Supporting information

Induced apoptosis in melanocytes cancer cell and oxidation in biomolecules through deuterium oxide generated from atmospheric pressure non-thermal plasma jet

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***In silico* molecular modelling and docking parameters**. To find the possible bioactive conformations of D_2O_2/H_2O_2 and actinomycin D, the Sybyl X 2.0 interfaced with Surflex-Dock module was used for molecular docking. The program automatically docks ligand into the binding pocket of an enzyme/receptor protein using a protomol-based algorithm and empirically produced scoring function. The X-ray crystallographic structures of skin cancer receptor protein targeting Erk2 (PDB:3SA0),[37] p53 (PDB:3ZME)[38] and caspase-8 (PDB:3KJQ)[39] protein receptor was taken from the protein data bank (PDB) and modified for docking calculations. Co-crystallized ligand was removed from the structure, water molecules were removed, H atoms were added and side chains were fixed during protein preparation. Protein structure minimization was performed by applying Tripos force field and partial atomic charges were calculated by the Gasteiger-Huckel method. The protomol is a very important and necessary factor for docking algorithm and works as a computational representation of the proposed ligand that interacts into the binding site. Surflex-Dock's scoring function has several factors that plays an important role in the ligand-receptor interaction, in terms of hydrophobic, polar, repulsive, entropic, and solvation, and it is a well-established and recognized method. The most standard docking protocols have ligand flexibility into the docking process, while count the protein as a rigid structure. Our molecular docking involves the following several steps: (1) the protein structure was imported into Surflex, and then hydrogens were added; (2) generation of protomol using a ligand-based strategy, and during this process, 2 parameters (first called protomol bloat, which determines how far the site should extend from a potential ligand; and another called protomol threshold,

which determines deepness of the atomic probes that is used to define the protomol penetration into the protein) must be specified to form the appropriate binding pocket. Thus, in the current study, protomol bloat was set to 0, and protomol threshold was set to 0.50, when a reasonable binding pocket was obtained; and (3) all of the compounds were docked into the binding pocket, and 20 possible active docking conformations with different scores were obtained for each compound. During the docking process, all of the other parameters were assigned to their default values. Surflex-Dock total scores, which express the -log 10 (Kd) units to represent binding affinities, were applied to estimate the ligand–receptor interactions of the newly designed molecules. The Program automatically docks ligand into the binding pocket of a target protein by using a protomol-based algorithm and empirically produced scoring function. Co-crystallized ligand was removed from the structure, water molecules were removed, H atoms were added and side chains were fixed during protein preparation. Protein structure minimization was performed by applying Tripos force field and partial atomic charges were calculated by the Gasteiger-Huckel method. In the reasonable binding pocket, all the species were docked into the binding pocket and 20 possible active docking conformations with different scores were obtained for each species. During the docking process, all of the other parameters were assigned their default values.

Titles and legends to figures

Figure S1. Plasma effects on cell death of normal human dermal fibroblast (NHDF) cells. Viability of NHDF cells treated with D_2O + N_2, plasma after incubation for 24, 48 and 72 h. for 0, 1, 3, and 5 (control without exposure is taken as 0 min). All values are expressed as (MFI) and ± SD in triplicates.

Figure S2. D_2O+ N_2 gas effects on cell death of melanocytes G361cells. Viability of G361 cells treated with D_2O + N_2, gas only after incubation for 24, 48 and 72 h for 0, 1, 3, and 5 (control without exposure is taken as 0 min). All values are expressed as (MFI) and ± SD in triplicates.

Figure S3. H_2O+ N_2 plasma effects on cell death of melanocytes G361cells. Viability of G361 cells treated with H_2O + N_2 plasma only after incubation for 24, 48 and 72 h for 0, 1, 3, and 5 (control without exposure is taken as 0 min). All values are expressed as (MFI) and ± SD in triplicates.

Figure S4. (D) Western blot analysis of the oxidative stress related protein phosphorylation of p-38 MAPK and p421/p44 MAPK (Erk 2). Actin was used as loading control.

Figure S5. The co-crystallize inhibitor was re-docked into the binding site of ERK2 with 0.5628 Å of RMSD between docked and crystallized conformation and total docking score of 4.4761.

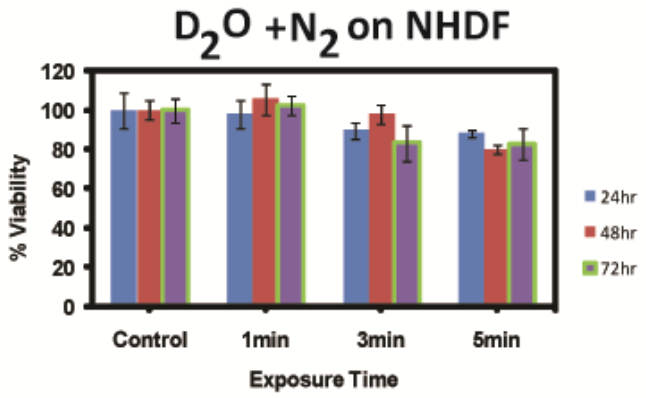

Figure S1

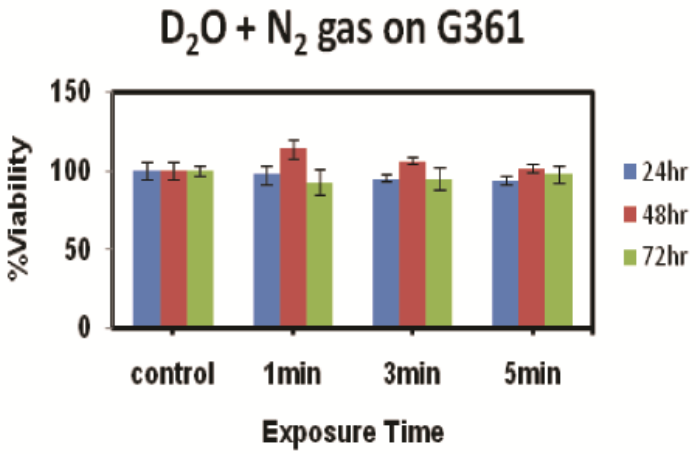

Figure S2

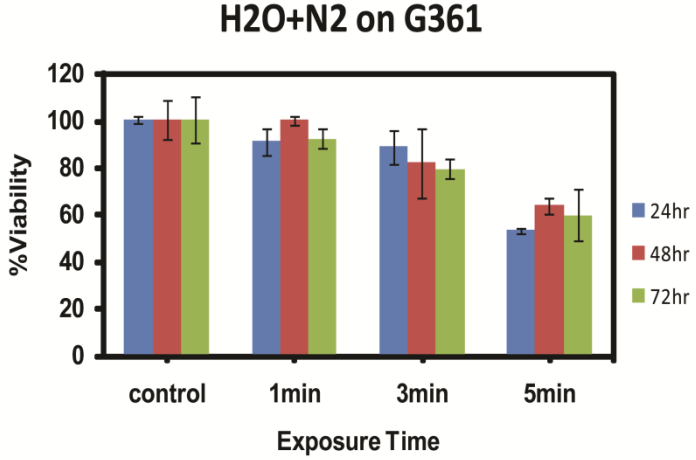

Figure S3

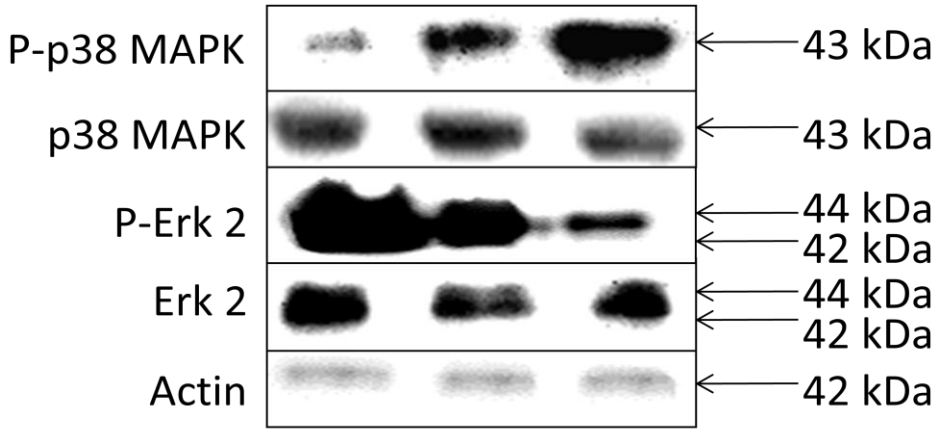

Figure S4

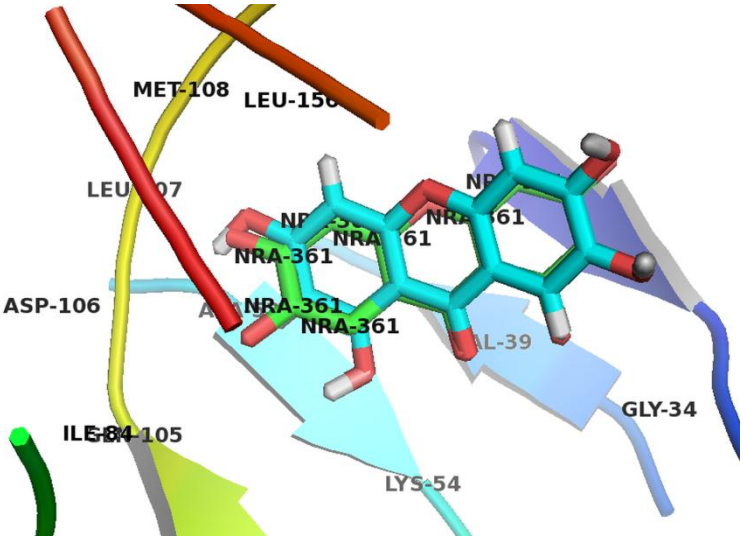

Figure S5