Supporting Information

Quercetin Loaded Nanoarchaeosomes for Breast Cancer Therapeutics: An ROS Mediated Cell Death Mechanism

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Zeta Potential of NAQ

We have evaluated the surface charge of the quercetin loaded Nanoarchaeosomes (NAQ) through zeta potential analysis. The zeta potential for NA, quercetin, and NAQ were found to be -55 ± 1 mV, +4.66 mV, and -42.1 mV (Fig. S1).

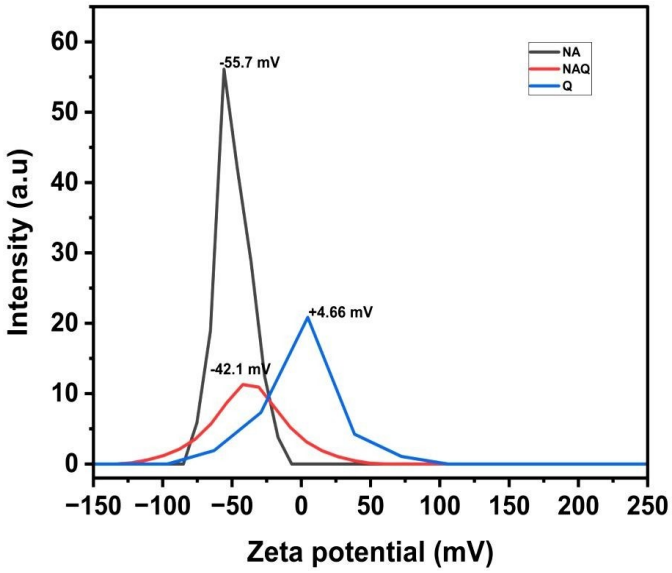


Fig. S1 Characterization of NAQ: Zeta potential of NA, NAQ and Quercetin (Q).

Atomic Force Microscopic (AFM) images of NA and NAQ

Atomic Force Microscopy is a powerful technique to evaluate the shape, structure, surface morphology, and size measurements of drug delivery systems. Hence, in our study, we have performed the structural characterization of NA and NAQ using AFM (Bruker, OTESPA-R3). For this study, NA and NAQ suspension was drop-casted on a precleaned glass slide (1×2 cm) and air-dried overnight. Then, dried samples were scanned by an Atomic Force Microscope (AFM) in tapping mode. Finally, images were analyzed using Nano analyzer 3.0 software. The results showed that NA (1mg/ml) and NAQ (100 µM) possess spherical shapes with height distributions of 86.2 nm and 56.8 nm, respectively (Fig. S2).

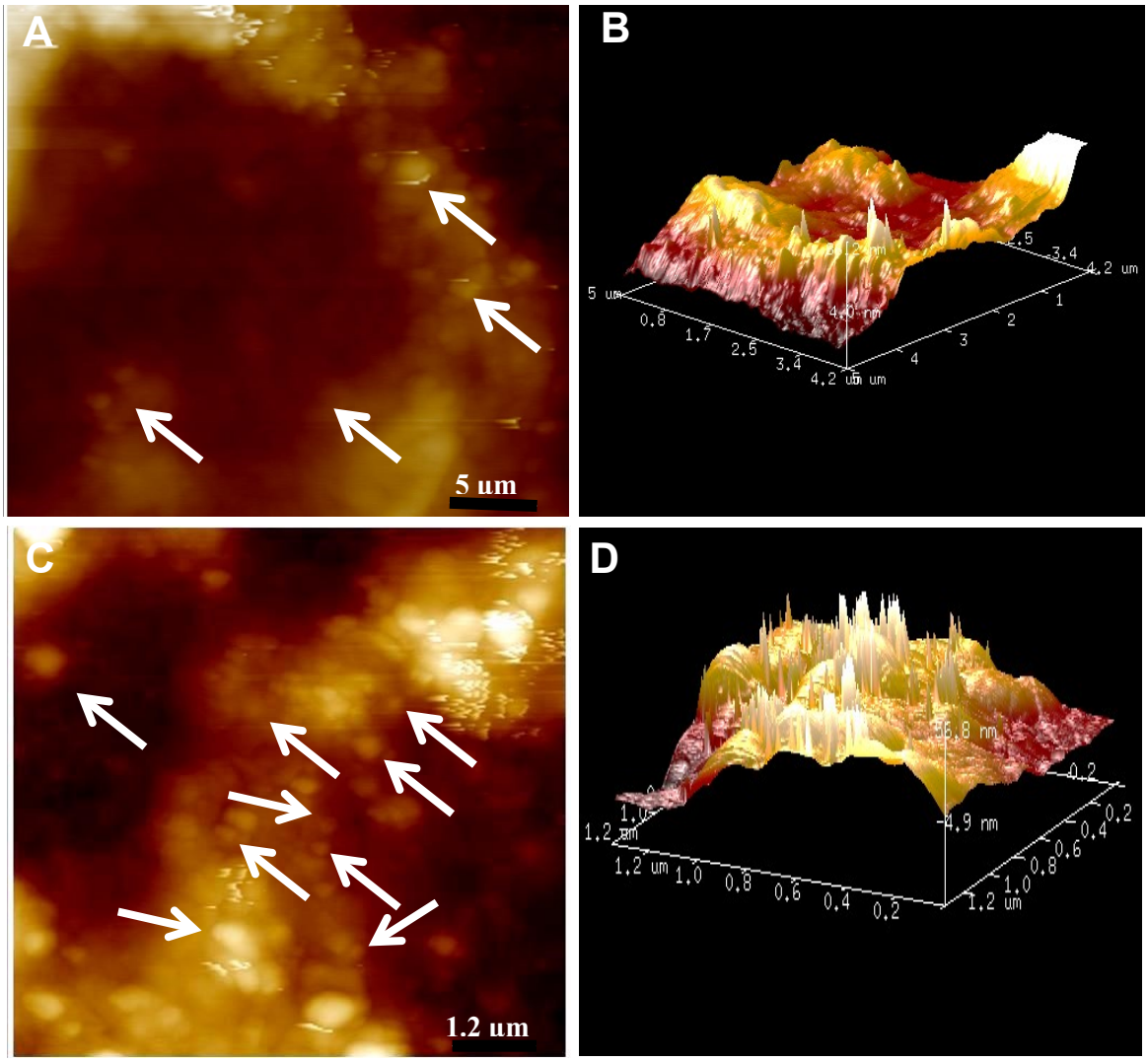


Fig. S2 AFM images of **A**) NA and **B)** 3D image of NA **C)** AFM illustration of NAQ **D)** 3D version of NAQ. (White arrow shows NA and NAQ).

Raman Spectroscopy analysis of NA and NAQ

We have analyzed the physical characterization of NA and NAQ using Raman Spectroscopy (WITec apha 300R). For this study, NA (1mg/ml) and NAQ (100 µM) suspension was placed on a sterile glass slide and allowed to air dry for 3 hours at room temperature. Then, the Raman spectrum of NA and NAQ was collected in the range fom100 cm^{-1} to 3500 cm^{-1}. The Raman spectra of NA were observed at 567cm^{-1}, 1098cm^{-1}, and 2897$cm^{-1,}$ respectively (Fig. S3A). The absorption at 1098cm^{-1} attributes C-C stretching, and 2894cm^{-1} corresponds to anti-symmetric CH_2 stretching vibration. Next, Raman spectra of NAQ were detected at 567cm^{-1}, 1098cm^{-1}, 1454 $cm^{-1,}$ and 2897$cm^{-1,}$ respectively, with considerable changes in Raman intensities of NA. In the Raman spectrum of NAQ, at 1454 cm^{-1} attributes OH phenolic bending (Fig. S3B). This confirms that quercetin has been successfully loaded into NA.

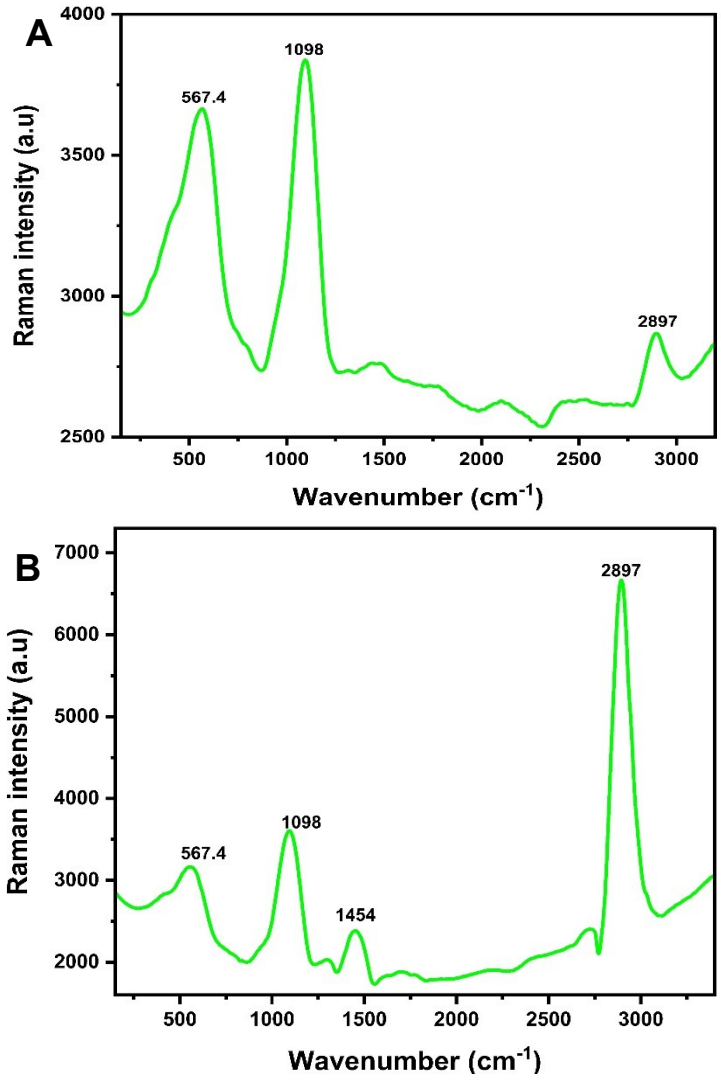
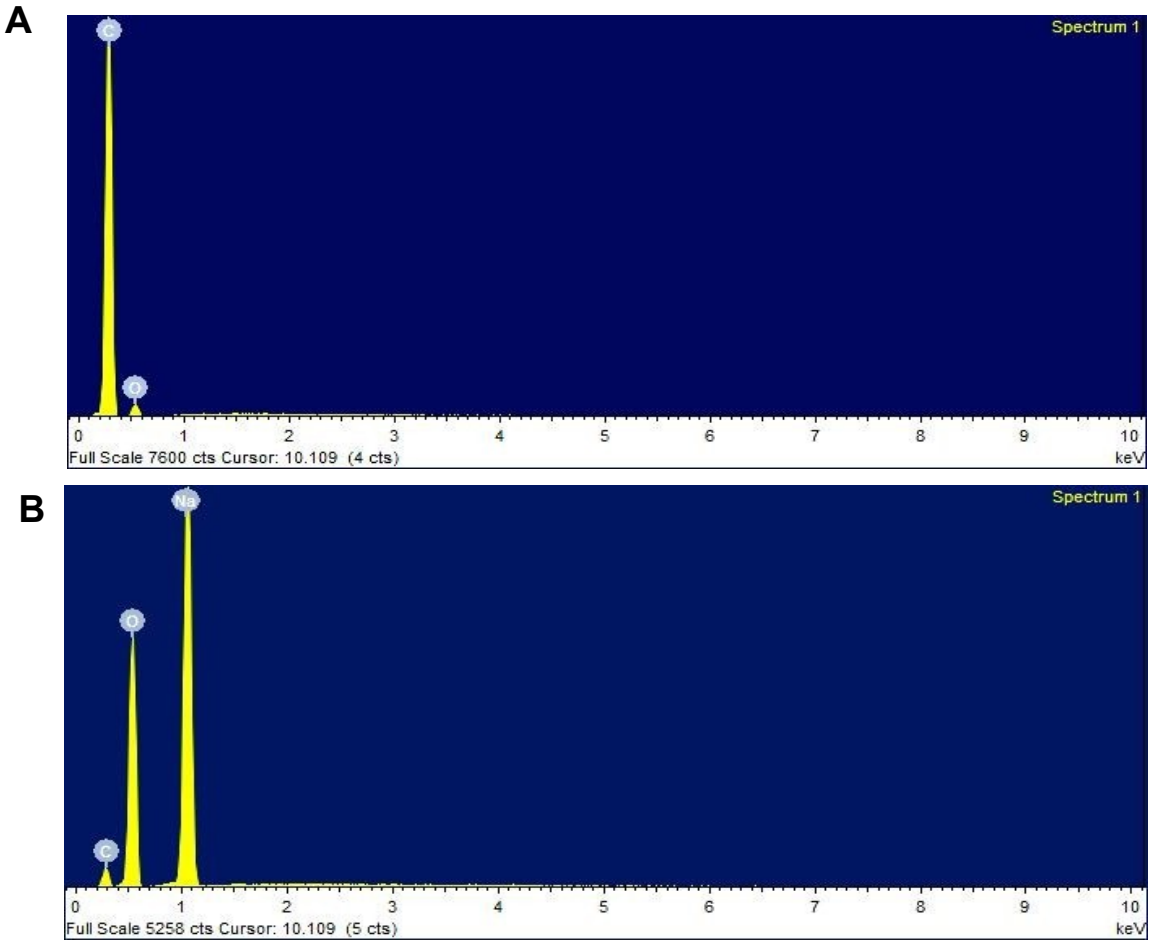


Fig. S3 Comparison of Raman spectra from **A**) NA and **B)** NAQ.

EDX analysis of NAQ

We have performed an EDX analysis of NA, NAQ, and quercetin to confirm the encapsulation of quercetin in NA. The weight % and atomic % of C, O and Na in NA, NAQ and Quercitin were represented in the Fig S4 and Table S1. The weight percentage of carbon (C) and oxygen (O) in NA were found to be 87.74% and 12.26%, respectively. The atomic percentage of C and O of 90.51% and 9.49% were found in NA. The weight percentage of C, O, and Na in quercetin was found to be 11.54%, 55.47%, and 32.99%, respectively, and atomic percentages of C, O and Na in quercetin were 16.39%, 59.14%, and 24.47%. Similarly, the weight percentage of C, O and Na in NAQ were 12.91%, 47.58%, and 39.51%, respectively, and atomic percentages of C, O, and Na in NAQ were 18.63%, 51.57%, and 29.80%, respectively. NAQ showed a higher weight and atomic percentage (47 to 52 %) of O elements and a reduced weight and atomic percentage (12 to 18%) of C elements compared to NA. We speculate that the shift in weight% and atomic % of elements in NAQ may be due to the presence of quercetin.



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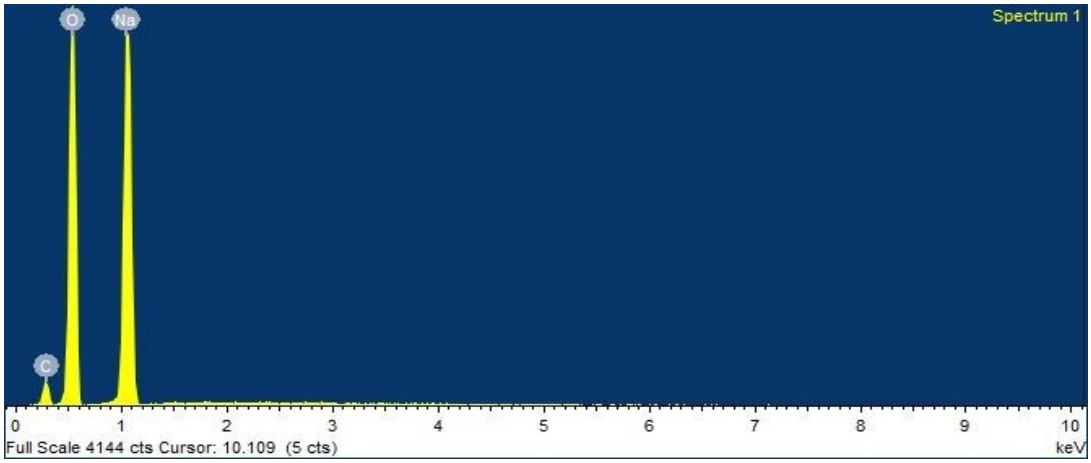

Fig. S4 Energy dispersive X-ray (EDX) of A) NA, B) NAQ, and C) Quercetin.

Table S1 Weight % and atomic % of elements present in NA, NAQ, and quercetin by Energy dispersive X-ray analysis (EDX)

Samples	**Elements**	**Weight%**	**Atomic%**
NA	C	87.74	90.51
	O	12.25	9.49
NAQ	C	12.91	18.63
	O	47.58	51.57
	Na	39.51	29.80
Quercetin	C	11.54	16.39
	O	55.47	59.14
	Na	32.99	24.47

Stability of NAQ in different physiological mediums

The stability of NAQ (100µM) in different physiological mediums at 37°C up to 72 hours was analyzed using DLS. The size and zeta potential of NAQ in the different physiological medium are depicted in Fig. S5. The size of NAQ in DMEM (Dulbecco's Modified Eagle's Medium), 10% FBS, 50% FBS, pH-7.4 PBS, and pH-4 PBS solution were found to be 42.8 ± 5.5 nm, 44.16 ± 5.57 nm, 54.64 ± 5.57 nm, 34.76 ± 4.11 nm, and 34.05 ± 5.1 nm respectively. Next, zeta potential was found to be -41.62 ± 2 mV, -46.2 ± 1.8mV, -55.7±0.8 mV, -44.18 ± 1.2 mV & -37.8±2 mV, respectively for in DMEM, 10% FBS, 50% FBS, pH-7.4 PBS, and pH-4 PBS medium. From this study, we could not find any significant size, zeta potential, and color changes in mediums and any aggregations.

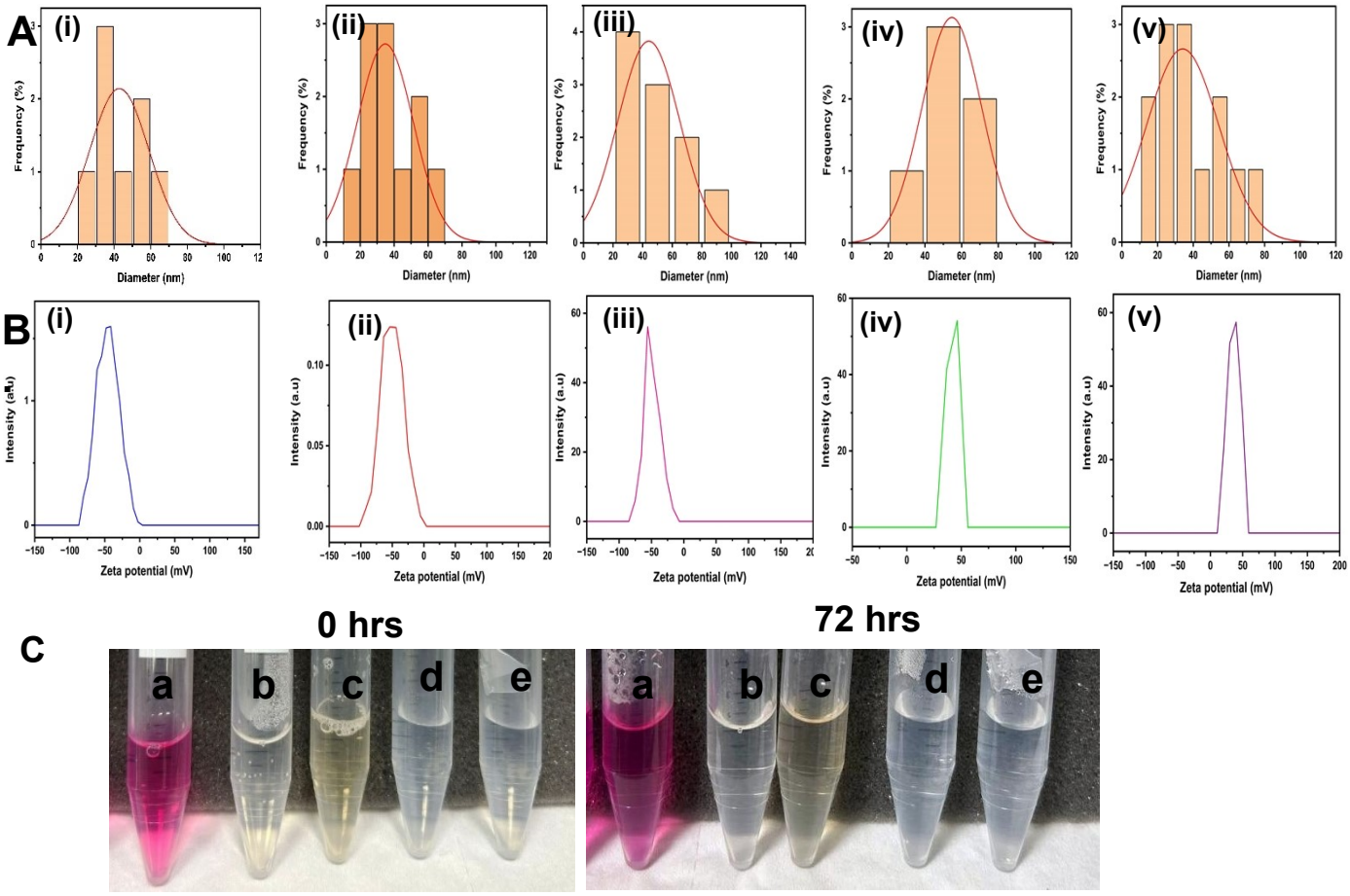

Fig. S5 Size **A)** and zeta potential **B)** of the NAQ in various physiological mediums. **A(i)** Size of the NAQ in DMEM solution, **(ii)** Size of the NAQ in 10 % FBS, **(iii)** Size of the NAQ in 50 % FBS, **(iv)** Size of the NAQ in pH-7.4 PBS, and **(v)** Size of the NAQ in pH-4 PBS solution. **B (i)** Zeta potential of the NAQ in DMEM solution, **(ii)** Zeta potential of the NAQ in 10 % FBS, **(iii)** Zeta potential of the NAQ in 50 % FBS, **(iv)** Zeta potential of the NAQ in pH-7.4 PBS, and **(v)**

Zeta potential of the NAQ in pH-4 PBS solution. **C**. Photograph of NAQ in various physiological mediums at 0 hours and 72 hours. **a**- NAQ in DMEM solution; **b**- NAQ in 10 % FBS; **c**- NAQ in 50 % FBS; **d**- NAQ in pH-7.4 PBS; **e**- NAQ in pH-4 PBS solution.

Hemolysis Assay

We have analyzed the hemolytic effect of NA and NAQ for 4 hrs. The results are represented in Fig. S6. Different concentrations of NA (0.01 & 0.02 mg) and NAQ (10, 50, & 100 µM) did not show any significant hemolytic effect as compared to positive control. At 4 hours, low concentrations of NA (0.01mg) and NAQ (10µM) showed 1.483 ± 0.1% and 1.60 ± 0.1% of hemolysis, respectively. High concentrations of NA (0.02) and NAQ (100 µM) showed below 5 % of hemolysis, which was 2.34 ± 0.2 and 2.96 ± 0.4, respectively. Further, the different concentrations of NA and NAQ showed significantly ($P \leq 0.05$) lesser hemolysis as compared to positive control.

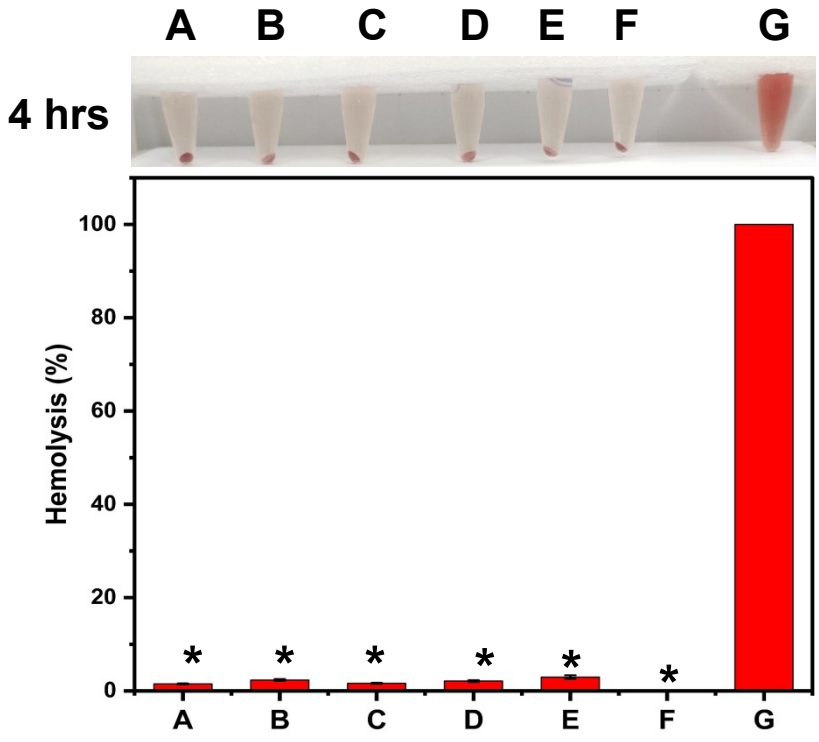

Fig. S6 The percent hemolysis effect of NA and NAQ at 4 hrs. The data represent the mean ± SD. * Represent significant difference at $P \leq 0.05$ as compared to positive control. **A)** 0.01mg of NA + RBC; **B)** 0.02 mg + RBC; **C)** 10 µM NAQ + RBC; **D)** 50 µM NAQ + RBC; **E)**100 µM NAQ + RBC; **F)** Negative control (1X PBS, (pH.7-4); **G)** Positive control (Distilled Water).

Cytoskeleton organization of NA on NIH 3T3 cells

We evaluated the cytoskeleton organization of NA (0.05 mg) treated NIH 3T3 cells and the results were shown in Fig. S7. 0.05 mg of NA-treated NIH 3T3 cells did not illustrate any disorganized cytoskeleton structure similar to control cells.

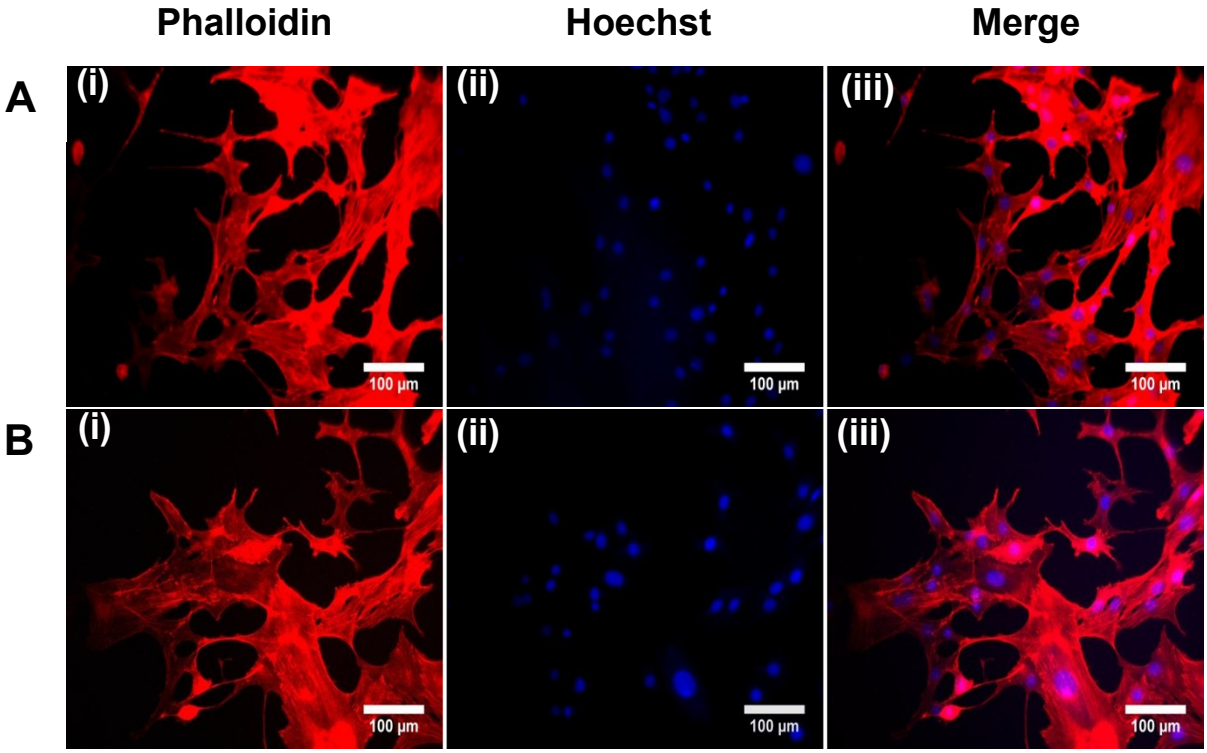

Fig. S7 The actin-cytoskeleton organization of NA treated NIH 3T3 cells A) Control, B) NA treated cells at 0.05mg with (i) Phalloidin stained (ii) Hoechst stained and (iii) Merged images.

Cytotoxicity analysis of free Quercetin on MCF-7 cells

We have evaluated the *in vitro* anticancer activity of quercetin at different concentrations (0.5, 1, 5, 10, 20, 40, 60, 80, 100, 250, and 500 µM) in MCF-7 breast cancer cells at 24 hours. The IC_{50} concentration of quercetin was found to be 88 ± 1.2 (Fig. S8).

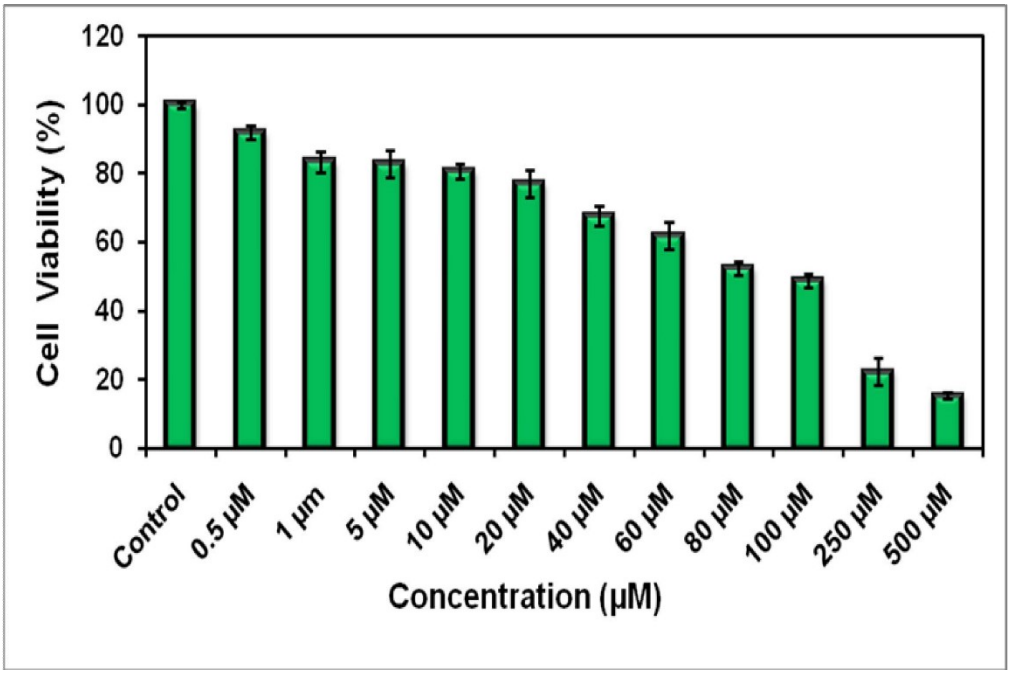

Fig. S8 Cytotoxicity effect of quercetin in MCF-7 breast cancer cells at various concentrations (0.5, 1, 5, 10, 20, 40, 60, 80, 100, 250, and 500 µM).

Cytotoxicity analysis of NA alone on MCF-7 cells

The cytotoxicity effect of NA at different concentrations 0.01, 0.025 and 0.05mg was assessed on MCF-7 cells. Fig. S9A showed cytotoxicity analysis of different concentrations of NA in MCF-7 cells by MTT method. From the results, we could not find any significant toxicity in NA-treated MCF-7 cells. Fig.S9B represents microscopic images of (i) untreated cells, and (ii) 0.05mg of NA treated MCF-7 cells. From the figure, we could not observe any significant morphological changes as well as cellular death in NA-treated MCF-7. It showed an intact nuclear structure that is very similar to that of control cells.

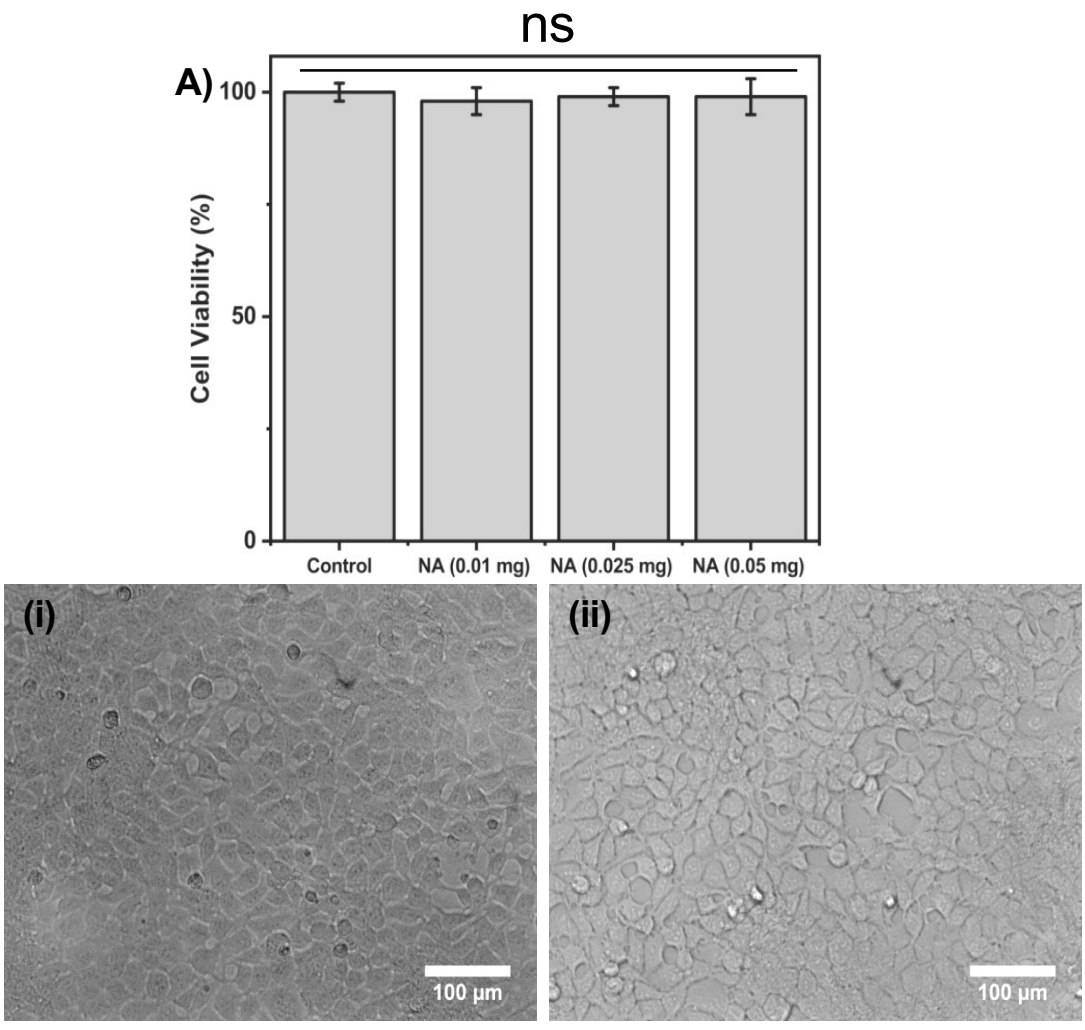

Fig. S9 A) Cytotoxic effect of various concentrations of NA (0.01, 0.025, and 0.05µM) on MCF-7 in 24 hrs by MTT assay. B) Optical microscopic images of (i) untreated (control) MCF-7 cells. (ii). 0.05mg of NA treated MCF-7 cells at 20 × magnifications. Data were expressed as mean ± SD (ns represents non-significant

results).

Morphological analysis of NAQ treated MCF-7 cells

We evaluated the *in vitro* anticancer effect of different concentrations of NAQ (0.05, 0.1, 0.4, 1, 2, 3, 4, 5, 7.5, and 10µM) in MCF-7 breast cancer cells at 24 hours. From the results, the Control cells showed normal intact nuclear structure with 100 % cell viability (Fig S10 a), whereas the MCF-7 cells treated at various concentrations exhibited cellular death and altered cellular morphology (Fig. S10 b to k).

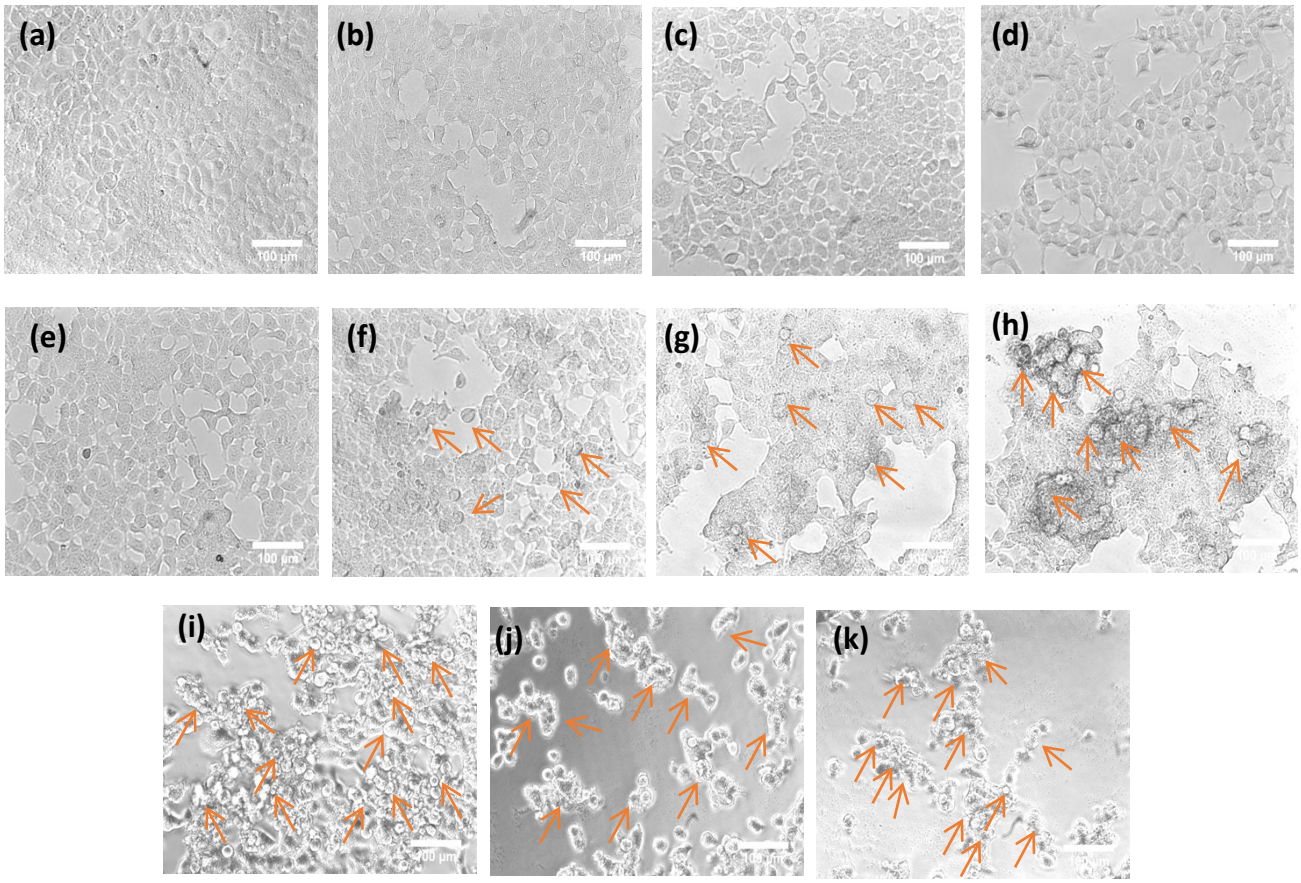

Fig. S10 Optical microscopic images of (a) control cells; NAQ treated cells at different concentrations of (b) 0.05 µM, (c) 0.1 µM, (d) 0.4 µM, (e) 1 µM, (f) 2 µM, (g) 3 µM, (h) 4 µM, (i)5 µM, (j) 7.5 µM and (k)10µM at 24 hrs.