# Genotoxicity and Toxicity Assessment of a Formulation Containing Silver Nanoparticles and Kaolin: An In Vivo Integrative Approach

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## SUPPLEMENTARY INFORMATION

**Table S1.** Instrumental parameters for determination of total silver by ICP-MS Agilent 7850.

| | ***Parameters*** | ***Setting Values*** | | ***Parameters*** | ***Setting Values*** |
|---|---|---|---|---|---|
| *Plasma* | Plasma mode | General Purpose | *Lens* | Deflect (V) | 2.2 |
| | RF power (V) | 1550 | | Cell Entrance (V) | -40 |
| | Nebulizer gas (L $min^{-1}$) | 1.06 | | Cell Exit (V) | -70 |
| | Auxiliary gas (L $min^{-1}$) | 0.90 | *Cell* | He Gas Flow (mL $min^{-1}$) | 4.5 |
| | Plasma gas (L $min^{-1}$) | 15.0 | | Energy discrimination (V) | 5.0 |
| | Sampling depth (mm) | 10.0 | | OctP Bias (V) | -18.0 |
| | Spray chamber temperature (ºC) | 2.0 | | OctP RF (V) | 190 |
| *Lens* | Extract 1 (V) | 0.0 | *QP* | Axis Offset | 0.00 |
| | Extract 2 (V) | -170.0 | | QP Bias (V) | -13.0 |
| | Omega bias (V) | -80 | *Torch* | Torch H (mm) | 0.5 |
| | Omega lens (V) | 9.3 | | Torch V (mm) | -0.1 |

## Dose-finding study

### Weight growth gain

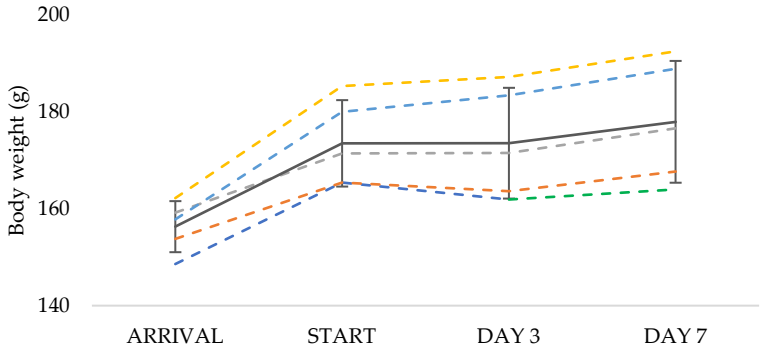



**Figure S1.** Results of the dose-finding study weight growth. The figure shows the weight growth recording for each study animal, expressed as body weight (g) during the 7 days of the study, represented in dotted lines (F1: green, F2: orange, F3: grey, F4: yellow, F5: blue) and the group mean weight ± the standard deviation (continuous grey line). Acclimatation period between arrival and start lasted 5 days. Start: first day of administration.

### Macroscopic alterations

**Table S2.** Results of the absolute organ weight for each animal of the dose-finding study (F1-F5). The table collects the individual weights (g) of each organ for each animal and the descriptive statistics of the whole group expressed as mean ± standard deviation. The normality ranges of each parameter (bold letter) were calculated from the laboratory histories (n = 57 animals). •: values outside the laboratory records.

| Animal ID | Spleen **0.263–0.592** | Heart **0.422–0.945** | Liver **5.068–8.498** | Thymus **0.135–0.568** | Right kidney | Left kidney | Kidneys sum **1.090–1.808** | Right ovary | Left ovary | Ovaries sum **0.060–0.190** |
|---|---|---|---|---|---|---|---|---|---|---|
| F1 | 0.350 | 0.564 | 4.760• | 0.361 | 0.579 | 0.584 | 1.163 | 0.029 | 0.042 | 0.071 |
| F2 | 0.390 | 0.498 | 4.792• | 0.401 | 0.557 | 0.529 | 1.086 | 0.034 | 0.033 | 0.067 |
| F3 | 0.400 | 0.534 | 5.710 | 0.447 | 0.702 | 0.673 | 1.375 | 0.065 | 0.069 | 0.134 |
| F4 | 0.483 | 0.592 | 6.879 | 0.541 | 0.761 | 0.659 | 1.420 | 0.064 | 0.053 | 0.117 |
| F5 | 0.411 | 0.569 | 5.348 | 0.562 | 0.700 | 0.702 | 1.402 | 0.075 | 0.066 | 0.141 |
| Mean ± SD | 0.407± 0.048 | 0.551± 0.036 | 5.498± 0.869 | 0.462± 0.087 | 0.660± 0.088 | 0.629± 0.071 | 1.289± 0.154 | 0.053± 0.021 | 0.053± 0.015 | 0.106± 0.035 |

**Table S3.** Results of the relative organ weight for each animal of the dose-finding study (F1-F5). The table collects the individual weights (g) of each organ for each animal and the descriptive statistics of the whole group expressed as mean ± standard deviation. The normality ranges of each parameter (bold letter) were calculated from the laboratory histories (n = 57 animals). •: values outside the laboratory records.

| Animal ID | Spleen **0.138–0.289** | Heart **0.198–0.489** | Liver **2.651–4.128** | Thymus **0.069–0.285** | Right kidney | Left kidney | Kidneys sum **0.546–0.906** | Right ovary | Left ovary | Ovaries sum **0.030–0.096** |
|---|---|---|---|---|---|---|---|---|---|---|
| F1 | 0.223 | 0.360 | 3.038 | 0.230 | 0.369 | 0.373 | 0.742 | 0.019 | 0.027 | 0.045 |
| F2 | 0.247 | 0.316 | 3.039 | 0.254 | 0.353 | 0.335 | 0.689 | 0.022 | 0.021 | 0.042 |
| F3 | 0.240 | 0.321 | 3.431 | 0.269 | 0.422 | 0.404 | 0.826 | 0.039 | 0.041 | 0.081 |
| F4 | 0.261 | 0.320 | 3.718 | 0.292• | 0.411 | 0.356 | 0.768 | 0.035 | 0.029 | 0.063 |
| F5 | 0.231 | 0.320 | 3.011 | 0.316• | 0.394 | 0.395 | 0.789 | 0.042 | 0.037 | 0.079 |
| Mean ± SD | 0.241± 0.015 | 0.327± 0.018 | 3.247± 0.316 | 0.272± 0.033 | 0.390± 0.029 | 0.373± 0.028 | 0.763± 0.052 | 0.031± 0.011 | 0.031± 0.008 | 0.062± 0.018 |

## Hematological parameters

**Table S4.** Results of the hematological parameters for each animal of the dose-finding study (F1–F5). The table collects the individual values of each parameter determined for each animal and the descriptive statistics of the whole group expressed as mean ± standard deviation. The normality ranges of each parameter (bold letter) were calculated from the laboratory histories (n = 240 animals).

| Animal ID | RBC (x10⁶cel/µL) **7.53–9.73** | WBC (x10³cel/µL) **3.51–12.05** | Hemoglobin (g/dL) **14.5–17.5** | Htc (%) **41.3–50.1** | MCV (fl) **46.2–59.8** | MCH (pg) **16.9–20.3** | CHMC (g/dL) **32.8–37.2** | Platelets (x10³cel/µL) **582–1203** |
|---|---|---|---|---|---|---|---|---|
| F1 | 8.52 | 3.73 | 15.5 | 43.9 | 51.5 | 18.2 | 35.3 | 844 |
| F2 | 8.68 | 9.42 | 15.9 | 43.9 | 50.6 | 18.3 | 36.2 | 843 |
| F3 | 8.05 | 8.64 | 15.8 | 45.8 | 56.9 | 19.6 | 34.5 | 839 |
| F4 | 8.07 | 6.24 | 15.3 | 43.3 | 53.7 | 19.0 | 35.3 | 673 |
| F5 | 8.31 | 8.28 | 15.7 | 44.4 | 53.4 | 18.9 | 35.4 | 861 |
| Mean ± SD | 8.33±0.28 | 7.26±2.3 | 15.6±0.2 | 44.3±0.9 | 53.2±2.4 | 18.8±.06 | 35.3±0.6 | 812±78 |

NC: negative control; RV: reversion. (): measurement unit. RBC: red blood cell count, WBC: white blood cell count, Hb: hemoglobin, Htc: hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, CHMC: corpuscular hemoglobin mean concentration.

## Biochemical parameters

**Table S5.** Results of the biochemical evaluation for each animal of the dose-finding study (F1-F5). The table collects the group mean and standard deviation of each parameter evaluated. Normality ranges (bold letter) were calculated from the laboratory histories (n = 240 animals). •: values outside the laboratory records.

| Animal ID | Albumin (g/dL) **4.0–5.3** | AST (U/L) **39–138** | ALT (U/L) **10–53** | ALP (U/L) **13–138** | Cholesterol (mg/dL) **43–109** | Creatinine (mg/dL) **0.22–0.56** | Total Protein (g/dL) **5.4–7.0** | Urea (mg/dL) **26–59** |
|---|---|---|---|---|---|---|---|---|
| F1 | 4.1 | 247• | 34 | 110 | 59 | 0.38 | 5.5 | 34 |
| F2 | 4.1 | 74 | 15 | 104 | 54 | 0.30 | 5.4 | 39 |
| F3 | 4.6 | 99 | 22 | 93 | 88 | 0.31 | 6.0 | 40 |
| F4 | 4.2 | 64 | 19 | 94 | 98 | 0.33 | 5.8 | 34 |
| F5 | 4.3 | 193• | 32 | 104 | 61 | 0.36 | 6.0 | 32 |
| Mean ± SD | 4.3±0.2 | 135±81 | 24±8 | 101±7 | 72±20 | 0.34±0.03 | 5.7±0.3 | 36±3 |

NC: negative control; RV: reversion; (): measurement unit. AST: aspartate transaminase, ALT: alanine transaminase, ALP: alkaline phosphatase.

# Repeated dose 28-day oral toxicity study

## Weight growth gain

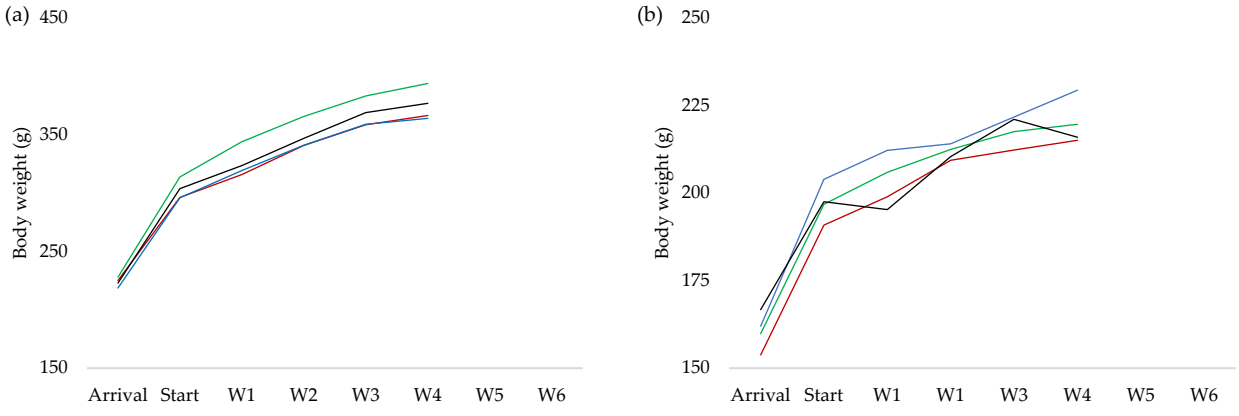



**Figure S2.** Results of the body weight growth from males (**a**) and females (**b**) of the 28-day oral toxicity study. Each figure shows in lines the mean body weight (g), for each principal study group, throughout the weeks (W) of the study (red: 2000 mg/kg b.w., blue: 300 mg/kg b.w., green 50 mg/kg b.w., black: negative control). Between arrival and start, 12 acclimatization days elapsed. Start: first day of administration.

## Hematological parameters

**Table S6.** Results of the hematological evaluation of the repeated-dose 28-day study. The table collects the group mean ± standard deviation and the statistical significance for each parameter evaluated. According to the levels of significance: (*: p <0.05).

| | RBC ($x10^6$ cel/ml) | WBC ($x10^3$ cel/ml) | Hb (g/dl) | Htc (%) | MCV (fl) | MCH (pg) | MCHC (g/dl) | Platelets ($x10^3$ cel/ml) | Ret (%) |
|---|---|---|---|---|---|---|---|---|---|
| Females | | | | | | | | | |
| NC | 8.24 ± 0.33 | 5.32 ± 1.24 | 15.0 ± 0.6 | 42.8 ± 1.9 | 51.9 ± 0.7 | 18.2 ± 0.1 | 35.0 ± 0.5 | 803 ± 98 | 2 ± 1 |
| 50 mg/kg | 8.05 ± 0.27 | 3.66 ± 0.92 | 14.9 ± 0.3 | 42.1 ± 0. | 52.3 ± 1.3 | 18.5 ± 0.5 | 35.3 ± 1.0 | 652 ± 159 | 1 ± 1 |
| 300 mg/kg | 8.12 ± 0.20 | 2.72 ± 1.18* | 14.9 ± 0.4 | 42.3 ± 1.1 | 52.1 ± 0.5 | 18.3 ± 0.5 | 35.2 ± 0.6 | 733 ± 129 | 1 ± 0 |
| 2000 mg/kg | 8.22 ± 0.50 | 3.71 ± 0.60 | 15.1 ± 0.7 | 43.1 ± 2.3 | 52.5 ± 1.3 | 18.4 ± 0.8 | 35.1 ± 1.4 | 655 ± 135 | 1 ± 1 |
| Males | | | | | | | | | |
| NC | 9.17 ± 0.35 | 5.66 ± 0.94 | 16.1 ± 0.3 | 44.6 ± 0.5 | 48.7 ± 1.4 | 17.5 ± 0.7 | 36.1 ± 0.7 | 712± 59 | 1 ± 1 |
| 50 mg/kg | 9.19 ± 0.51 | 6.24 ± 1.06 | 16.3 ± 0.7 | 45.4 ± 2.4 | 49.5 ± 0.4 | 17.8 ± 0.2 | 35.9 ± 0.4 | 832 ± 72 | 2 ± 1 |
| 300 mg/kg | 8.87 ± 0.46 | 6.68 ± 0.21 | 16.4 ± 0.6 | 44.9 ± 1.6 | 50.6 ± 1.8 | 18.5 ± 0.6* | 36.6 ± 0.2 | 830 ± 118 | 1 ± 1 |
| 2000 mg/kg | 9.44 ± 0.44 | 6.60 ± 1.09 | 16.7 ± 0.4 | 47.2 ± 1.6 | 50.1 ± 1.4 | 17.7 ± 0.5 | 35.4 ± 0.8 | 683 ± 193 | 1 ± 1 |

NC: negative control, (): measurement unit. RBC: red blood cell count, WBC: white blood cell count, Hb: hemoglobin, Htc: hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, CHMC: corpuscular hemoglobin mean concentration.

**Table S7.** Results of the absolute and differential count of the repeated-dose 28-day study. The table collects the group mean ± standard deviation and the statistical significance for each parameter evaluated. According to the levels of significance: (*: p <0.05).

| | Eosinophils | | Neutrophils | | Lymphocytes | | Monocytes | | Basophils | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $x10^3$ cell/mL | % | $x10^3$ cell/mL | % | $x10^3$ cell/mL | % | $x10^3$ cell/mL | % | $x10^3$ cell/mL | % |
| Females | | | | | | | | | | |
| NC | 0.06 ± 0.02 | 1.4 ± 0.7 | 0.58 ± 0.24 | 12.4 ± 4,6 | 4.00 ± 1,33 | 82.1 ± 6,3 | 0.21 ± 0.17 | 4.1 ± 2.8 | 0.00 ± 0.00 | 0.0 ± 0.0 |
| 50 mg/kg | 0.04 ± 0.01* | 1.2 ± 0.4 | 0.83 ± 0.06 | 25.4 ± 2,9 | 2.31 ± 0.48* | 69.6 ± 3,4 | 0.13 ± 0.04 | 3.9 ± 1.1 | 0.00 ± 0.00 | 0.0 ± 0.0 |
| 300 mg/kg | 0.04 ± 0.01 | 2.0 ± 1.0 | 0.49 ± 0.10 | 22.3 ± 14,2 | 2.07 ± 1,12* | 71.5 ± 16,0 | 0.11 ± 0.04 | 4.3 ± 1.4 | 0.00 ± 0.00 | 0.0 ± 0.0 |
| 2000 mg/kg | 0.07 ± 0.02 | 1.7 ± 0.4 | 0.64 ± 0.12 | 16.1 ± 2,8 | 3.07 ± 0.31 | 78.0 ± 3,5 | 0.17 ± 0.02 | 4.2 ± 0.8 | 0.00 ± 0.00 | 0.0 ± 0.0 |
| Males | | | | | | | | | | |
| NC | 0.12 ± 0.11 | 1.9 ± 1.8 | 1.19 ± 0.42 | 19.5 ± 5.7 | 4.60 ± 1.17 | 73.6 ± 6.4 | 0.30 ± 0.12 | 5.0 ± 1.9 | 0.00 ± 0.00 | 0.0 ± 0.0 |
| 50 mg/kg | 0.08 ± 0.05 | 1.2 ± 0.6 | 1.42 ± 0.64 | 20.9 ± 8.2 | 4.78 ± 0.99 | 71.8 ± 9.9 | 0.40 ± 0.12 | 6.0 ± 1.5 | 0.00 ± 0.00 | 0.0 ± 0.0 |
| 300 mg/kg | 0.06 ± 0.02 | 1.0 ± 0.5 | 0.86 ± 0.12 | 14.2 ± 3.1 | 5.01 ± 1.05 | 79.8 ± 3.3 | 0.31 ± 0.06 | 3.9 ± 2.1 | 0.00 ± 0.00 | 0.0 ± 0.0 |
| 2000 mg/kg | 0.09 ± 0.06 | 1.5 ± 0.9 | 0.96 ± 0.32 | 16.6 ± 5.5 | 4.62 ± 0.99 | 77.8 ± 7.0 | 0.23 ± 0.16 | 4.3 ± 3.4 | 0.00 ± 0.00 | 0.0 ± 0.0 |

NC: negative control; (): measurement unit.

## Biochemical parameters

**Table S8.** Results of the biochemical analysis of the repeated-dose 28-day study. The table collects the group mean ± standard deviation and the statistical significance of each parameter evaluated. According to the levels of significance: (*: p <0.05), (**: p<0.01), (****: p<0.001).

| | Females | | | |
|---|---|---|---|---|
| | NC | 50 mg/kg | 300 mg/kg | 2000 mg/kg |
| ALB (g/dL) | 4.3 ± 0.2 | 4.7 ± 0.4 | 4.2 ± 0.2 | 4.3 ± 0.2 |
| Urea (mg/dL) | 33 ± 4 | 38 ± 4 | 34 ± 12 | 30 ± 2 |
| AST (U/L) | 77 ± 10 | 82 ± 6 | 89 ± 4* | 92 ± 3** |
| ALT (U/L) | 23 ± 2 | 18 ± 2** | 22 ± 2 | 22 ± 4 |
| ALP (U/L) | 108 ± 15 | 47 ± 6** | 106 ± 24 | 104 ± 17 |
| BIL-T (mg/dL) | 0.11 ± 0.03 | 0.09 ± 0.02 | 0.10 ± 0.02 | 0.11 ± 0.03 |
| CHOL (mg/dL) | 77 ± 11 | 74 ± 16 | 70 ± 5 | 78 ± 7 |
| GLU (mg/dL) | 107 ± 11 | 111 ± 22 | 115 ± 22 | 102 ±16 |
| CREA (mg/dL) | 0.34 ± 0.02 | 0.40 ± 0.08 | 0.35 ± 0.05 | 0.34 ± 0.05 |
| TP (g/dL) | 6.2 ± 0.3 | 6.2 ± 0.3 | 6.0 ± 0.2 | 6.0 ± 0.3 |
| CPK (U/L) | 546 ± 219 | 525 ± 76 | 756 ± 100 | 726 ± 102 |
| Ca (mg/dL) | 9.94 ± 0.31 | 10.20 ± 0.25 | 10.19 ± 0.18 | 10.15 ± 0.16 |
| TG (mg/dL) | 36 ± 11 | 54 ± 3 | 35 ± 10* | 37 ± 7 |
| Cl (mg/dL) | 93.86 ± 1.24 | 101.9 ± 1.0**** | 102.3 ± 0.6**** | 105.5 ± 5.4**** |
| K (mg/dL) | 4.28 ± 0.20 | 4.50 ± 0.31 | 4.56 ± 0.31 | 4.94 ± 0.24 |
| Na (mg/dL) | 132 ± 9 | 145 ± 2* | 140 ± 8 | 145 ± 8* |
| Glob (g/dL) | 2.0 ± 0.2 | 1.7 ± 0.1 | 1.8 ± 0.2 | 1.7 ± 0.1 |

NC: negative control; (): measurement unit. ALB: Albumin, AST: aspartate transaminase, ALT: alanine transaminase, ALP: alkaline phosphatase, BIL-T total bilirubin, CHOL: total cholesterol, GLU: glucose, CREA: creatinine. TP: total protein, CPK: creatine phosphokinase, Ca: calcium, TG: triglycerides, Cl: chlorine, K: potassium, Na: sodium, Glob: globulin.

**Table S8. Cont.** Results of the biochemical analysis of the repeated-dose 28-day study. The table collects the group mean ± standard deviation and the statistical significance of each parameter evaluated. According to the levels of significance: (*: p <0.05), (**: p<0.01), (***: p <0.005), (****: p<0.001).

| | Males | | | |
|---|---|---|---|---|
| | NC | 50 mg/kg | 300 mg/kg | 2000 mg/kg |
| ALB (g/dL) | 4.7 ± 0.3 | 4.3 ± 0.1 | 4.8 ± 0.3 | 4.8 ± 0.4 |
| Urea (mg/dL) | 40 ± 7 | 37 ± 2 | 37 ± 4 | 38 ± 4 |
| AST (U/L) | 84 ± 17 | 93 ± 8 | 72 ± 10* | 91 ± 24** |
| ALT (U/L) | 21 ± 4 | 22 ± 5 | 18 ± 3 | 18 ± 5 |
| ALP (U/L) | 48 ± 8 | 92 ± 17**** | 58 ± 14 | 47 ± 9 |
| BIL-T (mg/dL) | 0.13 ± 0.02 | 0.11 ± 0.01 | 0.10 ± 0.02 | 0.11 ± 0.02 |
| CHOL (mg/dL) | 69 ± 4 | 81 ± 11 | 75 ± 16 | 68 ± 18 |
| GLU (mg/dL) | 95 ± 12 | 107 ± 17 | 103 ± 17 | 84 ± 10 |
| CREA (mg/dL) | 0.43 ± 0.05 | 0.40 ± 0.08** | 0.43 ± 0.05 | 0.43 ± 0.05 |
| TP (g/dL) | 6.4 ± 0.3 | 6.2 ± 0.2 | 6.3 ± 0.4 | 6.5 ± 0.2 |
| CPK (U/L) | 504 ± 147 | 770 ± 153 | 407 ± 84 | 633 ± 293 |
| Ca (mg/dL) | 9.72 ± 0.43 | 10.38 ± 0.42 | 10.39 ± 0.56 | 10.24 ± 0.12 |
| TG (mg/dL) | 36 ± 11 | 54 ±13* | 54 ± 13 | 54 ± 13 |
| Cl (mg/dL) | 95.6 ± 0.8 | 101.86 ±1.36**** | 104.72 ± 1.63*** | 103.94 ± 2.61** |
| K (mg/dL) | 4.02 ± 0.29 | 3.91 ± 0.13 | 4.19 ± 0.28 | 4.28 ± 0.21 |
| Na (mg/dL) | 133 ± 3 | 142 ±1** | 135 ± 3 | 138 ± 4 |
| Glob (g/dL) | 1.7 ± 0.2 | 1.9 ± 0.1 | 1.6 ± 0.2 | 1.7 ± 0.2 |

NC: negative control; (): measurement unit. ALB: Albumin, AST: aspartate transaminase, ALT: alanine transaminase, ALP: alkaline phosphatase, BIL-T total bilirubin, CHOL: total cholesterol, GLU: glucose, CREA: creatinine. TP: total protein, CPK: creatine phosphokinase, Ca: calcium, TG: triglycerides, Cl: chlorine, K: potassium, Na: sodium, Glob: globulin.

## Coagulation parameters

**Table S9.** Results from the coagulation analysis of the repeated-dose 28-day study. The table collects the group mean ± standard deviation and the statistical significance of each parameter evaluated. According to the levels of significance: (*: p <0.05), (**: p<0.01).

| | Males | | | Females | | |
|---|---|---|---|---|---|---|
| Group | Fibrinogen (mg/dL) | PT (sg) | aPTT (sg) | Fibrinogen (mg/dL) | PT (sg) | aPTT (sg) |
| NC | 209.6 ± 9.8 | 17.0 ± 0.8 | 29.0 ± 4.9 | 149.2 ± 9.5 | 16.4 ± 1.1 | 29.8 ± 5.3 |
| 50 mg/kg | 215.7 ± 10.6 | 14.9 ± 0.3** | 28.6 ± 1.3 | 179.2 ± 23.2* | 13.9 ± 0.3 | 31.1 ± 3.5 |
| 300 mg/kg | 233.6 ± 17.7 | 16.3 ± 3.0 | 30.4 ± 13.0 | 186.0 ± 12.2** | 14.4 ± 0.6 | 24.7 ± 3.4 |
| 2000 mg/kg | 220.3 ± 19.4 | 14.8 ± 1.1* | 24.6 ± 3.3 | 139.5 ± 39.4 | 12.8 ± 1.8* | 24.4 ± 9.3 |

NC: negative control; (): measurement unit. PT prothrombin time, aPTT: activated partial thromboplastin time.