

## Supplementary Material

# Polyphenol microbial metabolites exhibit gut and blood-brain barrier permeability and protect murine microglia against LPS-induced inflammation

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Received: date; Accepted: date; Published: date

**Table S1.** Effect of isoflavones (GEN, DAI, EQ) on NOS production. One-way ANOVA followed by Tukey's multiple comparison procedure. GEN 20  $\mu$ M comparisons in bold.

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Control vs. LPS (1 $\mu$ g/mL)	-36.28	-43.18 to -29.38	Yes	****	<0.0001
Control vs. GEN (20 $\mu$ M)	-10.11	-17.27 to -2.941	Yes	**	0.0011
Control vs. GEN (10 $\mu$ M)	-21.87	-29.04 to -14.7	Yes	****	<0.0001
Control vs. DAI (20 $\mu$ M)	-27.05	-34.22 to -19.89	Yes	****	<0.0001
Control vs. DAI (10 $\mu$ M)	-32.15	-39.31 to -24.98	Yes	****	<0.0001
Control vs. EQ (20 $\mu$ M)	-27.81	-34.97 to -20.64	Yes	****	<0.0001
Control vs. EQ (10 $\mu$ M)	-30.06	-37.23 to -22.9	Yes	****	<0.0001
LPS (1 $\mu$ g/mL) vs. GEN (20 $\mu$ M)	26.17	18.64 to 33.7	Yes	****	<0.0001
LPS (1 $\mu$ g/mL) vs. GEN (10 $\mu$ M)	14.41	6.88 to 21.93	Yes	****	<0.0001
LPS (1 $\mu$ g/mL) vs. DAI (20 $\mu$ M)	9.223	1.697 to 16.75	Yes	**	0.0069
LPS (1 $\mu$ g/mL) vs. DAI (10 $\mu$ M)	4.129	-3.397 to 11.66	No	ns	0.6667
LPS (1 $\mu$ g/mL) vs. EQ (20 $\mu$ M)	8.47	0.9441 to 16	Yes	*	0.0173
LPS (1 $\mu$ g/mL) vs. EQ (10 $\mu$ M)	6.216	-1.311 to 13.74	No	ns	0.1766
<b>GEN (20 <math>\mu</math>M) vs. GEN (10 <math>\mu</math>M)</b>	<b>-11.76</b>	<b>-19.54 to -3.991</b>	<b>Yes</b>	<b>***</b>	<b>0.0004</b>
<b>GEN (20 <math>\mu</math>M) vs. DAI (20 <math>\mu</math>M)</b>	<b>-16.95</b>	<b>-24.72 to -9.174</b>	<b>Yes</b>	<b>****</b>	<b>&lt;0.0001</b>
<b>GEN (20 <math>\mu</math>M) vs. DAI (10 <math>\mu</math>M)</b>	<b>-22.04</b>	<b>-29.81 to -14.27</b>	<b>Yes</b>	<b>****</b>	<b>&lt;0.0001</b>
<b>GEN (20 <math>\mu</math>M) vs. EQ (20 <math>\mu</math>M)</b>	<b>-17.7</b>	<b>-25.47 to -9.927</b>	<b>Yes</b>	<b>****</b>	<b>&lt;0.0001</b>
<b>GEN (20 <math>\mu</math>M) vs. EQ (10 <math>\mu</math>M)</b>	<b>-19.95</b>	<b>-27.73 to -12.18</b>	<b>Yes</b>	<b>****</b>	<b>&lt;0.0001</b>
GEN (10 $\mu$ M) vs. DAI (20 $\mu$ M)	-5.184	-12.96 to 2.59	No	ns	0.425
GEN (10 $\mu$ M) vs. DAI (10 $\mu$ M)	-10.28	-18.05 to -2.504	Yes	**	0.0027
GEN (10 $\mu$ M) vs. EQ (20 $\mu$ M)	-5.936	-13.71 to 1.837	No	ns	0.2577
GEN (10 $\mu$ M) vs. EQ (10 $\mu$ M)	-8.191	-15.96 to -0.418	Yes	*	0.0322
DAI (20 $\mu$ M) vs. DAI (10 $\mu$ M)	-5.094	-12.87 to 2.679	No	ns	0.4477

DAI (20 $\mu$ M) vs. EQ (20 $\mu$ M)	-0.7528	-8.526 to 7.02	No	ns	>0.9999
DAI (20 $\mu$ M) vs. EQ (10 $\mu$ M)	-3.008	-10.78 to 4.766	No	ns	0.9217
DAI (10 $\mu$ M) vs. EQ (20 $\mu$ M)	4.341	-3.432 to 12.11	No	ns	0.6467
DAI (10 $\mu$ M) vs. EQ (10 $\mu$ M)	2.086	-5.687 to 9.859	No	ns	0.9893
EQ (20 $\mu$ M) vs. EQ (10 $\mu$ M)	-2.255	-10.03 to 5.518	No	ns	0.9832

**Table S2.** Effect of isoflavones (GEN, DAI, EQ) on TNF- $\alpha$  concentration. One-way ANOVA followed by Tukey's multiple comparison procedure. GEN 20  $\mu$ M comparisons in bold.

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Control vs. LPS (1 $\mu$ g/mL)	-572.2	-608.4 to -536	Yes	****	<0.0001
Control vs. EST (20 $\mu$ M)	-539.1	-581.6 to -496.7	Yes	****	<0.0001
Control vs. EST (10 $\mu$ M)	-545.6	-588.1 to -503.1	Yes	****	<0.0001
Control vs. GEN (20 $\mu$ M)	-372.4	-414.9 to -330	Yes	****	<0.0001
Control vs. GEN (10 $\mu$ M)	-458.4	-500.9 to -415.9	Yes	****	<0.0001
Control vs. DAI (20 $\mu$ M)	-501.7	-544.1 to -459.2	Yes	****	<0.0001
Control vs. DAI (10 $\mu$ M)	-514.3	-556.8 to -471.9	Yes	****	<0.0001
Control vs. EQ (20 $\mu$ M)	-499.5	-541.9 to -457	Yes	****	<0.0001
Control vs. EQ (10 $\mu$ M)	-515.2	-557.7 to -472.7	Yes	****	<0.0001
LPS (1 $\mu$ g/mL) vs. EST (20 $\mu$ M)	33.1	-9.363 to 75.55	No	ns	0.2428
LPS (1 $\mu$ g/mL) vs. EST (10 $\mu$ M)	26.62	-15.84 to 69.08	No	ns	0.5312
LPS (1 $\mu$ g/mL) vs. GEN (20 $\mu$ M)	199.8	157.3 to 242.2	Yes	****	<0.0001
LPS (1 $\mu$ g/mL) vs. GEN (10 $\mu$ M)	113.8	71.38 to 156.3	Yes	****	<0.0001
LPS (1 $\mu$ g/mL) vs. DAI (20 $\mu$ M)	70.55	28.09 to 113	Yes	****	<0.0001
LPS (1 $\mu$ g/mL) vs. DAI (10 $\mu$ M)	57.91	15.46 to 100.4	Yes	**	0.0019
LPS (1 $\mu$ g/mL) vs. EQ (20 $\mu$ M)	72.78	30.32 to 115.2	Yes	****	<0.0001
LPS (1 $\mu$ g/mL) vs. EQ (10 $\mu$ M)	57.03	14.57 to 99.49	Yes	**	0.0023
<b>GEN (20 <math>\mu</math>M) vs. GEN (10 <math>\mu</math>M)</b>	<b>-85.94</b>	<b>-133.8 to -38.04</b>	<b>Yes</b>	<b>****</b>	<b>&lt;0.0001</b>
<b>GEN (20 <math>\mu</math>M) vs. DAI (20 <math>\mu</math>M)</b>	<b>-129.2</b>	<b>-177.1 to -81.34</b>	<b>Yes</b>	<b>****</b>	<b>&lt;0.0001</b>
<b>GEN (20 <math>\mu</math>M) vs. DAI (10 <math>\mu</math>M)</b>	<b>-141.9</b>	<b>-189.8 to -93.97</b>	<b>Yes</b>	<b>****</b>	<b>&lt;0.0001</b>
<b>GEN (20 <math>\mu</math>M) vs. EQ (20 <math>\mu</math>M)</b>	<b>-127</b>	<b>-174.9 to -79.11</b>	<b>Yes</b>	<b>****</b>	<b>&lt;0.0001</b>
<b>GEN (20 <math>\mu</math>M) vs. EQ (10 <math>\mu</math>M)</b>	<b>-142.8</b>	<b>-190.6 to -94.85</b>	<b>Yes</b>	<b>****</b>	<b>&lt;0.0001</b>
GEN (10 $\mu$ M) vs. DAI (20 $\mu$ M)	-43.29	-91.19 to 4.608	No	ns	0.1051
GEN (10 $\mu$ M) vs. DAI (10 $\mu$ M)	-55.92	-103.8 to -8.024	Yes	*	0.0118
GEN (10 $\mu$ M) vs. EQ (20 $\mu$ M)	-41.06	-88.96 to 6.837	No	ns	0.1464
GEN (10 $\mu$ M) vs. EQ (10 $\mu$ M)	-56.81	-104.7 to -8.906	Yes	*	0.01
DAI (20 $\mu$ M) vs. DAI (10 $\mu$ M)	-12.63	-60.53 to 35.27	No	ns	0.9959
DAI (20 $\mu$ M) vs. EQ (20 $\mu$ M)	2.229	-45.67 to 50.13	No	ns	>0.9999
DAI (20 $\mu$ M) vs. EQ (10 $\mu$ M)	-13.51	-61.41 to 34.39	No	ns	0.9933
DAI (10 $\mu$ M) vs. EQ (20 $\mu$ M)	14.86	-33.04 to 62.76	No	ns	0.987
DAI (10 $\mu$ M) vs. EQ (10 $\mu$ M)	-0.8819	-48.78 to 47.02	No	ns	>0.9999
EQ (20 $\mu$ M) vs. EQ (10 $\mu$ M)	-15.74	-63.64 to 32.16	No	ns	0.9808

**Table S3.** Effect of isoflavones (GEN, DAI, EQ) on IL-6 concentration. One-way ANOVA followed by Tukey's multiple comparison procedure. GEN 20  $\mu$ M comparisons in bold.

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Control vs. LPS (1 $\mu$ g/mL)	-103.1	-112.4 to -93.84	Yes	****	<0.0001
Control vs. EST (20 $\mu$ M)	-83.66	-94.53 to -72.8	Yes	****	<0.0001
Control vs. EST (10 $\mu$ M)	-89.07	-99.94 to -78.21	Yes	****	<0.0001
Control vs. GEN (20 $\mu$ M)	-19.72	-30.59 to -8.858	Yes	****	<0.0001
Control vs. GEN (10 $\mu$ M)	-67.96	-78.82 to -57.09	Yes	****	<0.0001
Control vs. DAI (20 $\mu$ M)	-82.42	-93.29 to -71.56	Yes	****	<0.0001
Control vs. DAI (10 $\mu$ M)	-85.84	-96.71 to -74.98	Yes	****	<0.0001
Control vs. EQ (20 $\mu$ M)	-83.63	-94.49 to -72.76	Yes	****	<0.0001
Control vs. EQ (10 $\mu$ M)	-92.72	-103.6 to -81.85	Yes	****	<0.0001
LPS (1 $\mu$ g/mL) vs. EST (20 $\mu$ M)	19.44	8.575 to 30.31	Yes	****	<0.0001
LPS (1 $\mu$ g/mL) vs. EST (10 $\mu$ M)	14.03	3.169 to 24.9	Yes	**	0.0037
LPS (1 $\mu$ g/mL) vs. GEN (20 $\mu$ M)	83.38	72.52 to 94.25	Yes	****	<0.0001
LPS (1 $\mu$ g/mL) vs. GEN (10 $\mu$ M)	35.15	24.28 to 46.01	Yes	****	<0.0001
LPS (1 $\mu$ g/mL) vs. DAI (20 $\mu$ M)	20.68	9.818 to 31.55	Yes	****	<0.0001
LPS (1 $\mu$ g/mL) vs. DAI (10 $\mu$ M)	17.26	6.397 to 28.13	Yes	***	0.0002
LPS (1 $\mu$ g/mL) vs. EQ (20 $\mu$ M)	19.48	8.614 to 30.34	Yes	****	<0.0001
LPS (1 $\mu$ g/mL) vs. EQ (10 $\mu$ M)	10.39	-0.4786 to 21.25	No	ns	0.0709
<b>GEN (20 <math>\mu</math>M) vs. GEN (10 <math>\mu</math>M)</b>	<b>-48.23</b>	<b>-60.49 to -35.98</b>	<b>Yes</b>	<b>****</b>	<b>&lt;0.0001</b>
<b>GEN (20 <math>\mu</math>M) vs. DAI (20 <math>\mu</math>M)</b>	<b>-62.7</b>	<b>-74.96 to -50.44</b>	<b>Yes</b>	<b>****</b>	<b>&lt;0.0001</b>
<b>GEN (20 <math>\mu</math>M) vs. DAI (10 <math>\mu</math>M)</b>	<b>-66.12</b>	<b>-78.38 to -53.86</b>	<b>Yes</b>	<b>****</b>	<b>&lt;0.0001</b>
<b>GEN (20 <math>\mu</math>M) vs. EQ (20 <math>\mu</math>M)</b>	<b>-63.9</b>	<b>-76.16 to -51.64</b>	<b>Yes</b>	<b>****</b>	<b>&lt;0.0001</b>
<b>GEN (20 <math>\mu</math>M) vs. EQ (10 <math>\mu</math>M)</b>	<b>-72.99</b>	<b>-85.25 to -60.74</b>	<b>Yes</b>	<b>****</b>	<b>&lt;0.0001</b>
GEN (10 $\mu$ M) vs. DAI (20 $\mu$ M)	-14.46	-26.72 to -2.207	Yes	*	0.0106
GEN (10 $\mu$ M) vs. DAI (10 $\mu$ M)	-17.89	-30.14 to -5.628	Yes	***	0.0007
GEN (10 $\mu$ M) vs. EQ (20 $\mu$ M)	-15.67	-27.93 to -3.411	Yes	**	0.0042
GEN (10 $\mu$ M) vs. EQ (10 $\mu$ M)	-24.76	-37.02 to -12.5	Yes	****	<0.0001
DAI (20 $\mu$ M) vs. DAI (10 $\mu$ M)	-3.421	-15.68 to 8.837	No	ns	0.9938
DAI (20 $\mu$ M) vs. EQ (20 $\mu$ M)	-1.204	-13.46 to 11.05	No	ns	>0.9999
DAI (20 $\mu$ M) vs. EQ (10 $\mu$ M)	-10.3	-22.55 to 1.961	No	ns	0.1647
DAI (10 $\mu$ M) vs. EQ (20 $\mu$ M)	2.216	-10.04 to 14.47	No	ns	0.9998
DAI (10 $\mu$ M) vs. EQ (10 $\mu$ M)	-6.876	-19.13 to 5.382	No	ns	0.6755
EQ (20 $\mu$ M) vs. EQ (10 $\mu$ M)	-9.092	-21.35 to 3.165	No	ns	0.3028

**Table S4.** Effect of lignans (SECO, ED, EL) on NOS production. One-way ANOVA followed by Tukey's multiple comparison procedure. EL 10  $\mu$ M comparisons in bold.

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Control vs. LPS (1 $\mu$ g/mL)	-39.03	-42.1 to -35.97	Yes	****	<0.0001
Control vs. SECO (10 $\mu$ M)	-34.38	-37.44 to -31.32	Yes	****	<0.0001
Control vs. ED (10 $\mu$ M)	-34.68	-37.74 to -31.62	Yes	****	<0.0001
Control vs. EL (10 $\mu$ M)	-23.7	-26.76 to -20.64	Yes	****	<0.0001
LPS (1 $\mu$ g/mL) vs. SECO (10 $\mu$ M)	4.653	1.591 to 7.715	Yes	**	0.0016
LPS (1 $\mu$ g/mL) vs. ED (10 $\mu$ M)	4.354	1.292 to 7.417	Yes	**	0.0031
LPS (1 $\mu$ g/mL) vs. EL (10 $\mu$ M)	15.34	12.27 to 18.4	Yes	****	<0.0001
SECO (10 $\mu$ M) vs. ED (10 $\mu$ M)	-0.2985	-3.361 to 2.764	No	ns	0.9995
<b>SECO (10 <math>\mu</math>M) vs. EL (10 <math>\mu</math>M)</b>	<b>10.68</b>	<b>7.62 to 13.75</b>	<b>Yes</b>	<b>****</b>	<b>&lt;0.0001</b>
<b>ED (10 <math>\mu</math>M) vs. EL (10 <math>\mu</math>M)</b>	<b>10.98</b>	<b>7.919 to 14.04</b>	<b>Yes</b>	<b>****</b>	<b>&lt;0.0001</b>

**Table S5.** Effect of lignans (SECO, ED, EL) on TNF- $\alpha$  production. One-way ANOVA followed by Tukey's multiple comparison procedure. EL 10  $\mu$ M comparisons in bold.

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Control vs. LPS (1 $\mu$ g/mL)	-491.3	-523.1 to -459.4	Yes	****	<0.0001
Control vs. SECO (10 $\mu$ M)	-470.7	-502.5 to -438.8	Yes	****	<0.0001
Control vs. ED (10 $\mu$ M)	-488.6	-520.5 to -456.8	Yes	****	<0.0001
Control vs. EL (10 $\mu$ M)	-338.5	-370.3 to -306.7	Yes	****	<0.0001
LPS (1 $\mu$ g/mL) vs. SECO (10 $\mu$ M)	20.62	-11.21 to 52.44	No	ns	0.3557
LPS (1 $\mu$ g/mL) vs. ED (10 $\mu$ M)	2.638	-29.18 to 34.46	No	ns	0.9992
LPS (1 $\mu$ g/mL) vs. EL (10 $\mu$ M)	152.8	120.9 to 184.6	Yes	****	<0.0001
SECO (10 $\mu$ M) vs. ED (10 $\mu$ M)	-17.98	-49.8 to 13.84	No	ns	0.4925
<b>SECO (10 <math>\mu</math>M) vs. EL (10 <math>\mu</math>M)</b>	<b>132.1</b>	<b>100.3 to 164</b>	<b>Yes</b>	<b>****</b>	<b>&lt;0.0001</b>
<b>ED (10 <math>\mu</math>M) vs. EL (10 <math>\mu</math>M)</b>	<b>150.1</b>	<b>118.3 to 181.9</b>	<b>Yes</b>	<b>****</b>	<b>&lt;0.0001</b>

**Table S6.** Effect of lignans (SECO, ED, EL) on IL-6 production. One-way ANOVA followed by Tukey's multiple comparison procedure. EL 10  $\mu$ M comparisons in bold.

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
LPS (1 $\mu$ g/mL) vs. SECO (10 $\mu$ M)	-7.108	-44.93 to 30.72	No	ns	0.9553
LPS (1 $\mu$ g/mL) vs. ED (10 $\mu$ M)	-24.9	-62.72 to 12.93	No	ns	0.2958
LPS (1 $\mu$ g/mL) vs. EL (10 $\mu$ M)	175.8	138 to 213.6	Yes	****	<0.0001
SECO (10 $\mu$ M) vs. ED (10 $\mu$ M)	-17.79	-55.61 to 20.03	No	ns	0.5803
<b>SECO (10 <math>\mu</math>M) vs. EL (10 <math>\mu</math>M)</b>	<b>182.9</b>	<b>145.1 to 220.7</b>	<b>Yes</b>	<b>****</b>	<b>&lt;0.0001</b>
<b>ED (10 <math>\mu</math>M) vs. EL (10 <math>\mu</math>M)</b>	<b>200.7</b>	<b>162.9 to 238.5</b>	<b>Yes</b>	<b>****</b>	<b>&lt;0.0001</b>