

**Paeoniflorin protects against acetaminophen-induced liver injury in mice via JNK signaling pathway**

Xinyu Deng<sup>1</sup>, Yubing Li<sup>1</sup>, Xing Li<sup>2</sup>, Zhenpeng Zhang<sup>1</sup>, Shu Dai<sup>1</sup>, Hefei Wu<sup>1</sup>, Fangling Zhang<sup>1</sup>,

Qichao Hu<sup>1</sup>, Yuan Chen<sup>1</sup>, Jinhao Zeng<sup>3</sup>, Xiao Ma<sup>1\*</sup>

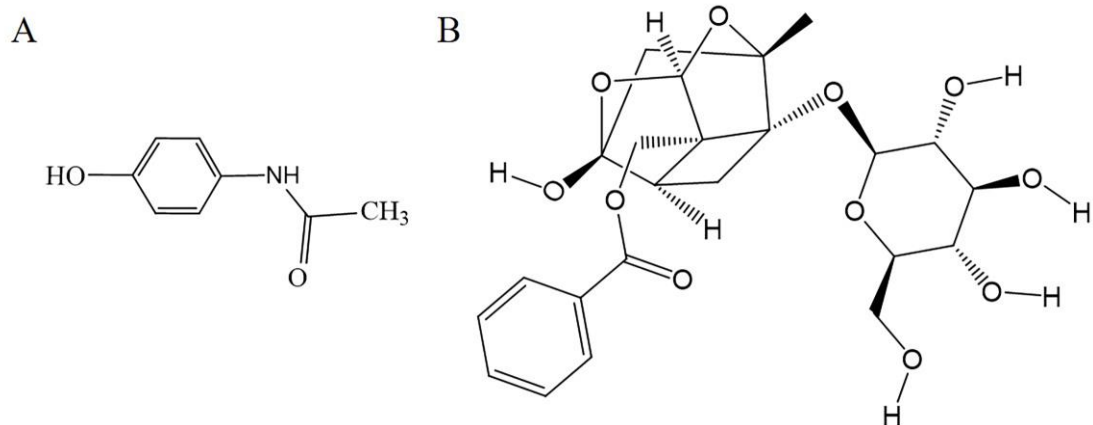
<sup>1</sup> State Key Laboratory of Southwestern Chinese Medicine Resources, School of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu, 611137, China

<sup>2</sup> Department of Pharmacy, Chinese PLA General Hospital, Beijing, 100039, China

<sup>3</sup> Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu 610072, China

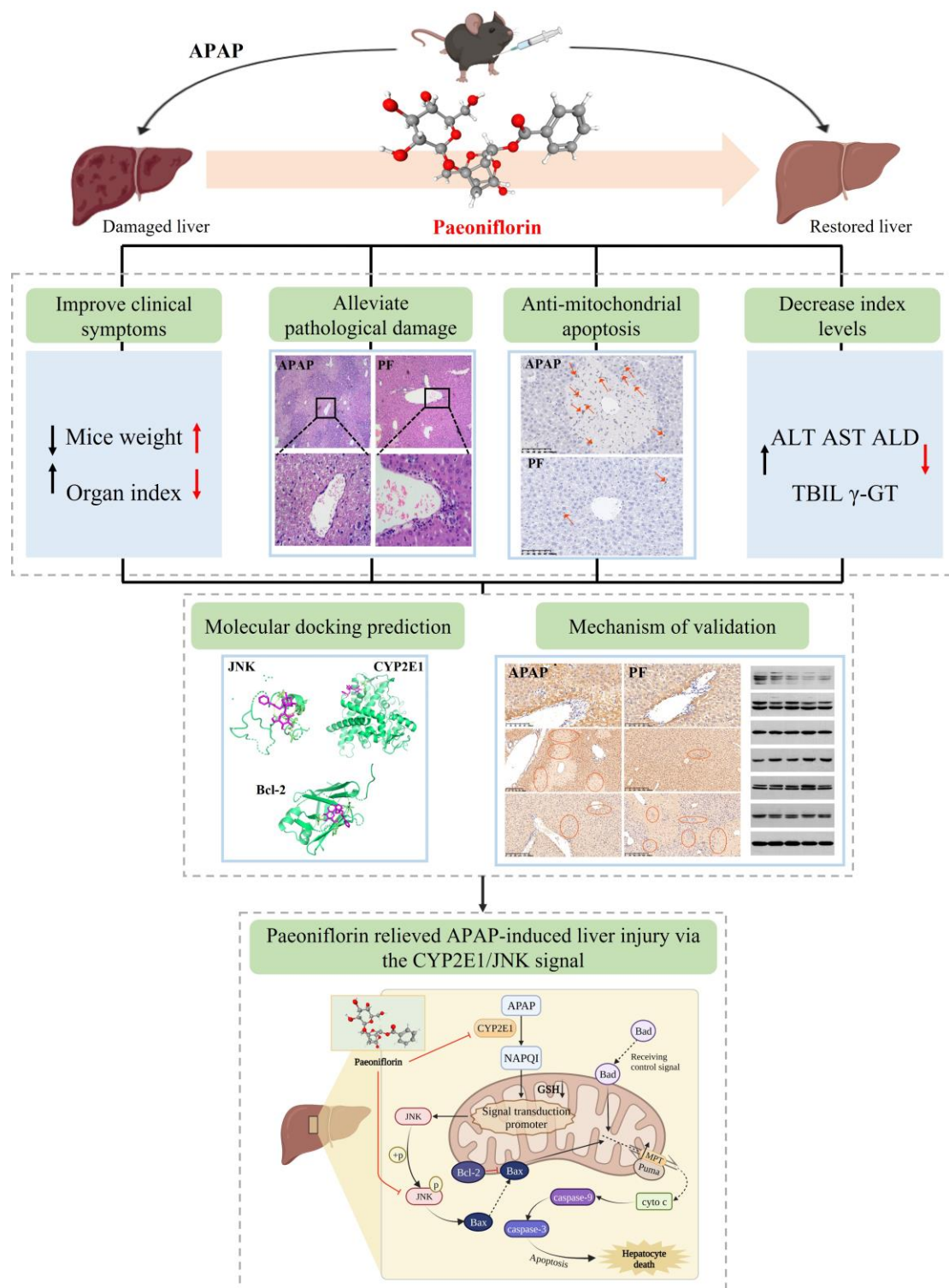
\* Corresponding authors: Jinhao Zeng, Email address: zengjinhao@cdutcm.edu.cn; Xiao Ma, Email address: tobymaxiao@cdutcm.edu.cn.

**Supplementary File**



**Figure S1. The chemical structures.**

**(A) the structure of acetaminophen. (B) the structure of paeoniflorin**



**Figure S2. The diagram of experiment**

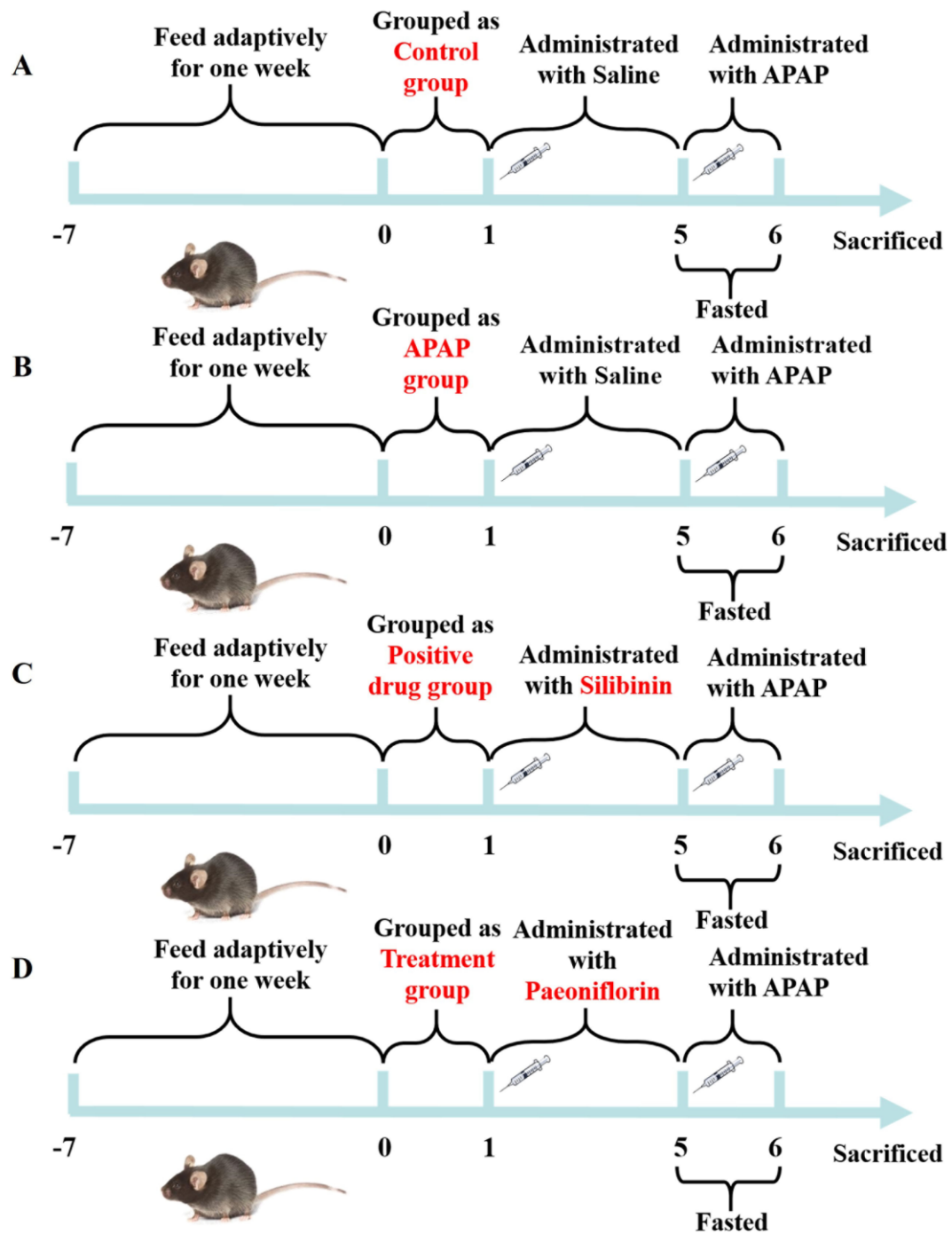


Figure S3. Experimental procedure of PF against APAP-induced liver injury

**Table S1 Effects of different doses of APAP on serum indices of C57BL/6 mice**

( $\bar{x} \pm s$ , n=6)

| Group          | Dosage (mg·kg <sup>-1</sup> ) | ALT level (U·L <sup>-1</sup> ) | AST level (U·L <sup>-1</sup> ) |
|----------------|-------------------------------|--------------------------------|--------------------------------|
| Ctrl           | —                             | 21.16±6.52                     | 60.57±11.08                    |
| Low dose APAP  | 250                           | 156.09±28.35**                 | 184.63±42.81*                  |
| High dose APAP | 400                           | 169.89±21.24**                 | 158.26±100.45*                 |

Notes: \* $P < 0.05$ , \*\* $P < 0.01$ , compared with Control group

**Table S2 Effects of different time of experimental period on serum indices in C57BL/6 mice**

( $\bar{x} \pm s$ , n=6)

| Period    | Group | Dosage (mg·kg <sup>-1</sup> ) | ALT level (U·L <sup>-1</sup> ) | AST level (U·L <sup>-1</sup> ) |
|-----------|-------|-------------------------------|--------------------------------|--------------------------------|
| Two days  | Ctrl  | —                             | 14.10±8.89                     | 39.37±18.41                    |
|           | APAP  | 250                           | 280.57±182.98**                | 136.35±73.42**                 |
|           | HPF   | 200                           | 402.88±36.38                   | 162.94±44.71                   |
| Five days | Ctrl  | —                             | 7.81±3.91                      | 21.55±7.12                     |
|           | APAP  | 250                           | 292.65±23.28**                 | 187.33±14.65**                 |
|           | HPF   | 200                           | 178.22±57.44##                 | 173.12±36.85                   |

Notes: Ctrl: Control group; APAP: APAP group; HPF: High-dose group of Paeoniflorin.

The periods of experiment are divided into the Two days and the Five days. \*\* $P < 0.01$ , compared with Control group; ## $P < 0.01$ , compared with APAP group

**Table S3 Effects of different varieties of positive drugs on serum indices in C57BL/6 mice**

( $\bar{x} \pm s$ , n=5)

| Group | Dosage (mg·kg <sup>-1</sup> ) | ALT level (U·L <sup>-1</sup> ) | AST level (U·L <sup>-1</sup> ) |
|-------|-------------------------------|--------------------------------|--------------------------------|
| Ctrl  | —                             | 26.00±8.39                     | 26.23±2.99                     |
| APAP  | 250                           | 505.99±51.38**                 | 212.59±15.73**                 |
| UDCA  | 55                            | 487.75±61.34                   | 202.64±18.14                   |
| Sli   | 60                            | 309.02±24.94##                 | 178.03±6.52##                  |

Notes: Ctrl: Control group; APAP: APAP group; UDCA: Ursodesoxycholic acid group; Sli:

Silibinin group. \*\*P < 0.01, compared with Control group; ## P < 0.01, compared with APAP group

**Table S4 Hepatotoxicity of paeoniflorin ( $\bar{x}\pm s$ , n=5)**

| Group | Dosage (mg·kg <sup>-1</sup> ) | ALT level (U·L <sup>-1</sup> ) | AST level (U·L <sup>-1</sup> ) |
|-------|-------------------------------|--------------------------------|--------------------------------|
| Ctrl  | —                             | 6.53±2.63                      | 22.42±3.69                     |
| HPF   | 200                           | 9.70±3.63                      | 21.89±6.42                     |

Notes: Ctrl: Control group; HPF: High-dose group of Paeoniflorin

**Table S5 Details of antibodies used in this study**

| Antibodies                                  | Host   | Dilution | Catalogue   | Manufacturer |
|---|--------|----------|-------------|--------------|
| <b>IHC</b>                                  |        |          |             |              |
| CYP2E1                                      | Rabbit | 1:100    | 19937-1-AP  | Proteintech  |
| Caspase-9                                   | Rabbit | 1:100    | 10380-1-AP  | Proteintech  |
| Caspase-3                                   | Rabbit | 1:100    | 19677-1-AP  | Proteintech  |
| <b>WB</b>                                   |        |          |             |              |
| JNK   | Rabbit | 1:2000   | 24164-1-AP  | Proteintech  |
| p-JNK                                       | Rabbit | 1:2000   | 80024-1-RR  | Proteintech  |
| CYP2E1                                      | Rabbit | 1:10000  | 19937-1-AP  | Proteintech  |
| Bad   | Mouse  | 1:3000   | 67830-1-Ig  | Proteintech  |
| Bcl-2                                       | Rabbit | 1:500    | 26593-1-AP  | Proteintech  |
| Bax   | Mouse  | 1:2000   | 60267-1-Ig  | Proteintech  |
| Caspase-9                                   | Rabbit | 1:300    | 10380-1-AP  | Proteintech  |
| Caspase-3                                   | Rabbit | 1:500    | 19677-1-AP  | Proteintech  |
| Beta actin                                  | Rabbit | 1:1000   | TA-09       | ZSGB-BIO     |
| Goat anti-rabbit IgG(H+L), HRP<br>conjugate |        | 1:10000  | 111-035-003 | Jackson      |
| Goat anti-mouse IgG(H+L), HRP<br>conjugate  |        | 1:10000  | 115-035-003 | Jackson      |