

SUPPORTING INFORMATION

Unique Catanionic Vesicles as a Potential “Nano-Taxi” for Drug Delivery System. *In Vitro*
and *In Vivo* Biocompatibility Evaluation

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Table S1. Raw Data for the Hemolytic Activity Experiments

| Concentration µg/mL | Abs | %Hemolysis |
|----------------------------|------------|-------------------|
| control | 0.018 | 0.80 |
| 0.0125 | 0.029 | 1.3 |
| 0.025 | 0.033 | 1.5 |
| 0.05 | 0.033 | 1.5 |
| 0.1 | 0.026 | 1.2 |
| 0.5 | 0.035 | 1.6 |
| 1 | 0.042 | 1.9 |
| 2 | 0.241 | 10.8 |

Abs at 541 nm is the average absorbance value of 6 experiments; % Hemolysis were calculated using the following equation:

$$\% \text{ hemolysis} = (Abs * 100) / Abs \text{ positive control}$$

The Abs of the positive control = 2.24

Table S2- Raw Data for the Trypan Blue Exclusion Method Experiments

| Concentration mg/mL | Cellular count | Number of Live Cells | Number of Death Cells | %viability |
|--------------------------------|---------------------------|---------------------------------|----------------------------------|-------------------|
| Negative Control | 753 | 690 | 63 | 91.6 |
| 0.025 | 813 | 727 | 86 | 89.4 |
| 0.05 | 689 | 590 | 99 | 85.6 |
| 1 | 915 | 585 | 330 | 63.9 |
| 2 | 898 | 418 | 480 | 46.5 |

Table S3- Raw Data for the Fibroblasts Mitochondrial Enzyme Activity Evaluation (MTT assay)

| Concentration µg/mL | System | Abs | %viability |
|--------------------------------|---------------|------------|-------------------|
| control | CONTROL | 1.36 | 100 |
| 50 | AOT-BHD | 1.099 | 80.87 |
| 25 | AOT-BHD | 1.31 | 96.75 |
| 2.5 | AOT-BHD | 1.54 | 113.62 |
| 0.025 | AOT-BHD | 1.32 | 97.25 |
| 2.5x10⁻⁵ | AOT-BHD | 1.57 | 115.75 |
| 50 | DOPC | 1.22 | 90.00 |
| 25 | DOPC | 1.41 | 103.62 |
| 2.5 | DOPC | 1.56 | 114.62 |
| 0.025 | DOPC | 1.56 | 114.62 |
| 2.5x10⁻⁵ | DOPC | 1.60 | 117.50 |

Abs at 540 nm is the average absorbance value of 8 experiments; Viability percentage were calculated using the following equation:

$$\% \text{ viability} = (Abs * 100) / Abs \text{ control}$$

Table S4- Raw Data for the Dose Lethal 50 experiments

| Log concentration | N | R | % Mortality | % Mortality Corrected |
|-------------------|----|----|-------------|-----------------------|
| 0 | 12 | 0 | 0 | 4.16 |
| 0.53 | 12 | 0 | 0 | 4.16 |
| 1.14 | 12 | 0 | 0 | 4.16 |
| 1.53 | 12 | 0 | 0 | 4.16 |
| 1.8 | 12 | 0 | 0 | 4.16 |
| 2.01 | 12 | 0 | 0 | 4.16 |
| 2.04 | 12 | 3 | 25 | 25 |
| 2.07 | 7 | 4 | 57.14 | 57.14 |
| 2.09 | 12 | 6 | 50 | 50 |
| 2.14 | 12 | 12 | 100 | 97.72 |

N is the total number of individuals; R is the number of dead or affected organism;

Mortality % = $\left(\frac{R}{N}\right) * 100$; %Mortality corrected is the value corrected using the data fitted with the Statistica V.7 software.

Table S5. Raw Data for the Chronic Study: Enzymatic Activity.

Transaminases GPT and GOT

| Concentration mg/Kg | Abs | GPT UI/L | GOT UI/L |
|---------------------|-------|-------------|-----------|
| control | 0.203 | 29.13 ± 3.8 | 5.8 ± 0.6 |
| 3.4 | 0.198 | 28.2 ± 4.5 | 5.7 ± 0.7 |
| 13.8 | 0.184 | 25.3 ± 3.3 | 5.2 ± 0.5 |

Abs at 505 nm is the average absorbance value of 8 experiments. GPT and GOT values were obtained using the calibration curve according to the following equation

$$Abs = a * Enzyme Concentration \left(\frac{UI}{L}\right) + b$$

GTP: b: 0.0638; a: 0.0047;

GOT: b: 0.03501; a: 0.0029

Alkaline phosphatase AP

| Concentration mg/Kg | Abs | AP UI/L |
|---------------------|--------|---------------|
| control | 0.4733 | 520.96 ± 47.2 |
| 3.4 | 0.5255 | 578.4 ± 35.7 |
| 13.8 | 0.5080 | 559.2 ± 28.8 |

Abs at 520 nm is the average absorbance value of 8 experiments. AP values were obtained using $AP\ UI/L = F * Abs$ with $F = (200\ UI/L) / Abs\ st$. Abs st represents the absorbance value of the standard provided by the Kit and the value is 0.1817

Lactate Deshidrogenase LDH

| Concentration mg/Kg | Abs | LDH UI/L |
|---------------------|--------|---------------|
| control | 0.023 | 222.9 ± 47.2 |
| 3.4 | 0.0206 | 199.79 ± 35.7 |
| 13.8 | 0.0219 | 212.85 ± 28.8 |

Abs at 340 nm is the average absorbance value obtained after 8 experiments.

$$LDH(UI/L) = (Abs) * F \text{ with } F = 9683 \text{ at } 37^{\circ}C \text{ and } \lambda = 340 \text{ nm}$$