Supporting Information

Anti-angiogenic vanadium pentoxide nanoparticles for the treatment of melanoma and their *in vivo* toxicity study

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Experimental:

1. X-ray diffraction (XRD) spectroscopy

The phase purity and crystallinity of V_2O_5 NPs was analyzed by X-ray diffraction (XRD) analysis [Bruker AXS D8 Advance Powder X-ray diffractometer (using CuKαλ=1.5406 A radiation) in range $2\theta = 20$ to 80^o].

2. Fourier transformed infrared (FTIR) spectroscopy

Fourier transformed infrared spectroscopy (FTIR: Thermo Nicolet Nexus 670 spectrometer) was utilized to determine the functional groups present in V_2O_5 NPs. The FT-IR spectra was performed at a resolution of 4 cm^{-1} in KBr pellet from range 400-4000 cm^{-1}.

3. Dynamic light scattering (DLS)

The size , shape and surface charge of as-synthesized of V_2O_5 NPs was detemined by dynamic light scattering (Malvern). In order to check the hydrodynamic diameter, 20 μL of NPs soultion was diluted in total 1 mL of mili-Q water and used in DLS measurement.

4. Transmission electron microscopy (TEM)

The morphology and shape of V_2O_5 NPs was observed using the TEM (TEM: Tecnai G2 F30 S-Twin Microscope, operated at 100 kV). The NPs solution in water was drop casted on the carbon-coated copper grid and allowed to dry in room temperature. This process was repeated for more than two times to coat it properly on the grid and subsequent analysis was carried out.

5. X-ray photoelectron specroscopy (XPS)

In order to know the elemental composition in the V_2O_5 NPs XPS analysis [KRATOS AXIS 165 with a dual anode (Mg and Al) apparatus using the Mg Kα anode] was performed.

6. Inductive coupled plasma optical emission spectroscopy (ICP-OES)

In order to know the uptake as well as clearance of vanadium from the cells and *in vivo* system; respectively, ICP-OES analysis [(IRIS intrepid II XDL, ThermoJarrel Ash] was carried out.

FIGURES

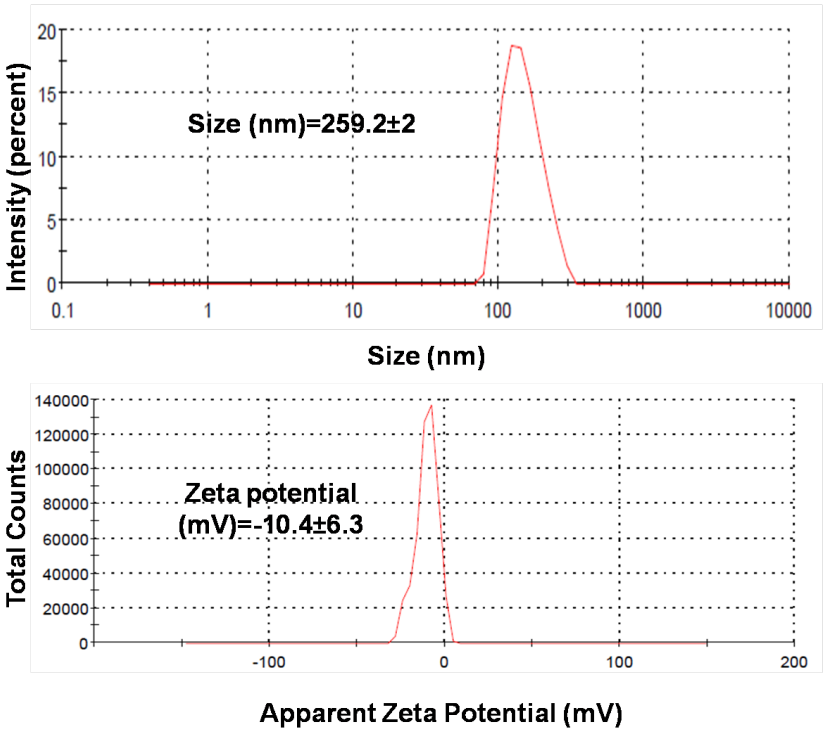

Figure S1: (a) size, and **(b)** zeta potential of the as synthesized V_2O_5 NPs measured by DLS.

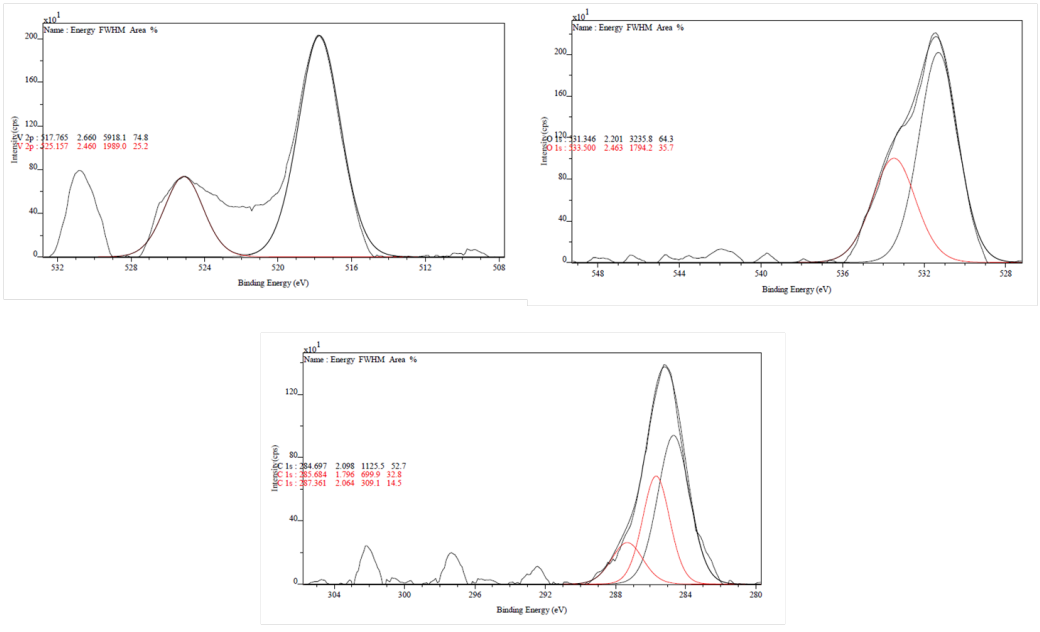

Figure S2: Different binding energy curves obtained from XPS analysis of the as synthesized V_2O_5 NPs.

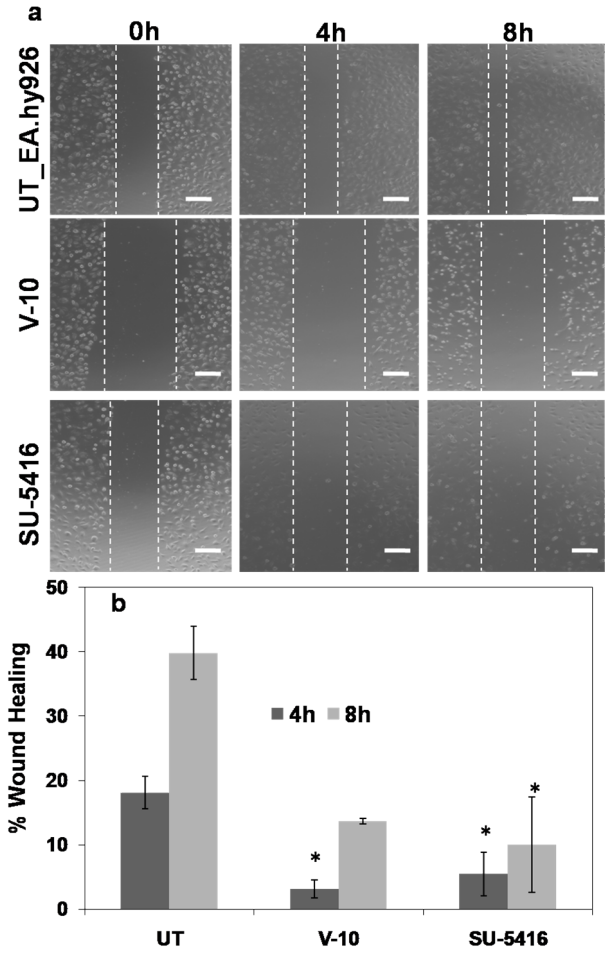

Figure S3: (a) Scratch wound healing assay in EA.hy926 within 0-8 h time points. The migration of cells was inhibited by V_2O_5 NPs (10 µg/mL: V-10) in a dose dependent fashion. **(b)** Histogram representation of the healing of the scratch area quantified using Image J software. These experiments were performed thrice and represented as mean±SD. Significant differences from untreated (UT) cells were observed (*$p < 0.05$). Scale bar=100 µm.

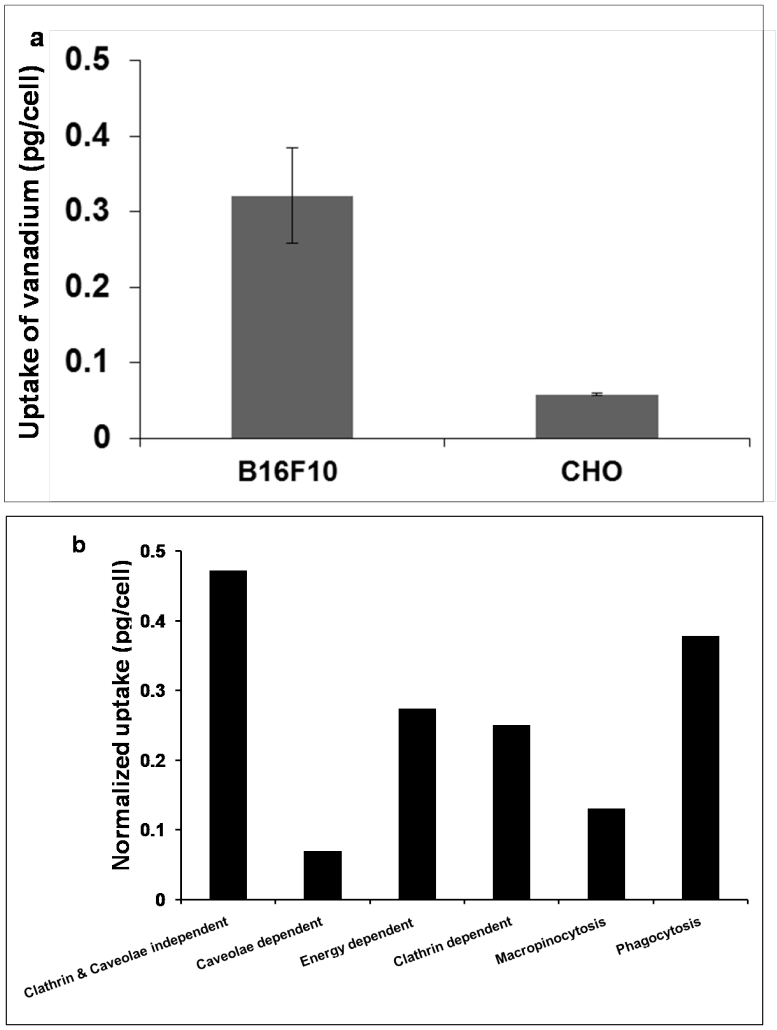

Figure S4: (a) Uptake variation of V_2O_5 NPs in terms of vanadium metal in cancer cell (B16F10) with respect to normal cell (CHO), measured using ICP-OES analysis. These experiments were performed thrice with similar results and no significant differences was observed (*$p < 0.05$). **(b)** Intracellular uptake pathway analysis of V_2O_5 NPs inside B16F10 cells under different endocytosis-inhibited conditions. Before 6 h incubation with V_2O_5 NPs (10 μg/mL) cells were incubated with specific endocytosis inhibitors, see the Experimental section for details. Data was represented as normalized uptake (pg/cell). It was a pulled from two independent experiment, so no statistical significance was provided.

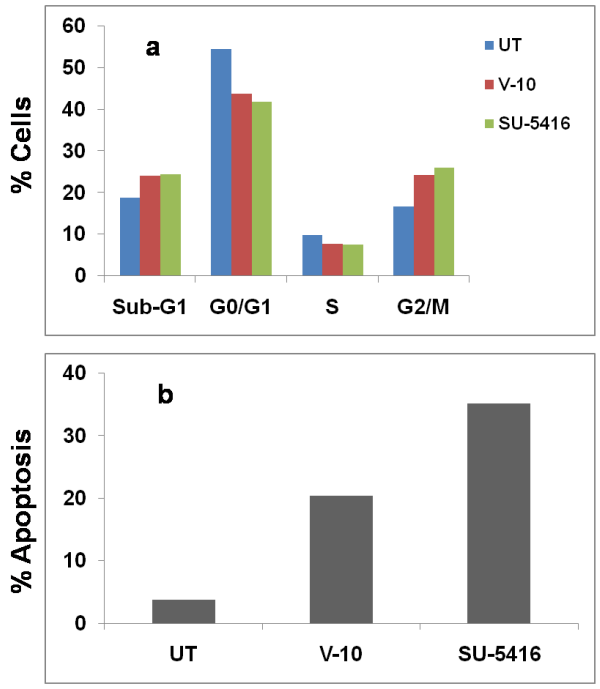

Figure S5: Histogram of **(a)** cell cycle and **(b)** apoptosis studies after various treatments [UT, V-10 (V_2O_5 NPs: 10 μg/mL), SU-5416]. B16F10 cells underwent G2/M phase arrest followed by apoptosis in late apoptotic region upon treatment with V_2O_5 NPs.

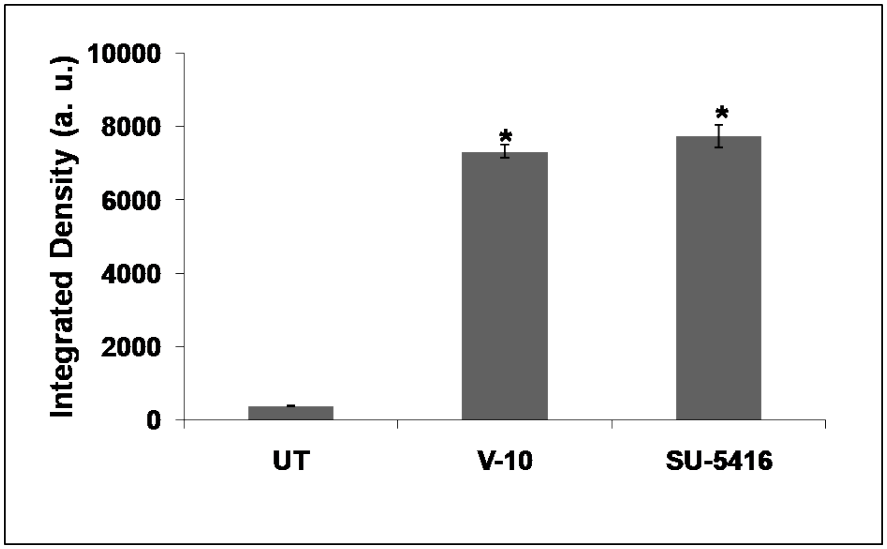

Figure S6: Quantification of the fluorescence intensity inside the cells corresponds to Fig. 10. These quantification were performed thrice and represented as mean±SD. Significant differences from UT cells were observed (*$p < 0.05$).

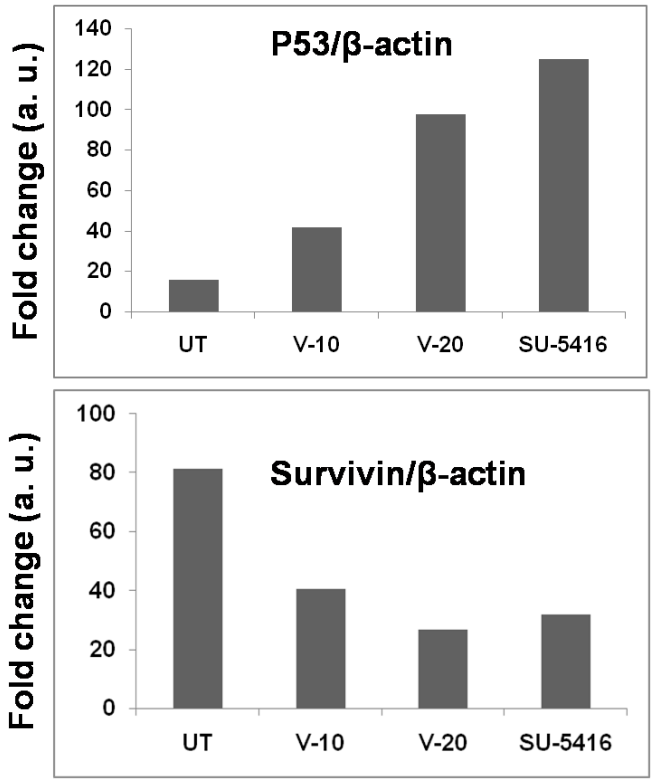

Figure S7: Quantification of the western blots data are represented as fold change vs treatments (UT, V-10, V-20, SU-5416). Beta actin was used as loading control. Upregulation of p53 expression and downregulation of survivin was observed in treatments compared to UT.

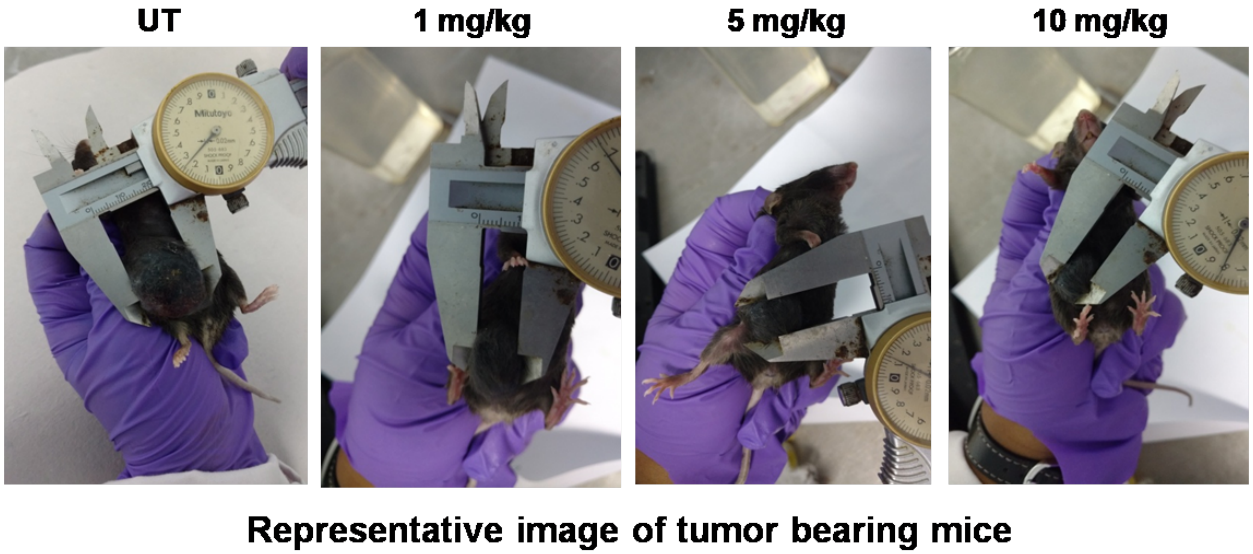

Figure S8: The representative images of tumor bearing mice of various groups (untreated (UT), V_2O_5 NPs: 1 mg/kg b.w, V_2O_5 NPs: 5 mg/kg b.w and V_2O_5 NPs: 10 mg/kg b.w).

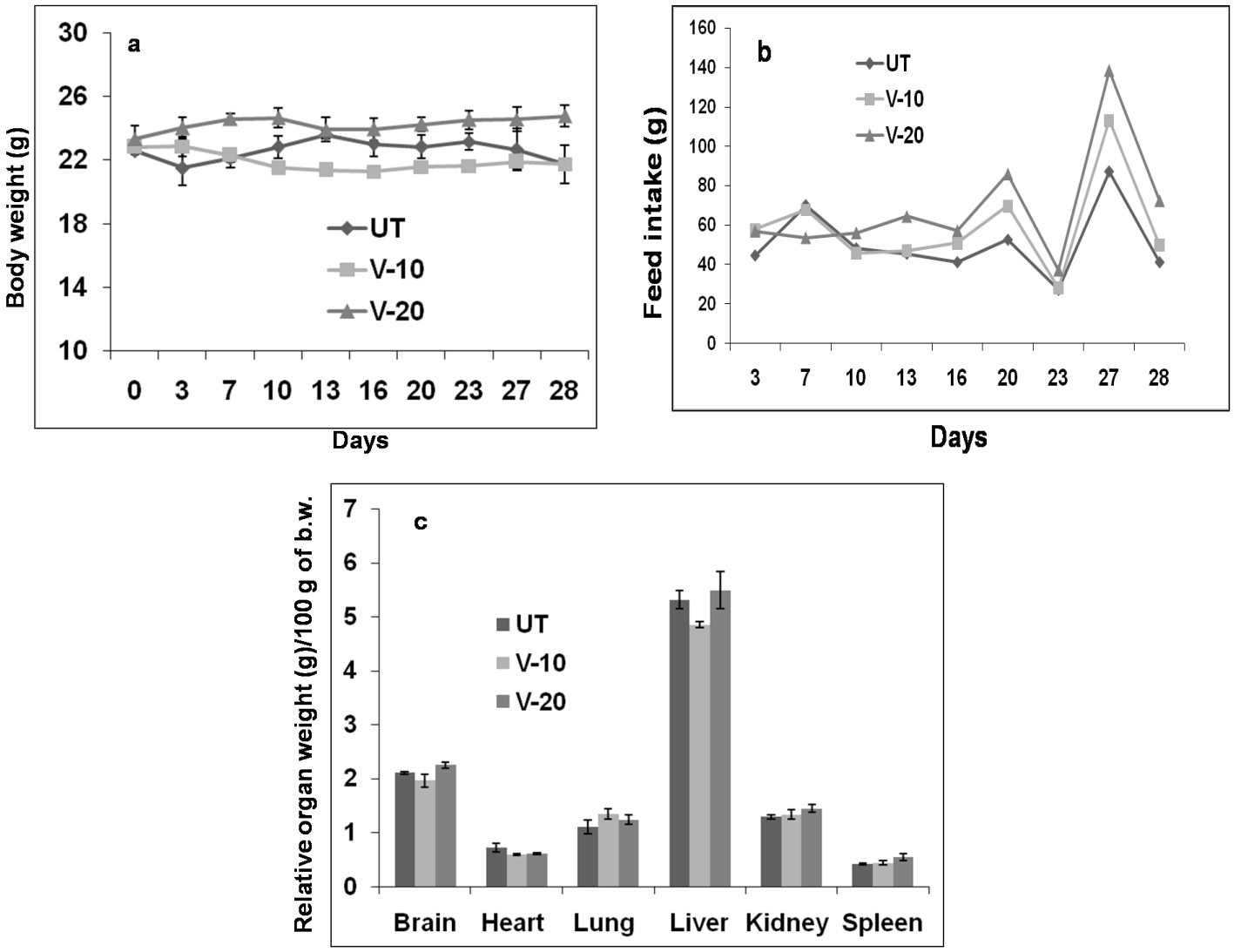

Figure S9: (a) Body weight data of mice after sub-chronic toxicity study. **(b)** Feed intake data of mice after sub-chronic toxicity study period. **(c)** Organ index of mice after sub-chronic toxicity study. Groups: UT, V-10 (V_2O_5 NPs: 10 mg/kg b.w), V-20 (V_2O_5 NPs: 20 mg/kg b.w). No significant difference in treated mice with respect to UT was observed with respect to body weight, feed intake or organ weight.

Pathways	Inhibitor name	Dose	Pre-incubation time
Energy dependent	NaN_3 (sodium azide)	0.1 %	1 h
Phagocytosis	Wortmanin	10 nM	1 h
Clathrin and caveloae independent	Monensin	30 µg/mL	30 min
Macropinocytosis	Amiloride	50 µM	30 min
Clathrin dependent	Chloropromazine hydrochloride	10 µg/mL	15 min
Caveloae dependent	Methyl-ß cyclodextrin	5 mM	15 min

Table S1: Cellular uptake pathway study of V_2O_5 NPs using various inhibitors.