

Original Article

Effects of glycyrrhizin, stevioside, and sucralose on the growth and metabolism of the omnipresent gut commensal *Escherichia coli*

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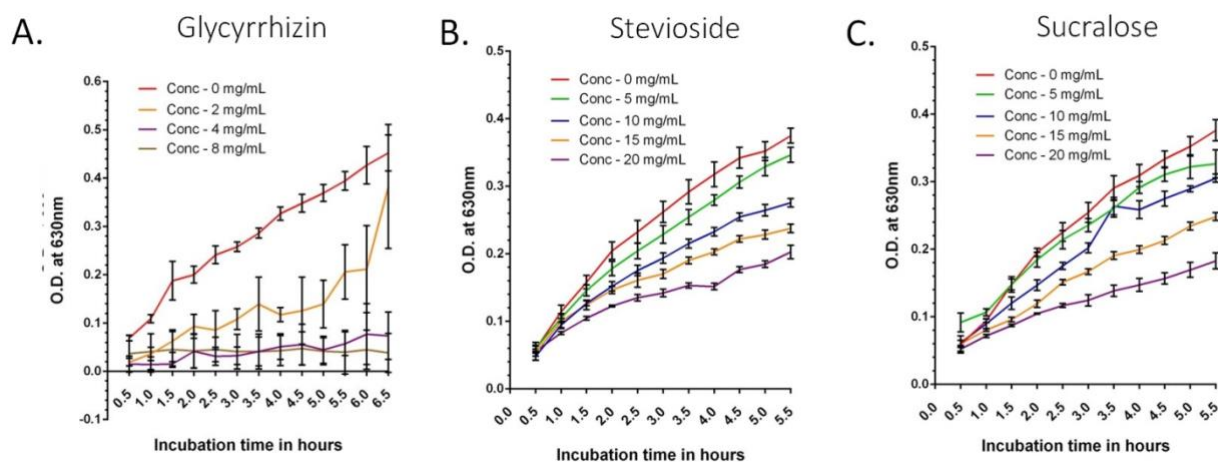
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SUPPLEMENTARY MATERIALS



Supplementary Figure 1. Growth of *E. coli* in LB media supplemented with a broad concentration range of NCS glycyrrhizin, stevioside, and sucralose. A. *E. coli* was cultured under a broad range of concentrations of glycyrrhizin (2 mg/ml, 4 mg/ml, and 8 mg/ml). At 2 mg/ml and above glycyrrhizin load, bacterial growth is inhibited with an increase in glycyrrhizin concentration. B. *E. coli* was cultured under a stevioside load with a broad range of concentrations (5 mg/ml, 10 mg/ml, 15 mg/ml, and 20 mg/ml). The higher the concentration of stevioside, the more bacterial growth was inhibited compared to the untreated control sample (0 mg/ml of stevioside). C. *E. coli* was cultured under a broad range of sucralose concentrations (5 mg/ml, 10 mg/ml, 15 mg/ml, and 20 mg/ml). The higher the concentration of the sweetener, the more bacterial growth was inhibited, with the least growth under 20 mg/ml sucralose load. Dunnett's multiple comparisons test was applied to compare the growth of NCS-treated cells at different time points relative to the untreated control cells. Data on statistical analyses are shown in supplementary tables 1, 2, and 3.

Supplementary Table 1. Growth of *E. coli* in LB medium supplemented with different concentrations of glycyrrhizin.

| Dunnett's multiple comparisons test | Mean Diff. | 95% CI of diff. | Significant? | Summary |
|---|------------|---------------------|--------------|---------|
| 1.0 hour | | | | |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 0.5 mg/mL | 0.01325 | -0.05114 to 0.07764 | No | ns |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 1 mg/mL | 0.01625 | -0.04814 to 0.08064 | No | ns |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 1.8 mg/mL | -0.0006667 | -0.06505 to 0.06372 | No | ns |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 2.5 mg/mL | -0.007000 | -0.07139 to 0.05739 | No | ns |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 5 mg/mL | -0.01275 | -0.07714 to 0.05164 | No | ns |
| 1.5 hours | | | | |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 0.5 mg/mL | 0.0395 | -0.02489 to 0.1039 | No | ns |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 1 mg/mL | 0.02025 | -0.04414 to 0.08464 | No | ns |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 1.8 mg/mL | 0.01967 | -0.04472 to 0.08405 | No | ns |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 2.5 mg/mL | 0.0055 | -0.05889 to 0.06989 | No | ns |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 5 mg/mL | -0.00175 | -0.06614 to 0.06264 | No | ns |
| 2.0 hours | | | | |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 0.5 mg/mL | 0.05325 | -0.01114 to 0.1176 | No | ns |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 1 mg/mL | 0.03025 | -0.03414 to 0.09464 | No | ns |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 1.8 mg/mL | 0.03017 | -0.03422 to 0.09455 | No | ns |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 2.5 mg/mL | -0.0130 | -0.07739 to 0.05139 | No | ns |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 5 mg/mL | 0.04675 | -0.01764 to 0.1111 | No | ns |
| 2.5 hours | | | | |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 0.5 mg/mL | 0.06125 | -0.003136 to 0.1256 | No | ns |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 1 mg/mL | 0.03225 | -0.03214 to 0.09664 | No | ns |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 1.8 mg/mL | 0.06017 | -0.004219 to 0.1246 | No | ns |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 2.5 mg/mL | 0.02850 | -0.03589 to 0.09289 | No | ns |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 5 mg/mL | 0.07725 | 0.01286 to 0.1416 | Yes | * |
| 3.0 hours | | | | |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 0.5 mg/mL | 0.03125 | -0.03314 to 0.09564 | No | ns |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 1 mg/mL | 0.02375 | -0.04064 to 0.08814 | No | ns |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 1.8 mg/mL | 0.08217 | 0.01778 to 0.1466 | Yes | ** |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 2.5 mg/mL | 0.07775 | 0.01336 to 0.1421 | Yes | * |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 5 mg/mL | 0.1073 | 0.04286 to 0.1716 | Yes | *** |
| 3.5 hours | | | | |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 0.5 mg/mL | -0.004000 | -0.06839 to 0.06039 | No | ns |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 1 mg/mL | 0.01025 | -0.05414 to 0.07464 | No | ns |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 1.8 mg/mL | 0.1094 | 0.04503 to 0.1738 | Yes | *** |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 2.5 mg/mL | 0.1063 | 0.04186 to 0.1706 | Yes | *** |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 5 mg/mL | 0.1253 | 0.06086 to 0.1896 | Yes | **** |
| 4.0 hours | | | | |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 0.5 mg/mL | -0.01975 | -0.08414 to 0.04464 | No | ns |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 1 mg/mL | -0.005250 | -0.06964 to 0.05914 | No | ns |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 1.8 mg/mL | 0.1363 | 0.07186 to 0.2006 | Yes | **** |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 2.5 mg/mL | 0.1338 | 0.06936 to 0.1981 | Yes | **** |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 5 mg/mL | 0.1523 | 0.08786 to 0.2166 | Yes | **** |
| 4.5 hours | | | | |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 0.5 mg/mL | -0.04350 | -0.1079 to 0.02089 | No | ns |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 1 mg/mL | -0.00925 | -0.07364 to 0.05514 | No | ns |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 1.8 mg/mL | 0.1262 | 0.06178 to 0.1906 | Yes | **** |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 2.5 mg/mL | 0.1253 | 0.06086 to 0.1896 | Yes | **** |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 5 mg/mL | 0.1543 | 0.08986 to 0.2186 | Yes | **** |

| 5.0 hours | | | | |
|---|----------|---------------------|-----|-----|
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 0.5 mg/mL | -0.0325 | -0.09689 to 0.03189 | No | ns |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 1 mg/mL | -0.04800 | -0.1124 to 0.01639 | No | ns |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 1.8 mg/mL | 0.1033 | 0.03886 to 0.1676 | Yes | *** |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 2.5 mg/mL | 0.1085 | 0.04411 to 0.1729 | Yes | *** |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 5 mg/mL | 0.1213 | 0.05686 to 0.1856 | Yes | *** |
| 5.5 hours | | | | |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 0.5 mg/mL | -0.0545 | -0.1189 to 0.009886 | No | ns |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 1 mg/mL | -0.04575 | -0.1101 to 0.01864 | No | ns |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 1.8 mg/mL | 0.08517 | 0.02078 to 0.1496 | Yes | ** |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 2.5 mg/mL | 0.1388 | 0.07436 to 0.2031 | Yes | *** |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 5 mg/mL | 0.1225 | 0.05811 to 0.1869 | Yes | *** |
| 6.0 hours | | | | |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 0.5 mg/mL | -0.05900 | -0.1234 to 0.005386 | No | ns |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 1 mg/mL | -0.05850 | -0.1229 to 0.005886 | No | ns |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 1.8 mg/mL | 0.1464 | 0.08203 to 0.2108 | Yes | *** |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 2.5 mg/mL | 0.1813 | 0.1169 to 0.2456 | Yes | *** |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 5 mg/mL | 0.1838 | 0.1194 to 0.2481 | Yes | *** |
| 6.5 hours | | | | |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 0.5 mg/mL | -0.09075 | -0.1551 to -0.02636 | Yes | ** |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 1 mg/mL | -0.07450 | -0.1389 to -0.01011 | Yes | * |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 1.8 mg/mL | 0.1671 | 0.1027 to 0.2315 | Yes | *** |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 2.5 mg/mL | 0.2140 | 0.1496 to 0.2784 | Yes | *** |
| Glycyrrhizin - 0 mg/mL vs. Glycyrrhizin - 5 mg/mL | 0.2408 | 0.1764 to 0.3051 | Yes | *** |

Supplementary Table 2. Growth of *E. coli* in LB medium supplemented with different concentrations of stevioside.

| Dunnett's multiple comparisons test | Mean Diff. | 95% CI of diff. | Significant? | Summary |
|---|------------|-----------------------|--------------|---------|
| 1.0 hour | | | | |
| Stevioside - 0 mg/mL vs. Stevioside - 1 mg/mL | -0.0145 | -0.03788 to 0.008880 | No | ns |
| Stevioside - 0 mg/mL vs. Stevioside - 2.5 mg/mL | -0.006250 | -0.02963 to 0.01713 | No | ns |
| Stevioside - 0 mg/mL vs. Stevioside - 5 mg/mL | 0.0005000 | -0.02288 to 0.02388 | No | ns |
| 1.5 hours | | | | |
| Stevioside - 0 mg/mL vs. Stevioside - 1 mg/mL | -0.007500 | -0.03088 to 0.01588 | No | ns |
| Stevioside - 0 mg/mL vs. Stevioside - 2.5 mg/mL | -0.008250 | -0.03163 to 0.01513 | No | ns |
| Stevioside - 0 mg/mL vs. Stevioside - 5 mg/mL | 0.004750 | -0.01863 to 0.02813 | No | ns |
| 2.0 hours | | | | |
| Stevioside - 0 mg/mL vs. Stevioside - 1 mg/mL | -0.007500 | -0.03088 to 0.01588 | No | ns |
| Stevioside - 0 mg/mL vs. Stevioside - 2.5 mg/mL | -0.009000 | -0.03238 to 0.01438 | No | ns |
| Stevioside - 0 mg/mL vs. Stevioside - 5 mg/mL | 0.003000 | -0.02038 to 0.02638 | No | ns |
| 2.5 hours | | | | |
| Stevioside - 0 mg/mL vs. Stevioside - 1 mg/mL | -0.008000 | -0.03138 to 0.01538 | No | ns |
| Stevioside - 0 mg/mL vs. Stevioside - 2.5 mg/mL | -0.01175 | -0.03513 to 0.01163 | No | ns |
| Stevioside - 0 mg/mL vs. Stevioside - 5 mg/mL | 0.003750 | -0.01963 to 0.02713 | No | ns |
| 3.0 hours | | | | |
| Stevioside - 0 mg/mL vs. Stevioside - 1 mg/mL | -0.01425 | -0.03763 to 0.009130 | No | ns |
| Stevioside - 0 mg/mL vs. Stevioside - 2.5 mg/mL | 0.0 | -0.02338 to 0.02338 | No | ns |
| Stevioside - 0 mg/mL vs. Stevioside - 5 mg/mL | 0.01225 | -0.01113 to 0.03563 | No | ns |
| 3.5 hours | | | | |
| Stevioside - 0 mg/mL vs. Stevioside - 1 mg/mL | -0.02325 | -0.04663 to 0.0001297 | No | ns |
| Stevioside - 0 mg/mL vs. Stevioside - 2.5 mg/mL | -0.006500 | -0.02988 to 0.01688 | No | ns |
| Stevioside - 0 mg/mL vs. Stevioside - 5 mg/mL | 0.005000 | -0.01838 to 0.02838 | No | ns |
| 4.0 hours | | | | |
| Stevioside - 0 mg/mL vs. Stevioside - 1 mg/mL | -0.01668 | -0.04005 to 0.006705 | No | ns |
| Stevioside - 0 mg/mL vs. Stevioside - 2.5 mg/mL | -0.003500 | -0.02688 to 0.01988 | No | ns |
| Stevioside - 0 mg/mL vs. Stevioside - 5 mg/mL | 0.008750 | -0.01463 to 0.03213 | No | ns |
| 4.5 hours | | | | |
| Stevioside - 0 mg/mL vs. Stevioside - 1 mg/mL | -0.02935 | -0.05273 to -0.005970 | Yes | ** |
| Stevioside - 0 mg/mL vs. Stevioside - 2.5 mg/mL | -0.005500 | -0.02888 to 0.01788 | No | ns |
| Stevioside - 0 mg/mL vs. Stevioside - 5 mg/mL | 0.006750 | -0.01663 to 0.03013 | No | ns |
| 5.0 hours | | | | |
| Stevioside - 0 mg/mL vs. Stevioside - 1 mg/mL | -0.01900 | -0.04238 to 0.004380 | No | ns |
| Stevioside - 0 mg/mL vs. Stevioside - 2.5 mg/mL | -0.008250 | -0.03163 to 0.01513 | No | ns |
| Stevioside - 0 mg/mL vs. Stevioside - 5 mg/mL | 0.004500 | -0.01888 to 0.02788 | No | ns |
| 5.5 hours | | | | |
| Stevioside - 0 mg/mL vs. Stevioside - 1 mg/mL | -0.01825 | -0.04163 to 0.005130 | No | ns |

| | | | | |
|---|-----------|----------------------|----|----|
| Stevioside - 0 mg/mL vs. Stevioside - 2.5 mg/mL | -0.01500 | -0.03838 to 0.008380 | No | ns |
| Stevioside - 0 mg/mL vs. Stevioside - 5 mg/mL | -0.004000 | -0.02738 to 0.01938 | No | ns |
| 6.0 hours | | | | |
| Stevioside - 0 mg/mL vs. Stevioside - 1 mg/mL | -0.02150 | -0.04488 to 0.001880 | No | ns |
| Stevioside - 0 mg/mL vs. Stevioside - 2.5 mg/mL | -0.01375 | -0.03713 to 0.009630 | No | ns |
| Stevioside - 0 mg/mL vs. Stevioside - 5 mg/mL | 0.002750 | -0.02063 to 0.02613 | No | ns |

Supplementary Table 3. Growth of *E. coli* in LB medium supplemented with different concentrations of sucralose.

| Dunnett's multiple comparisons test | Mean Diff. | 95% CI of diff. | Significant? | Summary |
|-------------------------------------|------------|-----------------------|--------------|---------|
| 1.0 hours | | | | |
| Conc - 0 mg/mL vs. Conc - 0.5 mg/mL | -0.01425 | -0.04144 to 0.01294 | No | ns |
| Conc - 0 mg/mL vs. Conc - 1 mg/mL | 0.004250 | -0.02294 to 0.03144 | No | ns |
| Conc - 0 mg/mL vs. Conc - 2.5 mg/mL | 0.009333 | -0.01786 to 0.03652 | No | ns |
| Conc - 0 mg/mL vs. Conc - 5 mg/mL | -0.002000 | -0.02919 to 0.02519 | No | ns |
| 1.5 hours | | | | |
| Conc - 0 mg/mL vs. Conc - 0.5 mg/mL | -0.003750 | -0.03094 to 0.02344 | No | ns |
| Conc - 0 mg/mL vs. Conc - 1 mg/mL | 0.01100 | -0.01619 to 0.03819 | No | ns |
| Conc - 0 mg/mL vs. Conc - 2.5 mg/mL | 0.0165 | -0.01069 to 0.04369 | No | ns |
| Conc - 0 mg/mL vs. Conc - 5 mg/mL | 0.01425 | -0.01294 to 0.04144 | No | ns |
| 2.0 hours | | | | |
| Conc - 0 mg/mL vs. Conc - 0.5 mg/mL | 0.002000 | -0.02519 to 0.02919 | No | ns |
| Conc - 0 mg/mL vs. Conc - 1 mg/mL | 0.0175 | -0.009689 to 0.04469 | No | ns |
| Conc - 0 mg/mL vs. Conc - 2.5 mg/mL | 0.02325 | -0.003939 to 0.05044 | No | ns |
| Conc - 0 mg/mL vs. Conc - 5 mg/mL | 0.01725 | -0.009939 to 0.04444 | No | ns |
| 2.5 hours | | | | |
| Conc - 0 mg/mL vs. Conc - 0.5 mg/mL | 0.008500 | -0.01869 to 0.03569 | No | ns |
| Conc - 0 mg/mL vs. Conc - 1 mg/mL | 0.0210 | -0.006189 to 0.04819 | No | ns |
| Conc - 0 mg/mL vs. Conc - 2.5 mg/mL | 0.03750 | 0.01031 to 0.06469 | Yes | ** |
| Conc - 0 mg/mL vs. Conc - 5 mg/mL | 0.01825 | -0.008939 to 0.04544 | No | ns |
| 3.0 hours | | | | |
| Conc - 0 mg/mL vs. Conc - 0.5 mg/mL | 0.007500 | -0.01969 to 0.03469 | No | ns |
| Conc - 0 mg/mL vs. Conc - 1 mg/mL | 0.01925 | -0.007939 to 0.04644 | No | ns |
| Conc - 0 mg/mL vs. Conc - 2.5 mg/mL | 0.03983 | 0.01264 to 0.06702 | Yes | ** |
| Conc - 0 mg/mL vs. Conc - 5 mg/mL | 0.02375 | -0.003439 to 0.05094 | No | ns |
| 3.5 hours | | | | |
| Conc - 0 mg/mL vs. Conc - 0.5 mg/mL | 0.004000 | -0.02319 to 0.03119 | No | ns |
| Conc - 0 mg/mL vs. Conc - 1 mg/mL | 0.02675 | -0.0004389 to 0.05394 | No | ns |
| Conc - 0 mg/mL vs. Conc - 2.5 mg/mL | 0.03767 | 0.01048 to 0.06486 | Yes | ** |
| Conc - 0 mg/mL vs. Conc - 5 mg/mL | 0.01700 | -0.01019 to 0.04419 | No | ns |
| 4.0 hours | | | | |
| Conc - 0 mg/mL vs. Conc - 0.5 mg/mL | 0.001250 | -0.02594 to 0.02844 | No | ns |
| Conc - 0 mg/mL vs. Conc - 1 mg/mL | 0.02200 | -0.005189 to 0.04919 | No | ns |
| Conc - 0 mg/mL vs. Conc - 2.5 mg/mL | 0.04392 | 0.01673 to 0.07111 | Yes | *** |
| Conc - 0 mg/mL vs. Conc - 5 mg/mL | 0.01900 | -0.008189 to 0.04619 | No | ns |
| 4.5 hours | | | | |
| Conc - 0 mg/mL vs. Conc - 0.5 mg/mL | -0.003500 | -0.03069 to 0.02369 | No | ns |

| | | | | |
|-------------------------------------|------------|----------------------|-----|-----|
| Conc - 0 mg/mL vs. Conc - 1 mg/mL | 0.0205 | -0.006689 to 0.04769 | No | ns |
| Conc - 0 mg/mL vs. Conc - 2.5 mg/mL | 0.04175 | 0.01456 to 0.06894 | Yes | *** |
| Conc - 0 mg/mL vs. Conc - 5 mg/mL | 0.01625 | -0.01094 to 0.04344 | No | ns |
| 5.0 hours | | | | |
| Conc - 0 mg/mL vs. Conc - 0.5 mg/mL | -0.002500 | -0.02969 to 0.02469 | No | ns |
| Conc - 0 mg/mL vs. Conc - 1 mg/mL | 0.01825 | -0.008939 to 0.04544 | No | ns |
| Conc - 0 mg/mL vs. Conc - 2.5 mg/mL | 0.04233 | 0.01514 to 0.06952 | Yes | *** |
| Conc - 0 mg/mL vs. Conc - 5 mg/mL | 0.01200 | -0.01519 to 0.03919 | No | ns |
| 5.5 hours | | | | |
| Conc - 0 mg/mL vs. Conc - 0.5 mg/mL | -0.003000 | -0.03019 to 0.02419 | No | ns |
| Conc - 0 mg/mL vs. Conc - 1 mg/mL | 0.01550 | -0.01169 to 0.04269 | No | ns |
| Conc - 0 mg/mL vs. Conc - 2.5 mg/mL | 0.03625 | 0.009061 to 0.06344 | Yes | ** |
| Conc - 0 mg/mL vs. Conc - 5 mg/mL | -0.0002500 | -0.02744 to 0.02694 | No | ns |
| 6.0 hours | | | | |
| Conc - 0 mg/mL vs. Conc - 0.5 mg/mL | -0.002250 | -0.02944 to 0.02494 | No | ns |
| Conc - 0 mg/mL vs. Conc - 1 mg/mL | 0.0280 | 0.0008111 to 0.05519 | Yes | * |
| Conc - 0 mg/mL vs. Conc - 2.5 mg/mL | 0.04317 | 0.01598 to 0.07036 | Yes | *** |
| Conc - 0 mg/mL vs. Conc - 5 mg/mL | 0.007750 | -0.01944 to 0.03494 | No | ns |