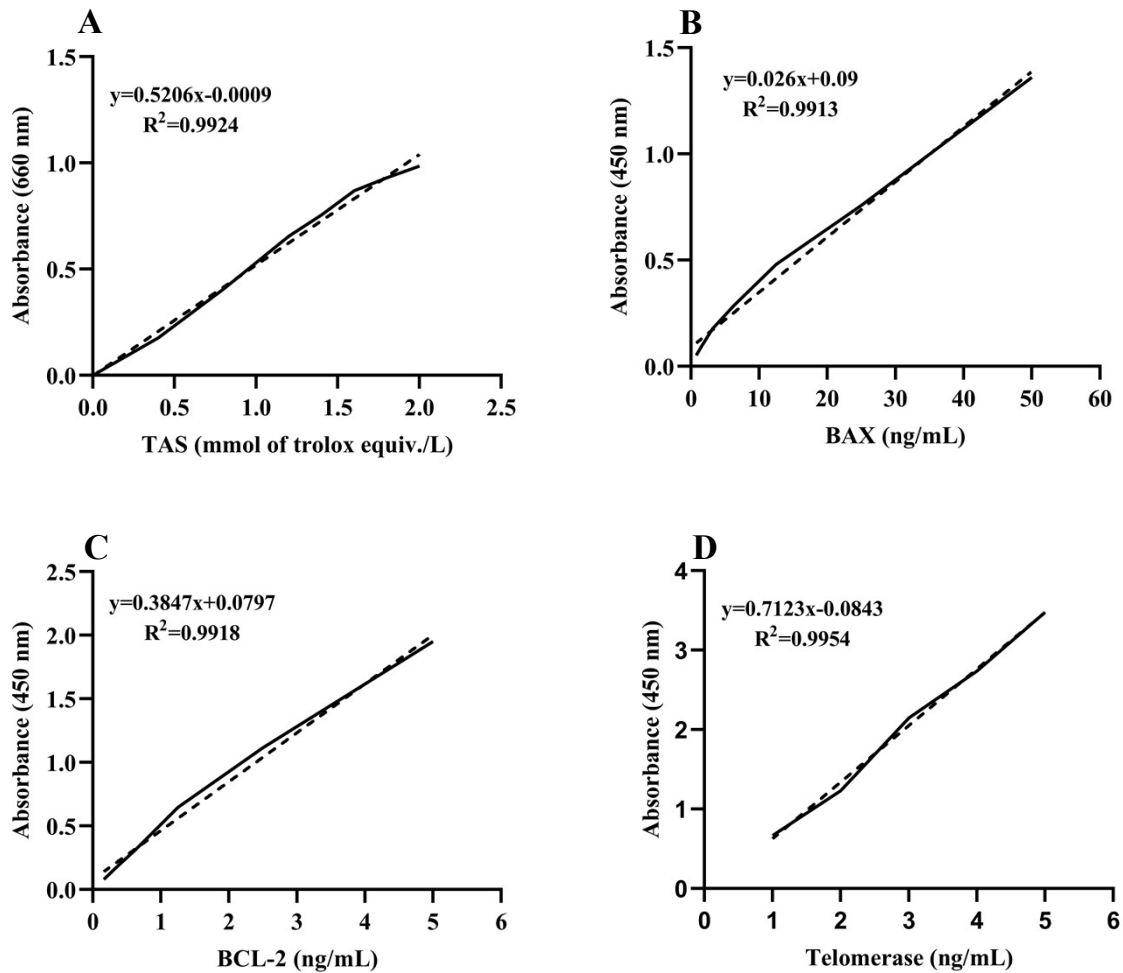


**Figure S2:** TEM images representing 2D morphology of p-MWCNT and MTX-MWCNT. p-MWCNT (**A**) show clear concentric graphene walls with hollow lumen while (**B**) MTX-MWCNT display roughened wall morphology consistent with drug attachment. Scale Bar- 100 nm



**Figure S3:** Cell viability (MTT) assay to determine the cytotoxicity of the synthesized nanoconjugate in non-cancerous (HEK 293T) cells. The % cell viability of **(A)** MTX-MWCNT and **(B)** MTX reveal that the synthesized nanoconjugate exhibited a significantly higher IC<sub>50</sub> value in HEK 293T cells compared to cancer cells, indicating lower cytotoxicity and good biocompatibility toward normal cells. Data are presented as mean ± SEM of experiment performed in triplicates, and statistical significance was determined by comparing the mean of treated groups with that of the vehicle control using one-way ANOVA followed by Dunnett's multiple comparison test (\*p<0.05, \*\*p< 0.01 and \*\*\*p<0.001)

**Figure S4**



**Figure S4:** Standard curves for TAS and sandwich ELISA-based quantification of protein levels. The standard curve for (A) TAS was generated by plotting standard curve at 660 nm while for (B) BAX, (C) BCL-2, and (D) telomerase, the standard curve was generated by plotting absorbance at 450 nm (Y-axis) against known concentrations of respective standards (X-axis). Each data point represents the mean  $\pm$  SEM of triplicate measurements, and linear regression analysis ( $Y = mX + c$ ) was used to obtain the best-fit line and coefficient of determination ( $R^2$ ) values which indicates significant linearity of the generated curves.