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## Supplementary Information

### Screening and verification of linearly dependent biomarkers with acute toxicity induced by Radix Aconiti based on liquid chromatography–mass spectrometry-based metabolite profiling†

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26 Part 1. Examples of substances identification

27 With the (tR=8.0129 min, m/z 570.355) as an example to explain the process of  
28 identification of compounds. In the HMDB database, we using m/z to search to get  
29 molecular formula of compounds may be C<sub>30</sub>H<sub>52</sub>NO<sub>7</sub>P, in addition, the mass spectra  
30 fragments of compounds, 552.3, 184.1 and 125.0 m/z, corresponding to the loss of -  
31 H<sub>2</sub>O, -C<sub>21</sub>H<sub>41</sub>NO<sub>3</sub>P, -C<sub>28</sub>H<sub>47</sub>NO<sub>3</sub>. According to the fragment information, eventually  
32 concluded that the compound was LPC (22:5).

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48 **Table S1** The results of experimental methodology.

<b>Experiment name</b>	<b>RSD (Retention time)</b>	<b>RSD (Peak area)</b>
Precision instrument	<0.25%	<7.26%
Method repeatability	<0.98%	<12.44%
Sample stability	<0.95%	<13.68%

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67 **Table S2**  $R^2$  and  $Q^2$  values of PLS-DA model in ethanol extraction

	A	B	C
R2X (cum)	0.293	0.34	0.245
R2Y (cum)	0.999	0.986	0.999
Q2 (cum)	0.555	0.968	0.822

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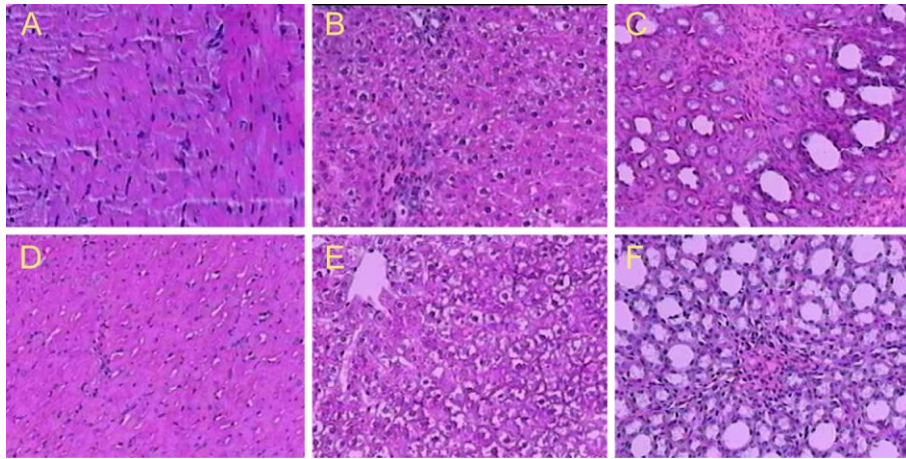
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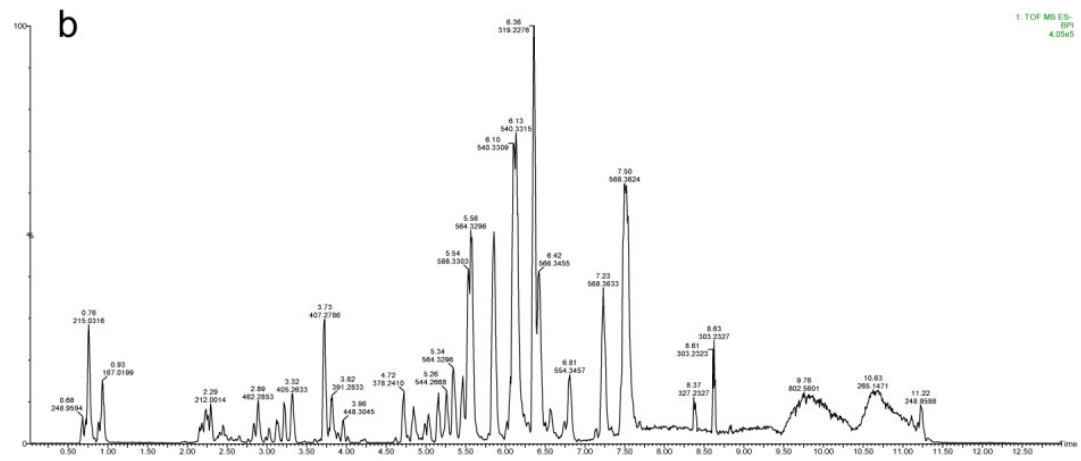
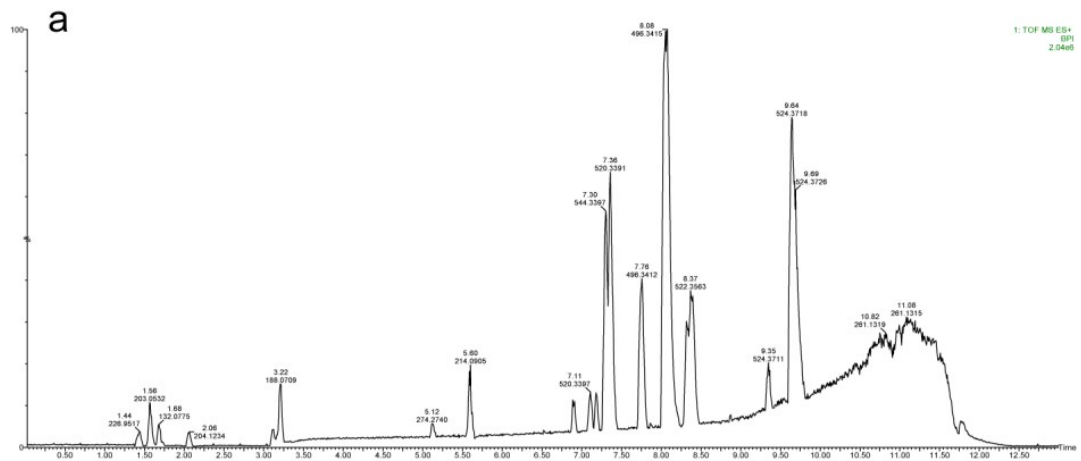
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87 **Fig S1** pathological examination of heart, kidney and liver tissue following the  
 88 administration of Radix Aconitit ethanol extraction at a dose of 10 g kg A:Healthy  
 89 heart control; B: healthy liver control; C: healthy renal control; D: histopathological  
 90 changes in the heart tissue: dilated blood vessels under subcapsular E:  
 91 histopathological changes in the liver tissue:partial swelling of liver cells F:  
 92 histopathological changes in the kidney tissue: slight edema on proximal tubule. All  
 93 photomicrographs showed no obvious fibrosis and inflammation magnification\*200.

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96 **Figure S2.** Basic peak ion (BPI) chromatograms of QC samples in (a) ESI<sup>+</sup> mode and  
 97 (b) ESI<sup>-</sup> mode

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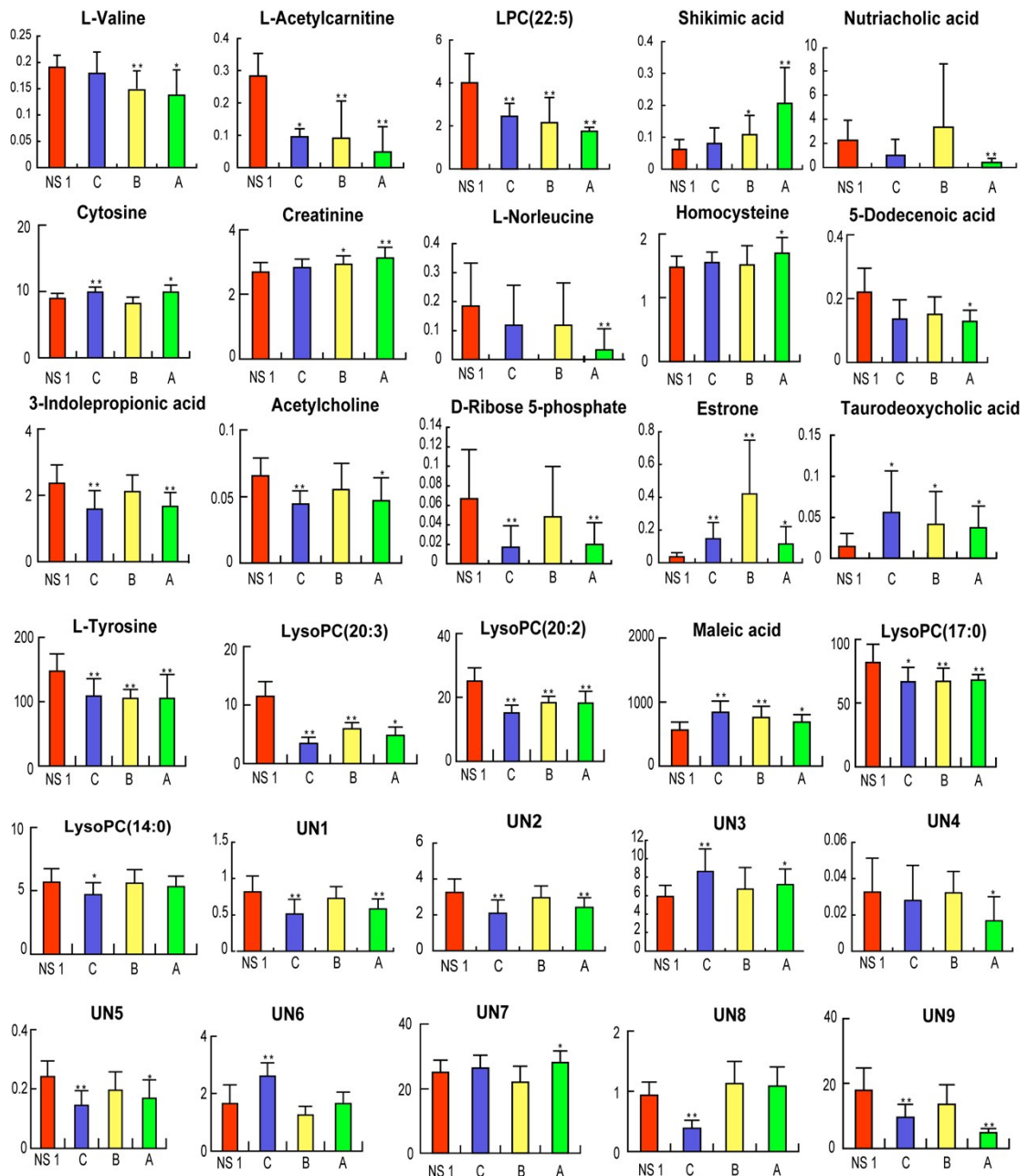
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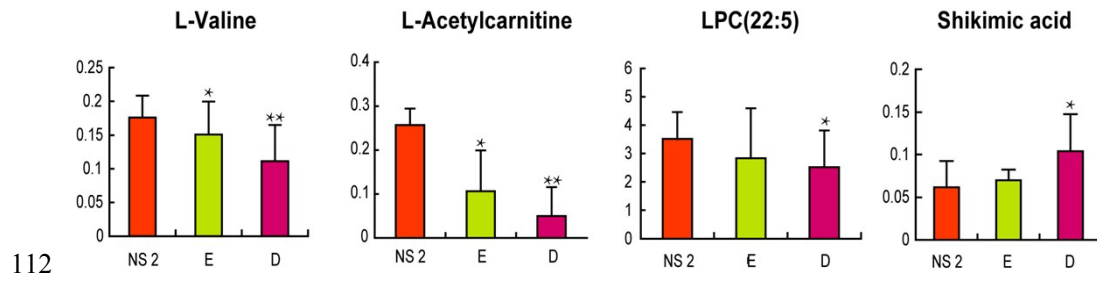


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106 **Figure S3** The trends in all potential biomarkers' relative content (peak area  
 107 intensity) changed with different concentrations of administration (ethanol extract  
 108 group). NS: Normal Saline; 2 g/kg ethanol extraction B: 5 g/kg ethanol extraction A:  
 109 10 g/kg ethanol extraction; significant difference from control: \* $p < 0.05$ , \*\* $p < 0.01$ .

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 113 **Figure S4** The peak area intensity variation of four substances in water extraction are  
 114 as same as in ethanol extraction NS 2:Normal Saline, D:10g/kg, E:5g/kg statistical  
 115 difference from control: \*p<0.05, \*\*p<0.01.

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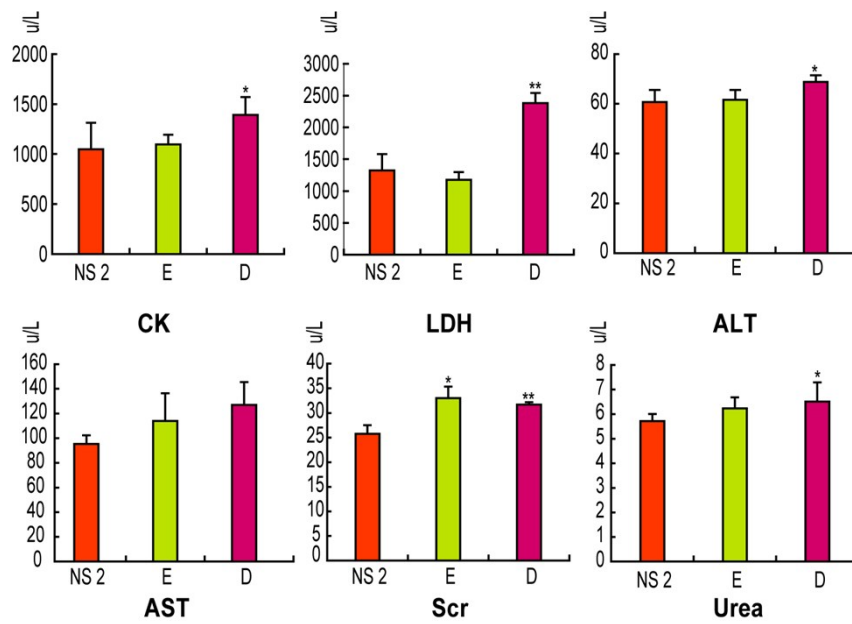
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129 **Figure S5** Various biochemical parameters in rats of administrating with *Radix*  
130 *Aconitit* water extraction. NS 2:Normal Saline, D:10g/kg, E:5g/kg statistical  
131 difference from control: \*p<0.05, \*\*p<0.01.

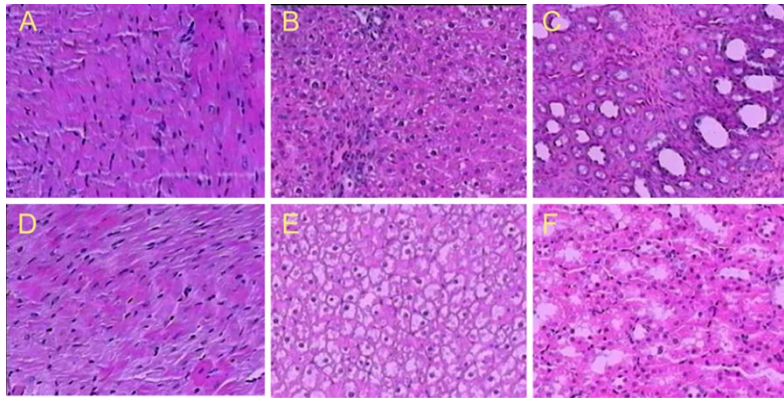
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138 **Fig S6** pathological examination of heart, kidney and liver tissue following the  
139 administration of *Radix Aconitit* water extraction at a dose of 10 g kg A:Healthy  
140 heart control; B: healthy liver control; C: healthy renal control; D: histopathological  
141 changes in the heart tissue: slightly irregular nuclear E: histopathological changes in  
142 the liver tissue:partial swelling of liver cells F: histopathological changes in the  
143 kidney tissue: partial distal convoluted tubule and collecting duct dilatation with  
144 epithelial atrophy. All photomicrographs showed no obvious fibrosis and  
145 inflammation magnification\*200.

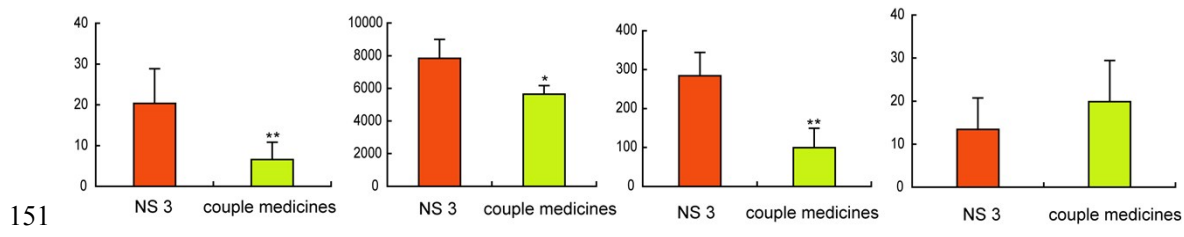
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152 **Figure S7** The peak area intensity variation of four substances in water extraction are  
 153 as same as in ethanol extraction NS 3:Normal Saline, couple medicines: processed  
 154 *Radix Aconiti-Pinellia ternata* couple medicines. statistical difference from control:  
 155 \* $p < 0.05$ , \*\* $p < 0.01$ .

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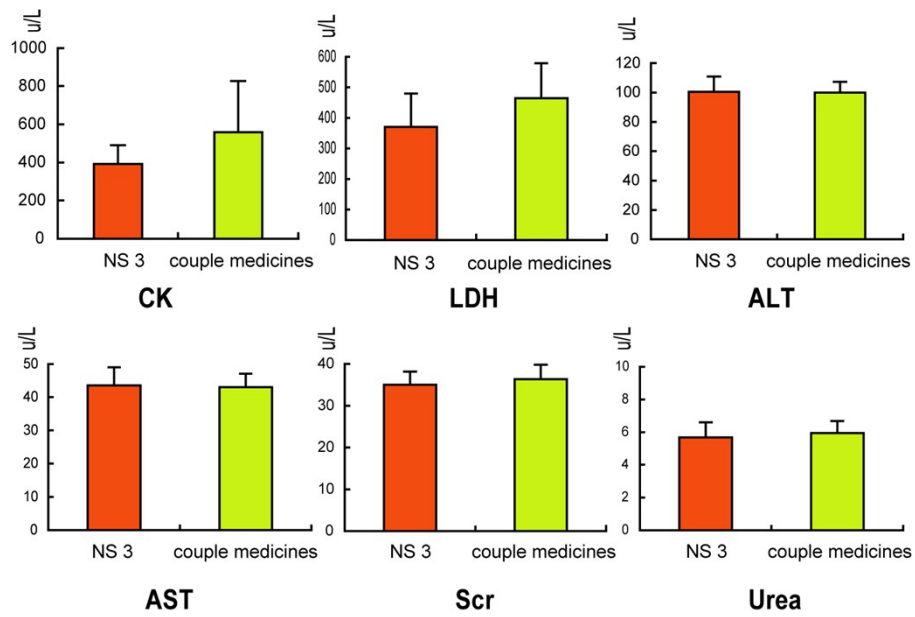
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166 **Figure S8** Various biochemical parameters in rats of administrating with processed  
 167 *Radix Aconiti-Pinellia ternata* couple medicines. NS 3:Normal Saline, couple  
 168 medicines: processed *Radix Aconiti-Pinellia ternata* couple medicines. No statistical  
 169 difference was shown from control

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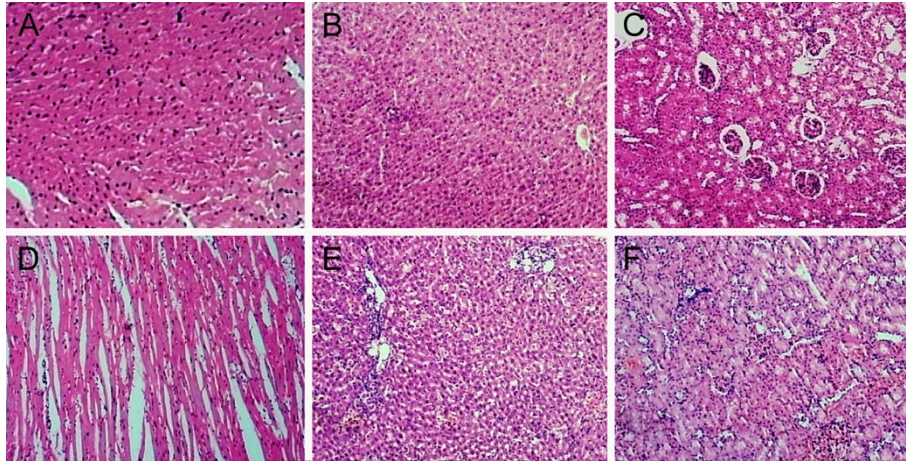
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181 **Fig S9** Pathological examination of heart, kidney and liver tissue following the  
182 administration of processed *Radix Aconiti-Pinellia ternata* couple medicines at a dose  
183 of 10 g/kg A:Healthy heart control; B: healthy liver control; C: healthy renal control;  
184 D: histopathological changes in the heart tissue: slightly irregular nuclear, peripheral  
185 lymphocytes and plasma cells increased E: histopathological changes in the liver  
186 tissue:partial swelling of liver cells, mitosis and apoptosis increased F:  
187 histopathological changes in the kidney tissue: partial distal convoluted tubule and  
188 collecting duct dilatation with epithelial atrophy. All photomicrographs showed no  
189 obvious fibrosis and inflammation magnification\*200.