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quercetin nanoparticles. Brie y, 50 mg of PLGA and 20 mg of quercetin was dissolved in acetone and injected in Millipore distilled water and was subjected to sonication with additional volume of water en the organic phase was removed by using Rota evaporator (IKA® RB 10) at 37°C under reduced pressure and then it was centrifuged repeatedly (Remi, C-24 BC) at 15000 rpm for 30 minutes and nally lyophilized (ermo sher, Germany & ermo Heto LL 3000) to obtain dry sample [7].

B **w**

e attachment of folate to the quercetin nanoparticles was done by applying EDC-NHS conjugation chemistry. For this, the previously suspended quercetin nanoparticles (1 mg ml^{-1}) in PBS (pH 5.6) were incubated with EDC in dark for half an hour and then immediately mixed with NHS for at least 6 hours [7,8] e obtained product is washed with water several times, ltered and mixed with folate at a concentration of 100 $\mu g ml^{-1}$ in PBS at pH 5.5 for overnight duration. Again, the product is washed with 1 ml PBS, pH 5.5 to remove the excessive reagents and further lyophilized. Finally, the FA-QuNPs were re-dispersed in water [9].

HaCaT cells, A431 cells and KB Cells were cultured separately in DMEME F-12HAM media supplemented with 10% FBS (fetal bovine serum) 100 U of streptomycin and 100 U of penicillin G at 37°C in 5% CO_2 Supply. All the cell lines were seeded at a density of 1×105 cells well. A er an exposure of 24 hours, the medium was replaced with DMEM containing FA-QuNP ranging from 0-75 µm in concentration [10]. All the samples were prepared in stock solution of DMSO. A er the treatment, cells were collected using a hem cytometer. Typhon Blue was dye was used for staining the cells.

e test was employed to measure the intracellular oxidative stress generated as a result of administration of FA-QuNPs, QuNPs and Free Quercetin by using 2',7'-Dichloro uoresin Diacetate (DCFH-DA) which quanti es intracellular hyper oxides [11,12]. e healthy cells were grown and experiment was carried out in eight sets consisting of

was taken every week from each group of animals and was embedded in para n a er xing in 10% bu ered formalin. en the sections were sliced with a semi-Automated Rotary Microtome (Leica®, Germany) and stained accordingly using Hematoxylin and Eosin (H and E) staining dyes. Finally, the stained specimens were observed under Fluorescence microscope (Nikon®, India), in order to determine the extent of damage caused to skin components.

Data are shown as means ± standard deviation (n=5). Statistical data were analyzed by the Student's t-test at the level of P=0.05.

C . (FA-Q -NP.)

Average size and size distribution of the drug-loaded nanoparticles were measured by the laser light scattering technique using a particle size analyzer (Malvern®,Zetasizer,ZF-90). Samples for measurement were prepared by diluting the material suspension with Milli Q water. Di erential scanning calorimeter (DSC) is a technique of employed to investigate the melting and recrystallization behavior of crystalline materials. e DSC thermo grams of pure Qu, FA, and physical mixture are shown in Figure 3A. e melting point of pure Qu was found to be 316°C. e DSC thermo gram of Qu and FA showed a sharp endothermic peak at 316°C and 250°C respectively. Physical mixture showed two mild peak changes in the position of endothermic peaks. us, there was a chemical interaction between Qu and FA. FA-Qu-NPs showed two endothermic peaks in DSC thermo gram, one at 250°C for ligand and another at around 155°C for mannitol (cry protectant used in lyophilization of FA-Qu-NPs) the peak for PLGA was not visible showing its amorphous nature, the peak of Qu and FA was clearly visible in FA-Qu-NPs. Although, the nal peaks of drug

signi cant at a concentration of 150 mg/l in both A431 and KB cell lines in 24 hours (Figure 4).

e ROS Study has shown that time exposure to folate conjugated quercetin nanoparticles increases the production of reactive oxygen species generation in all the three cell lines. In case of FA-Qu-NPs, there was tremendous increase in ROS production in all three cell lines.

e conjugation of folate with quercetin potentiate the generation of

Figure 5: a) **b)**

nanoparticles. **c)**

Note: 60Mins 120Mins 180Mins 240Mins

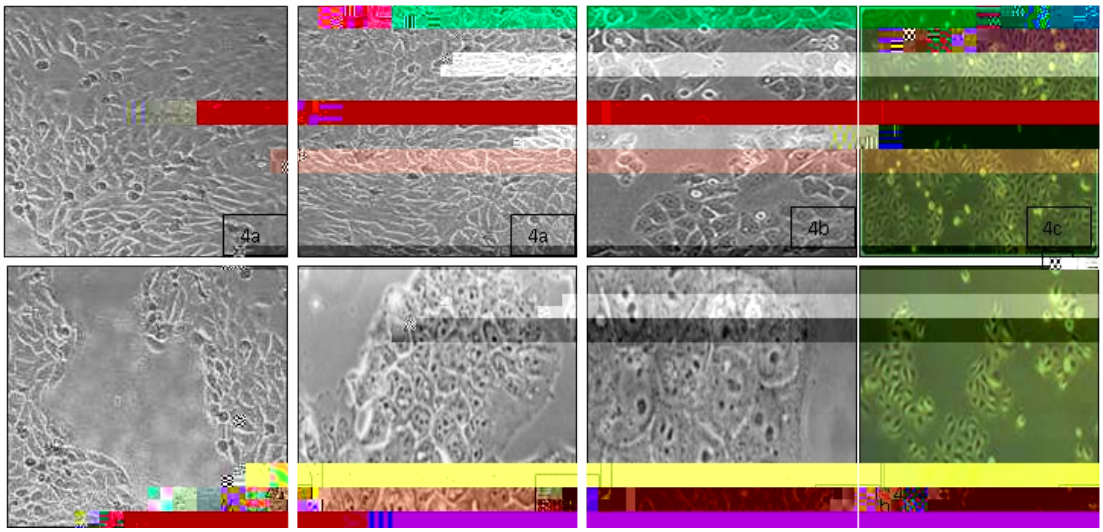

Figure 6: a) **b)**

c)

nanoparticles.

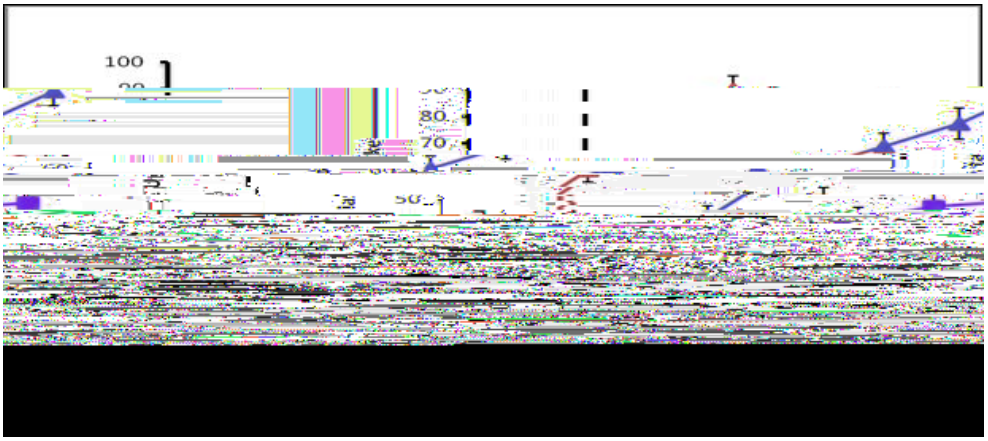

Figure 7a: .

Note: Free-Qu Qu- NPs FA-Qu-NPs

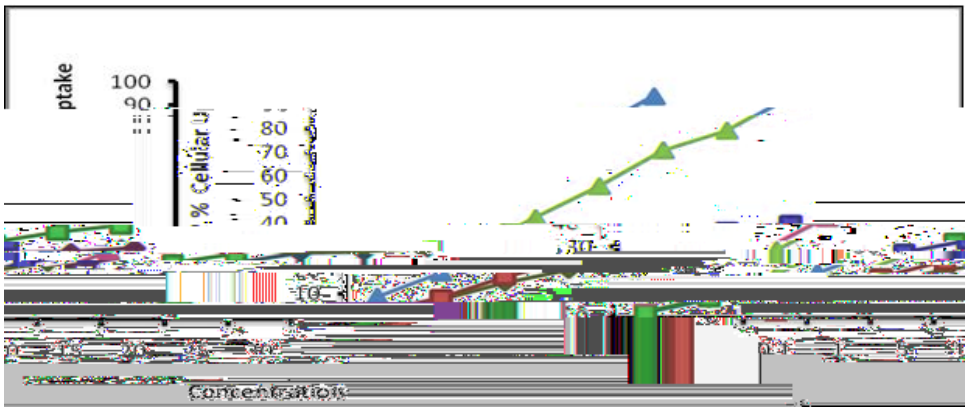

Figure 7b: shows cellular uptake of folate conjugated quercetin nanoparticles, non-conjugated quercetin nanoparticles and free quercetin in KB cell lines.
Note: Free-Qu Qu- NPs FA-Qu-NPs

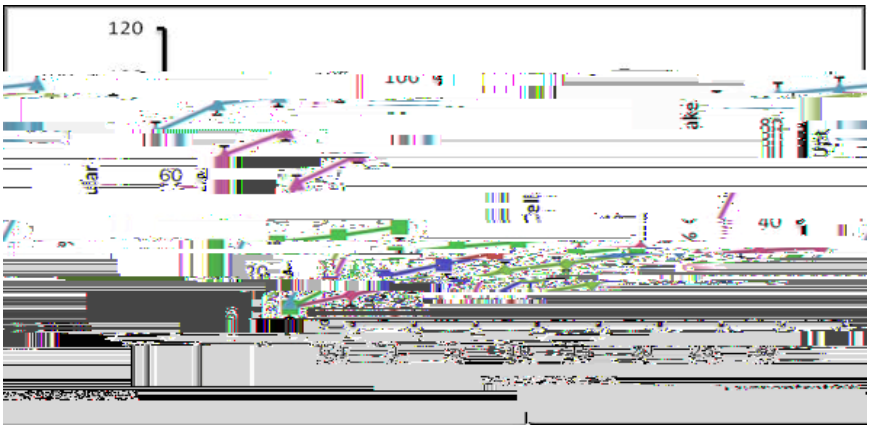

Figure 7c: C
Note: Free-Qu Qu- NPs FA-Qu-NPs

the probe containing 0.03% of Rhoda mine were applied homogeneously and non-occlusive to the skin. e experiments were carried out employing Franz di usion cells with the receiver chamber lled with phosphate bu er pH 5.5 solutions. A er 24 h, the skin was removed and washed with phosphate bu er. e skin was then rapidly frozen by liquid nitrogen and a skin surface perpendicular rectangular piece was taken from the site of drug application with the help of a sharp blade.

is tissue was xed on the sample holder with the help of a Tissue frozen medium gel. (Gung, Leica, Germany). e skin perpendicular sections (dermis to horny layer) of (250 µm) full thickness were cut with the help of cry microtome (Leica, Germany). e treated area was cut out and tested for probe penetration. e full skin thickness was optically scanned at 15-30 nm increments through the Z-axis of a Leica DMIRE2 confocal laser scanning microscope (Germany) attached to a Leica TCS SP2 uorescence microscope. e gures are shown in Figure 8.

e skin retention studies of di erent formulations were performed in order to analyse the content of quercetin in the skin a er 24 h of di usion (Table 3). e study showed that percentage drug retention of formulations was found higher for FA-Qu-NPs loaded novel lotions (NL-4) (Table 6). e % retention was near about similar for Marketed formulation, 0.58 ± 0.30 and that of FA-Qu-NPs loaded novel lotion 0.78± 0.5 (Figure 8).

In vivo

e main objective of our study was to determine the targeting ability of folate conjugated quercetin nanoparticles (NL-4) dermally and to evaluate its topical and targeted e ect on skin. e macroscopic e ects of UV radiation on the animal's skin were highly distinguishing for evaluation (Table 4). e non-irradiated skin was free from any type of lesion formation while the irradiated groups developed lesion from the fourth week onwards in nearly 50% of the test animals. At the end of week 6 about 85% of animals developed photo carcinoma lesions (extensive degradation). e cellular components like collagen, elastin, matrix proteins network was found damaged. e animals treated with free quercetin lotion developed lesser skin lesions in comparison to the irradiated group. e animals treated with market formulation Fivocil®, Alkem, India, showed minimal lesion formation at the last week of the irradiation. On discontinuation of the UV irradiations the formulation showed healing e ect. e test sample showed nominal skin degradation and negligible lesion formation (Table 4).

In above study, Figure 9 shows the skin histology of that group

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