**Supplemental data**

**Identification of serum-based metabolic feature and characteristic metabolites in paraquat intoxicated mouse models**

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**Table S1.** Differential metabolites between PQ3d group and ctrl group using univariate statistical analysis.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Class | Name | HMDB ID | Kegg ID | FC | *P*-value |
| Amino Acid | **Ratio of Glycine/L-Serine** | **HMDB00123/HMDB00187** | **C00037/C00065** | **0.565** | **3.50E-04** |
|  | **Ratio of L-Serine/Glycine** | **HMDB00187/HMDB00123** | **C00065/C00037** | **1.774** | **6.00E-04** |
|  | **Ratio of Beta-Alanine/L-Aspartic acid** | **HMDB00056/HMDB00191** | **C00099/C00049** | **0.548** | **2.10E-03** |
|  | **Glycine** | **HMDB00123** | **C00037** | **0.643** | **2.80E-03** |
|  | **Acetylglycine** | **HMDB00532** | **NA** | **8.000** | **4.10E-03** |
|  | **2-Hydroxybutyric acid** | **HMDB00008** | **C05984** | **20.432** | **1.00E-02** |
|  | L-Lysine | HMDB00182 | C00047 | 0.682 | 2.00E-02 |
|  | **Ornithine** | **HMDB00214** | **C00077** | **0.508** | **2.50E-02** |
|  | **Ratio of Sarcosine/Glycine** | **HMDB00271/HMDB00123** | **C00213/C00037** | **3.199** | **2.50E-02** |
|  | **Beta-Alanine** | **HMDB00056** | **C00099** | **0.511** | **3.20E-02** |
|  | Ratio of Urea/L-Arginine | HMDB00294/HMDB00517 | C00086/C00062 | 1.619 | 5.50E-02 |
|  | 5-Hydroxylysine | HMDB00450 | C16741 | 1.520 | 5.90E-02 |
|  | L-Alanine | HMDB00161 | C00041 | 0.789 | 6.00E-02 |
|  | Ratio of L-Tyrosine/L-Phenylalanine | HMDB00158/HMDB00159 | C00082/C00079 | 0.468 | 6.70E-02 |
|  | Methylcysteine | HMDB02108 | NA | 1.531 | 7.90E-02 |
|  | L-Cystine | HMDB00192 | C00491 | 0.324 | 9.00E-02 |
|  | L-Arginine | HMDB00517 | C00062 | 0.561 | 9.80E-02 |
| Carbohydrates | **L-Arabitol** | **HMDB01851** | **C00532** | **0.437** | **4.20E-05** |
|  | **Sorbitol** | **HMDB00247** | **C00794** | **0.057** | **1.30E-03** |
|  | D-Threitol | HMDB04136 | C16884 | 0.751 | 1.60E-02 |
|  | **Rhamnose** | **HMDB00849** | **C00507** | **1.559** | **3.00E-02** |
|  | D-Galactose | HMDB00143 | C00984 | 0.826 | 5.50E-02 |
|  | Mannitol | HMDB00765 | C00392 | 0.661 | 5.90E-02 |
|  | 1,5-Anhydrosorbitol | HMDB02712 | C07326 | 0.715 | 6.90E-02 |
| **Fatty Acids** | **Docosahexaenoic acid** | **HMDB02183** | **C06429** | **2.083** | **9.40E-03** |
|  | **Oleic acid** | **HMDB00207** | **C00712** | **3.204** | **9.80E-03** |
|  | **Linoleic acid** | **HMDB00673** | **C01595** | **2.159** | **1.30E-02** |
|  | **Myristic acid** | **HMDB00806** | **C06424** | **2.663** | **4.00E-02** |
| **Indoles** | **3-Indolepropionic acid** | **HMDB02302** | **NA** | **0.228** | **1.20E-04** |
| **Lipids** | **Cholesterol** | **HMDB00067** | **C00187** | **1.789** | **1.00E-02** |
|  | O-Phosphoethanolamine | HMDB00224 | C00346 | 1.135 | 6.70E-02 |
| **Nucleotide** | **Guanosine** | **HMDB00133** | **C00387** | **0.263** | **2.20E-03** |
|  | **Uracil** | **HMDB00300** | **C00106** | **2.812** | **1.80E-02** |
|  | Ratio of Inosine/Adenosine | HMDB00195/HMDB00050 | C00294/C00212 | 4.491 | 5.90E-02 |
|  | Inosine | HMDB00195 | C00294 | 6.474 | 6.00E-02 |
| **Organic Acids** | **Hypotaurine** | **HMDB00965** | **C00519** | **0.548** | **2.10E-02** |
|  | **2-Hydroxy-3-methylbutyric acid** | **HMDB00407** | **NA** | **5.028** | **2.30E-02** |
|  | **L-Lactic acid** | **HMDB00190** | **C00186** | **1.841** | **4.00E-02** |
|  | Ratio of Pyruvic acid/L-Lactic acid | HMDB00243/HMDB00190 | C00022/C00186 | 0.362 | 6.30E-02 |
|  | Ratio of Oxoglutaric acid/Isocitric acid | HMDB00208/HMDB00193 | C00026/C00311 | 0.490 | 7.00E-02 |
|  | Oxalic acid | HMDB02329 | C00209 | 1.355 | 7.20E-02 |
|  | Glycolic acid | HMDB00115 | C00160 | 0.800 | 7.60E-02 |

Bolded metabolites with significant *P*-value < 0.05 and > 1.5 fold increase or decrease.

*P*-values were calculated from the Student’s *t*-test.

**Table S2.** Differential metabolites between PQ30d group and ctrl group using univariate statistical analysis.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Class | Name | HMDB ID | Kegg ID | FC | *P*-value |
| Alcohols | 2-Hydroxypyridine | HMDB13751 | C02502 | 1.088 | 2.00E-02 |
| Amino Acid | **Ratio of L-Tyrosine/L-Phenylalanine** | **HMDB00158/HMDB00159** | **C00082/C00079** | **1.531** | **4.10E-04** |
|  | **Ratio of L-Valine/Alpha-ketoisovaleric acid** | **HMDB00883/HMDB00019** | **C00183/C00141** | **0.576** | **7.80E-03** |
|  | **L-Leucine** | **HMDB00687** | **C00123** | **0.649** | **8.80E-03** |
|  | L-Valine | HMDB00883 | C00183 | 0.673 | 1.00E-02 |
|  | L-Alloisoleucine | HMDB00557 | NA | 0.691 | 1.50E-02 |
|  | **Ratio of Alpha-ketoisovaleric acid/L-Valine** | **HMDB00019/HMDB00883** | **C00141/C00183** | **1.738** | **1.60E-02** |
|  | **Aminomalonic acid** | **HMDB01147** | **C00872** | **1.722** | **1.90E-02** |
|  | Alpha-ketoisovaleric acid | HMDB00019 | C00141 | 1.425 | 5.50E-02 |
|  | L-Lysine | HMDB00182 | C00047 | 0.795 | 7.10E-02 |
|  | L-Proline | HMDB00162 | C00148 | 0.683 | 8.50E-02 |
| Carbohydrates | Threonic acid | HMDB00943 | C01620 | 1.202 | 3.10E-02 |
|  | Mannitol | HMDB00765 | C00392 | 1.534 | 6.10E-02 |
| Lipids | **MG182** | **HMDB11568** | **NA** | **1.970** | **1.60E-02** |
| Nucleotide | Inosine | HMDB00195 | C00294 | 6.992 | 9.70E-02 |
| Organic Acids | Pyruvic acid | HMDB00243 | C00022 | 0.731 | 1.90E-02 |
|  | Isocitric acid | HMDB00193 | C00311 | 1.369 | 3.60E-02 |
|  | Glyceric acid | HMDB00139 | C00258 | 1.063 | 6.70E-02 |
|  | Citric acid | HMDB00094 | C00158 | 1.814 | 7.40E-02 |

Bolded metabolites with significant *P*-value < 0.05 and >1.5 fold increase or decrease.

*P*-values were calculated from the Student’s *t*-test.

**Table S3.** Differential metabolites between PQ3d group and PQ30d group using univariate statistical analysis.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Class | Name | HMDB ID | Kegg ID | FC | *P*-value |
| Alcohols | 2-Hydroxypyridine | HMDB13751 | C02502 | 0.801 | 9.20E-02 |
| Amino Acid | **Ratio of L-Serine/Glycine** | **HMDB00187/HMDB00123** | **C00065/C00037** | **2.031** | **8.70E-05** |
|  | **Ratio of Glycine/L-Serine** | **HMDB00123/HMDB00187** | **C00037/C00065** | **0.494** | **4.80E-04** |
|  | **Ratio of L-Tyrosine/L-Phenylalanine** | **HMDB00158/HMDB00159** | **C00082/C00079** | **0.306** | **1.50E-03** |
|  | **Ratio of L-Valine/Alpha-ketoisovaleric acid** | **HMDB00883/HMDB00019** | **C00183/C00141** | **1.578** | **5.70E-03** |
|  | **L-Leucine** | **HMDB00687** | **C00123** | **1.783** | **2.70E-02** |
|  | Glycine | HMDB00123 | C00037 | 0.676 | 4.00E-03 |
|  | **Acetylglycine** | **HMDB00532** | **NA** | **8.338** | **4.70E-03** |
|  | **2-Hydroxybutyric acid** | **HMDB00008** | **C05984** | **24.915** | **9.90E-03** |
|  | Ratio of Oxoglutaric acid/L-Glutamic acid | HMDB00208/HMDB00148 | C00026/C00025 | 0.681 | 2.70E-02 |
|  | **Beta-Alanine** | **HMDB00056** | **C00099** | **0.552** | **3.00E-02** |
|  | Ratio of Beta-Alanine/L-Aspartic acid | HMDB00056/HMDB00191 | C00099/C00049 | 0.699 | 3.10E-02 |
|  | **L-Valine** | **HMDB00883** | **C00183** | **1.527** | **4.60E-02** |
|  | L-Alloisoleucine | HMDB00557 | NA | 1.323 | 4.30E-02 |
|  | **Ratio of Alpha-ketoisovaleric acid/L-Valine** | **HMDB00019/HMDB00883** | **C00141/C00183** | **0.638** | **3.50E-02** |
|  | **L-Cystine** | **HMDB00192** | **C00491** | **0.351** | **3.60E-02** |
|  | **Ratio of Urea/L-Arginine** | **HMDB00294/HMDB00517** | **C00086/C00062** | **2.001** | **3.70E-02** |
|  | **L-Phenylalanine** | **HMDB00159** | **C00079** | **1.537** | **4.30E-02** |
|  | L-Cysteine | HMDB00574 | NA | 0.706 | 4.60E-02 |
|  | Ratio of Sarcosine/Glycine | HMDB00271/HMDB00123 | C00213/C00037 | 2.481 | 5.50E-02 |
|  | Ratio of L-Glutamic acid/Oxoglutaric acid | HMDB00148/HMDB00208 | C00025/C00026 | 1.463 | 5.90E-02 |
|  | Urea | HMDB00294 | C00086 | 1.513 | 7.60E-02 |
| Carbohydrates | **Sorbitol** | **HMDB00247** | **C00794** | **0.055** | **1.00E-03** |
|  | **L-Arabitol** | **HMDB01851** | **C00532** | **0.489** | **2.70E-03** |
|  | **Mannitol** | **HMDB00765** | **C00392** | **0.431** | **1.20E-02** |
| Fatty Acids | **Linoleic acid** | **HMDB00673** | **C01595** | **2.152** | **1.60E-02** |
|  | **Oleic acid** | **HMDB00207** | **C00712** | **2.892** | **2.50E-02** |
|  | **Docosahexaenoic acid** | **HMDB02183** | **C06429** | **1.620** | **4.20E-02** |
|  | Arachidonic acid | HMDB01043 | C00219 | 1.536 | 7.30E-02 |
|  | Myristic acid | HMDB00806 | C06424 | 2.205 | 9.00E-02 |
| Indols | **3-Indolepropionic acid** | **HMDB02302** | **NA** | **0.205** | **1.30E-03** |
|  | Serotonin | HMDB00259 | C00780 | 0.638 | 8.00E-02 |
| Lipids | **MG182** | **HMDB11568** | **NA** | **0.324** | **3.20E-03** |
|  | **Cholesterol** | **HMDB00067** | **C00187** | **1.612** | **1.70E-02** |
|  | Glycerol 3-phosphate | HMDB00126 | C00093 | 0.722 | 1.80E-02 |
| Nucleotide | **Uracil** | **HMDB00300** | **C00106** | **2.734** | **1.00E-02** |
|  | **Guanosine** | **HMDB00133** | **C00387** | **0.284** | **2.20E-02** |
|  | Adenosine | HMDB00050 | C00212 | 1.547 | 5.00E-02 |
| Organic Acids | **Ratio of Fumaric acid/Succinic acid** | **HMDB00134/HMDB00254** | **C00122/C00042** | **0.483** | **8.40E-04** |
|  | **2-Hydroxy-3-methylbutyric acid** | **HMDB00407** | **NA** | **3.373** | **3.20E-02** |
|  | Succinic acid | HMDB00254 | C00042 | 2.184 | 6.10E-02 |
|  | Malic acid | HMDB00744 | C00711 | 0.638 | 7.80E-02 |
|  | Hypotaurine | HMDB00965 | C00519 | 0.669 | 8.40E-02 |
|  | L-Lactic acid | HMDB00190 | C00186 | 1.603 | 8.60E-02 |
| Vitamin | **Pantothenic acid** | **HMDB00210** | **C00864** | **0.330** | **4.70E-03** |

Bolded metabolites with significant *P*-value < 0.05 and >1.5 fold increase or decrease.

*P*-values were calculated from the Student’s *t*-test.

**Table S4.** Comparison results of PQ 3d group vs ctrl group by Bonferroni correction or FDR correction following *t*-test and Lasso regression.

|  |  |
| --- | --- |
| **Bonferroni correction** | ***P*-value** |
| 3-Indolepropionic.acid | 0.04001822 |
| **FDR correction** | **Adjusted *P*-value** |
| 2.Hydroxybutyric.acid | 0.0004159187 |
| Glycine | 0.0023082716 |
| Acetylglycine | 0.0031099548 |
| L.Arabitol | 0.0004372888 |
| Oleic.acid | 0.0026464254 |
| 3.Indolepropionic.acid | 0.0002286756 |
| Inosine | 0.0013022046 |
| 2.Hydroxy.3.methylbutyric.acid | 0.0034191633 |
| Ratio.of.Beta.Alanine./L.Aspartic.acid | 0.0020643432 |
| Ratio.of.L.Serine./Glycine | 0.0006006160 |
| Ratio.of.Glycine./L.Ser | 0.0003512792 |
| **Lasso regression** | **Coefficients** |
| 2.Hydroxybutyric.acid | 0.193785404 |
| L.Lysine | -0.691470557 |
| L.Arabitol | -0.004685646 |
| 3.Indolepropionic.acid | -0.766348084 |
| 2.Hydroxy.3.methylbutyric.acid | 0.000835890 |
| Ratio.of.Glycine. /L.Serine | -0.468387540 |

**Table S5.** Comparison results of PQ 30d group vs ctrl group by Lasso regression.

|  |  |
| --- | --- |
| **Lasso regression** | **Coefficients** |
| L.Valine | -0.036519100 |
| Aminomalonic.acid | 0.327417951 |
| Threonic.acid | 0.095408105 |
| Inosine | 0.423017363 |
| Isocitric.acid | 0.198673323 |
| Ratio.of.L.Tyrosine./L.Phenylalanine | 0.794599030 |

**Table S6.** Comparison results of PQ 3d group vs PQ 30d group by Bonferroni correction or FDR correction following *t*-test and Lasso regression.

|  |  |
| --- | --- |
| **Bonferroni correction** | ***P*-value** |
| 3-Indolepropionic.acid | 0.02366588 |
| Ratio.of.L.Serine./Glycine | 0.01520688 |
| **FDR correction** | **Adjusted *P*-value** |
| 2.Hydroxybutyric.acid | 3.488447e-04 |
| Glycine | 1.251415e-03 |
| L.Arabitol | 1.462788e-03 |
| Mannitol | 4.317037e-04 |
| Sorbitol | 3.527095e-03 |
| 3.Indolepropionic.acid | 1.352336e-04 |
| Pantothenic.acid | 2.651249e-03 |
| Ratio.of.L.Serine./Glycine | 8.689643e-05 |
| Ratio.of.Glycine./L.Serine | 4.790217e-04 |
| Ratio.of.L.Tyrosine./L.Phenylalanine | 1.495456e-03 |
| Ratio.of.Fumaric.acid./Succinic.acid | 8.399055e-04 |
| **Lasso regression** | **Coefficients** |
| 2.Hydroxybutyric.acid | 1.70125514 |
| Glycine  | -0.26502190 |
| Pantothenic.acid | -0.07054445 |
| Ratio.of.L.Serine./Glycine | 1.43264376 |
| Ratio.of.Fumaric.acid./Succinic.acid | -0.02031136 |

**Table S7.** Results from pathway analysis of PQ 3d group vs ctrl group.\*

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| pathway | Total | Expected | Hits | Raw *P* | -log(*P*) | Holm adjust | FDR | Impact |
| **Glycine, serine and threonine metabolism** | **48** | **0.15953** | **3** | **0.000389** | **7.8524** | **0.030716** | **0.015552** | **0.32378** |
| Aminoacyl-tRNA biosynthesis | 75 | 0.24927 | 3 | 0.001455 | 6.5331 | 0.11345 | 0.038786 | 0.11268 |
| Pantothenate and CoA biosynthesis | 27 | 0.089738 | 2 | 0.003256 | 5.7274 | 0.25068 | 0.056003 | 0.07286 |
| beta-Alanine metabolism | 28 | 0.093062 | 2 | 0.0035 | 5.6549 | 0.26601 | 0.056003 | 0.25694 |
| Methane metabolism | 34 | 0.113 | 2 | 0.005143 | 5.2701 | 0.38572 | 0.062234 | 0.01751 |
| Propanoate metabolism | 35 | 0.11633 | 2 | 0.005446 | 5.213 | 0.40297 | 0.062234 | 0.085 |
| Nitrogen metabolism | 39 | 0.12962 | 2 | 0.006737 | 5.0002 | 0.49177 | 0.067366 | 0.00067 |
| Cysteine and methionine metabolism | 56 | 0.18612 | 2 | 0.013608 | 4.2971 | 0.97978 | 0.12096 | 0.01197 |
| Purine metabolism | 92 | 0.30577 | 2 | 0.034823 | 3.3575 | 1 | 0.27859 | 0.00425 |

**Table S8.** Results from pathway analysis of PQ 30d group vs ctrl group.\*

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| pathway | Total | Expected | Hits | Raw *P* | -log(*P*) | Holm adjust | FDR | Impact |
| Phenylalanine, tyrosine and tryptophan biosynthesis | 27 | 0.078521 | 2 | 0.002459 | 6.0081 | 0.19424 | 0.098347 | 0.008 |
| Phenylalanine metabolism | 45 | 0.13087 | 2 | 0.006763 | 4.9963 | 0.52075 | 0.13526 | 0.11906 |

\*Metabolic pathways with Raw *P* > 0.05, Impact factor > 0 were listed.

**Figure S1.** Correlation analysis of PQ3d and PQ30d showed positive correlation between 2-hydroxybutyric acid and Ratio of L.serine/glycine.

