Supplementary Materials: Pueraria *mirifica* Exerts Estrogenic Effects in the Mammary Gland and Uterus and Promotes Mammary Carcinogenesis in   
Donryu Rats

Anna Kakehashi, Midori Yoshida, Yoshiyuki Tago, Naomi Ishii, Min Gi and Hideki Wanibuchi

**Table S1.** Final body, relative organ weights and chemicals intakes of Donryu rats in Exp. 3.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Treament** | **No. Rats a** | **Final Body Weight (g)** | **Food Consumption (g/rat/day)** | **Chemical Intake** | | | | **Total Chemical Intake (g/rat)** |
| **(mg/rat/day)** | | **(mg/kg b.w./day)** | |
| DMBA, ENNG | 21 | 375.5 ± 89.4 | 17.5 ± 3.6 | 0 ± 0 | | 0 ± 0 | | 0 ± 0 |
| DMBA, ENNG → PM, 0.03% | 21 | 344.1 ± 77.6 | 17.5 ± 5.8 | 5 ± 2 | | 21 ± 13 | | 1.4 |
| DMBA, ENNG → PM, 0.3% | 21 | 306.8 ± 82.1 \* | 19.2 ± 8.4 | 58 ± 25 | | 263 ± 176 | | 14.9 |
| DMBA, ENNG → PM 1% | 21 | 263.3 ± 49.0 \*\*\* | 17.9 ± 2.5 | 179 ± 25 | | 814 ± 171 | | 45.1 |
| DMBA, ENNG → IA, 0.2% | 21 | 323.8 ± 65.7 | 18.2 ± 4.9 | 36 ± 10 | | 160 ± 95 | | 9.4 |
| Vehicle | 5 | 372.1 ± 42.9 | 19.6 ± 3.4 | 0 ± 0 | | 0 ± 0 | | 0 ± 0 |
| Vehicle → PM, 1% | 6 | 237.3 ± 30.9 # | 15.4 ± 3.6 | 159 ± 23 | | 728 ± 104 | | 40.1 |
| **Treatment** | **No. Rats** | **Uterus (%)** | **Liver (%)** | **Kidneys (%)** | **Spleen (%)** | | **Thymus (%)** | **Adrenals (%)** |
| DMBA, ENNG | 21 | 0.33 ± 0.03 | 4.20 ± 1.92 | 0.64 ± 0.17 | 0.51 ± 1.37 | | 0.075 ± 0.091 | 0.026 ± 0.023 |
| DMBA, ENNG → PM, 0.03% | 21 | 0.41 ± 0.07 | 3.70 ± 0.79 | 0.64 ± 0.11 | 0.34 ± 0.28 | | 0.186 ± 0.144 \*\* | 0.022 ± 0.006 |
| DMBA, ENNG → PM, 0.3% | 21 | 0.47 ± 0.06 \* | 3.84 ± 0.74 \* | 0.73 ± 0.14 | 0.22 ± 0.06 | | 0.046 ± 0.021 | 0.024 ± 0.007 |
| DMBA, ENNG → PM 1% | 21 | 1.35 ± 0.52 \* | 3.73 ± 0.60 \*\*\* | 0.79 ± 0.14 \*\*\* | 0.37 ± 0.48 | | 0.048 ± 0.018 | 0.031 ± 0.016 |
| DMBA, ENNG → IA, 0.2% | 21 | 0.36 ± 0.04 | 3.82 ± 1.51 | 0.66 ± 0.10 | 0.35 ± 0.31 | | 0.054 ± 0.019 | 0.023 ± 0.005 |
| Vehicle | 5 | 0.27 ± 0.02 | 2.66 ± 0.26 | 0.58 ± 0.05 | 0.17 ± 0.03 | | 0.063 ± 0.022 | 0.016 ± 0.003 |
| Vehicle → PM, 1% | 6 | 0.99 ± 0.71 | 3.20 ± 0.16 | 0.81 ± 0.07 # | 0.19 ± 0.03 | | 0.065 ± 0.014 | 0.022 ± 0.003 ## |

\* *p* < 0.05; \*\* *p* < 0.01; \*\*\* *p* < 0.001 vs. DMBA, ENNG group; # *p* < 0.05; ## *p* < 0.01 vs. vehicle control group.

**Table S2.** Results of the hematological and blood biochemical analyses.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Test Compound** | **DMBA, ENNG** | | | | | **Vehicle** | |
| **PM** | | | | **IA** | **Vehicle** | **PM** |
| Dose of test compound (%) | 0 | 0.03 | 0.3 | 1 | 0.2 | 0 | 1 |
| No. of animals examined | 17 | 15 | 13 | 19 | 15 | 5 | 6 |
| WBC (/μL) | 4550 ± 1777 | 6479 ± 8488 | 4525 ± 2235 | 4141 ± 2463 | 7547 ± 12,949 | 3600 ± 1706 | 3320 ± 996 |
| RBC (×104/μL) | 593.0 ± 95.6 # | 589.4 ± 112.3 | 635.1 ± 125.7 | 612.7 ± 113.8 | 625.9 ± 185.1 | 770.4 ± 32.6 | 728.6 ± 31.9 |
| Hb (g/dL) | 11.7 ± 1.6 # | 11.6 ± 2.6 | 11.94 ± 2.0 | 11.6 ± 2.0 | 11.8 ± 3.4 | 14.1 ± 0.6 | 13.9 ± 1.1 |
| Ht (%) | 38.0 ± 4.7 # | 35.7 ± 7.3 | 37.9 ± 5.8 | 36.6 ± 6.0 | 37.6 ± 10.1 | 44.7 ± 0.9 | 42.1 ± 1.8 # |
| MCV (fL) | 64.6 ± 4.2 | 60.6 ± 5.0 | 60.4 ± 4.6 | 60.1 ± 3.3 | 62.3 ± 8.2 | 58.2 ± 1.8 | 57.8 ± 0.8 |
| MCH (pg) | 19.9 ± 1.0 | 19.9 ± 5.7 | 18.9 ± 1.0 | 19.0 ± 0.8 | 19.3 ± 2.4 | 18.4 ± 0.8 | 19.1 ± 1.1 |
| MCHC (g/dL) | 30.8 ± 0.8 | 32.8 ± 8.0 | 31.4 ± 1.2 | 31.7 ± 0.9 | 31.1 ± 1.9 | 31.6 ± 0.9 | 32.9 ± 2.0 |
| Platelets (×1010/L) | 76.7 ± 13.0 ## | 57.4 ± 24.2 \* | 39.1 ± 31.9 \*\* | 54.2 ± 25.5 \*\* | 56.2 ± 28.5 | 38.5 ± 20.0 | 56.4 ± 15.8 |
| Neutrophils (×103/L) | 32.2 ± 17.0 | 42.1 ± 23.9 | 25.8 ± 15.1 | 34.3 ± 14.4 | 31.9 ± 15.7 | 30.0 ± 5.6 | 42.8 ± 8.1 # |
| Band neutrophils (×103/L) | 1.4 ± 0.6 | 1.1 ± 0.4 | 1.2 ± 0.4 | 1.1 ± 0.2 | 1.4 ± 0.9 | 1.0 ± 0.0 | 1.0 ± 0.0 |
| Eosinophils (×103/L) | 1.2 ± 1.2 | 0.9 ± 1.2 | 0.7 ± 1.0 | 0.7 ± 1.6 | 0.7 ± 1.3 | 1.0 ± 0.7 | 0.6 ± 0.5 |
| Basophils (×103/L) | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Monocytes (×103/L) | 2.9 ± 1.4 | 2.4 ± 1.5 | 3.5 ± 1.3 | 3.9 ± 2.3 | 2.8 ± 1.8 | 3.0 ± 1.6 | 3.8 ± 1.5 |
| Lymphocytes (×103/L) | 62.3 ± 16.7 | 53.5 ± 24.0 | 68.8 ± 15.8 | 60.1 ± 14.0 | 62.7 ± 16.2 | 65.0 ± 6.7 | 51.6 ± 9.8 # |
| T-protein (g/dL) | 7.3 ± 1.0 | 6.7 ± 0.6 | 7.1 ± 0.5 | 6.8 ± 0.8 | 7.2 ± 0.4 | 7.5 ± 0.4 | 7.6 ± 0.5 |
| Albumin (g/dL) | 5.4 ± 0.5 | 4.8 ± 0.8 | 5.2 ± 0.6 | 4.9 ± 0.8 | 5.3 ± 0.5 | 5.1 ± 0.4 | 5.4 ± 0.4 |
| A/G ratio | 3.2 ± 0.9 | 2.6 ± 0.8 | 3.0 ± 0.9 | 2.6 ± 0.7 | 3.0 ± 0.7 | 2.1 ± 0.4 | 2.6 ± 0.5 |
| T-BiL (mg/dL) | 0.10 ± 0.00 | 0.10 ± 0.00 | 0.10 ± 0.00 | 0.11 ± 0.02 | 0.11 ± 0.03 | 0.14 ± 0.1 | 0.10 ± 0.00 |
| AST (IU/L) | 428.1 ± 216.9 | 425.1 ± 528.6 | 378.2 ± 295.2 | 263.7 ± 132.6 \* | 375.2 ± 296.6 | 467.0 ± 105.7 | 223.2 ± 19.4 ## |
| ALT (IU/L) | 62.7 ± 39.8 | 62.8 ± 77.6 | 87.4 ± 84.0 | 55.6 ± 28.8 | 88.8 ± 89.1 | 96.2 ± 41.1 | 55.7 ± 12.0 # |
| ALP (IU/L) | 62.0 ± 34.0 | 147.7 ± 253.6 | 73.6 ± 18.3 | 99.7 ± 58.6 \* | 77.5 ± 38.3 | 163.9 ± 98.1 | 125.3 ± 51.0 |
| γ-GTP (IU/L) | 2.1 ± 3.1 | 3.0 ± 2.9 | 2.5 ± 1.6 | 5.5 ± 11.7 \*\* | 3.9 ± 2.9 \*\* | 1.0 ± 0.0 | 2.2 ± 1.0 # |
| T-Cholesterol (mg/dL) | 141.5 ± 35.4 # | 123.5 ± 48.2 | 123.0 ± 30.0 | 105.3 ± 27.9 \* | 130.0 ± 36.9 | 191.6 ± 39.0 | 116.7 ± 31.8 ## |
| TG (mg/dL) | 90.6 ± 51.5 # | 76.7 ± 44.7 | 65.5 ± 60.2 | 68.1 ± 46.3 | 111.9 ± 169.2 | 47.4 ± 19.2 | 39.7 ± 28.4 |
| BUN (mg/dL) | 22.3 ± 4.1 # | 25.1 ± 7.2 | 24.2 ± 4.0 | 27.1 ± 9.9 | 22.0 ± 7.0 | 17.2 ± 2.2 | 23.7 ± 4.4 # |
| Creatinine (mg/dL) | 0.31 ± 0.07 | 0.33 ± 0.05 | 0.35 ± 0.06 | 0.29 ± 0.08 | 0.29 ± 0.04 | 0.37 ± 0.05 | 0.36 ± 0.03 |
| Na (mEq/L) | 143.9 ± 1.4 | 143.9 ± 1.3 | 143.0 ± 2.7 | 143.4 ± 1.8 | 143.4 ± 1.8 | 143.6 ± 1.9 | 144.7 ± 1.6 |
| K (mEq/L) | 5.0 ± 0.9 | 5.1 ± 0.5 | 5.6 ± 3.7 | 4.8 ± 0.9 | 4.9 ± 0.7 | 4.8 ± 0.3 | 4.6 ± 0.4 |
| Cl (mEq/L) | 100.1 ± 1.7 | 100.5 ± 1.7 | 99.8 ± 1.6 | 100.2 ± 2.0 | 100.5 ± 2.1 | 98.4 ± 1.9 | 99.2 ± 1.8 |
| Ca (mEq/L) | 9.9 ± 0.4 | 9.7 ± 0.4 | 9.7 ± 0.4 | 9.4 ± 0.4 \*\*\* | 9.6 ± 0.3 | 10.2 ± 0.5 | 9.7 ± 0.4 |
| IP (mEq/L) | 6.8 ± 1.6 | 7.2 ± 1.4 | 6.3 ± 2.5 | 5.9 ± 1.2 | 5.5 ± 1.7 | 7.9 ± 3.2 | 6.4 ± 1.1 |

Values are means ± SD; \* *p* < 0.05;\*\* *p* < 0.01; \*\*\* *p* < 0.001 vs. DMBA, ENNG initiation control group; # *p* < 0.05; ## *p* < 0.01 vs. vehicle control group; TG, triglycerides; T-Bil, T-bilirubin; IP, inorganic phosphorus.