

Hepatoprotective effects of ginsenoside Rk3 in acetaminophen-induced liver injury in mice by activation of autophagy

Lin-lin Qu^{1, 2, 3, #}, Rong-zhan Fu^{1, 2, 3, #}, Xiaoxuan Ma^{1, 2, 3}, Dai-di Fan^{1, 2, 3, *}

¹Shaanxi Key Laboratory of Degradable Biomedical Materials, School of Chemical Engineering, Northwest University, Taibai North Road 229, Xi'an, Shaanxi 710069, China.

²Shaanxi R&D Center of Biomaterials and Fermentation Engineering, School of Chemical Engineering, Northwest University, Taibai North Road 229, Xi'an, Shaanxi 710069, China.

³Biotech. & Biomed. Research Institute, Northwest University, Taibai North Road 229, Xi'an, Shaanxi 710069, China.

These author contributed equally to this work.

* Address correspondence to: Dai-di Fan, E-mail: fandaidi@nwu.edu.cn, Tel./fax: +86-29-88305118

Table S1 Information of antibodies for western blot

Antibody	Working Dilution (IF)	Working Dilution (IHC)	Working Dilution (WB)	Manufacturer	Molecular Weight (kDa)	Cat. Number
LC3B	1:1000	1:1000	1:1000	Abcam	15	ab63817
Nrf2	---	---	1:500	Abcam	68	ab62352
CYP2E1	1:500	1:500	1:10000	PTG	57	19937-1-AP
ATG5	---	1:500	1:2000	PTG	32	10181-2-AP
ATG7	---	1:500	1:2000	PTG	78	10088-2-AP
ATG12	---	1:200	1:1000	PTG	15	11122-1-AP
Beclin1	---	1:500	1:5000	PTG	60	11306-1-AP
P62	1:200	1:200	1:4000	PTG	62	18420-1-AP
TNF- α	---	1:1000	1:4000	PTG	50	60291-1-Ig
IL-6	---	1:500	1:1000	PTG	23	21865-1-AP
IL-1 β	---	1:500	1:6000	PTG	32	66737-1-Ig
3-NT	---	1:500	---	Bioss	---	bs-8551R
TLR4	1:200	1:500	1:2000	Bioss	96	bs-1021R
NLRP3	---	1:500	1:1000	Bioss	110	bs-10021R
Caspase1	---	1:500	1:2000	Bioss	45	bs-10442R
NQO1	1:200	---	1:2000	Bioss	31	bs-2184R
HO-1	---	---	1:1000	CST	33	#86806
Lamin B1	---	---	1:2000	CST	67	#17416
GAPDH	---	---	1:1000	CST	36	#5174

Table S2 Sequences of primers used in qRT-PCR

Genes	Forward primer (5'-3')	Reverse primer (5'-3')
GAPD H	GCTGAGTATGTCGTGGAGT	G TTCACACCCATCACAAAC
TNF- α	GTCTACTGAACTTCGGGGTGAT	GGCTACAGGCTTGTCACTC G
IL-6	CTCTGCAAGAGACTTCCATCC	GAATTGCCATTGCACAAC C
IL-1 β	CCAACAAGTGATATTCTCCATG AG	ACTCTGCAGACTCAAAC CA

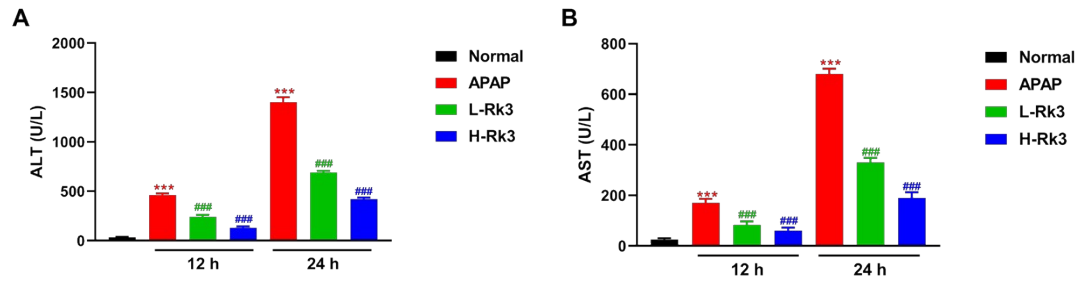


Figure S1. Effect of ginsenoside Rk3 on APAP-induced liver injury. Mice were treated 300 mg/kg APAP, after 2 h, mice in ginsenoside Rk3 groups were treated ginsenoside Rk3, (A) serum ALT and (B) AST levels in different time were measured using ELISA. Data are presented as mean \pm SD with 6 mice per group. *** $P < 0.001$ compared with the Normal group, # $P < 0.05$ compared with the APAP group, ## $P < 0.01$ compared with the APAP group, ### $P < 0.001$ compared with the APAP group.

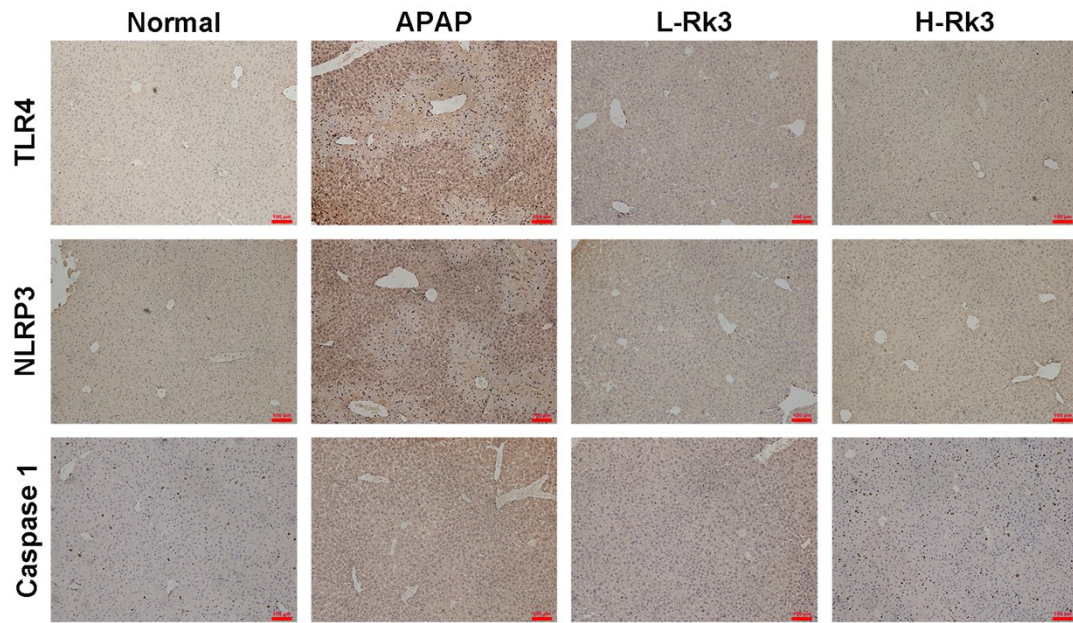


Figure S2 Immunohistochemical staining of TLR4, NLRP3, and Caspase 1 in liver.

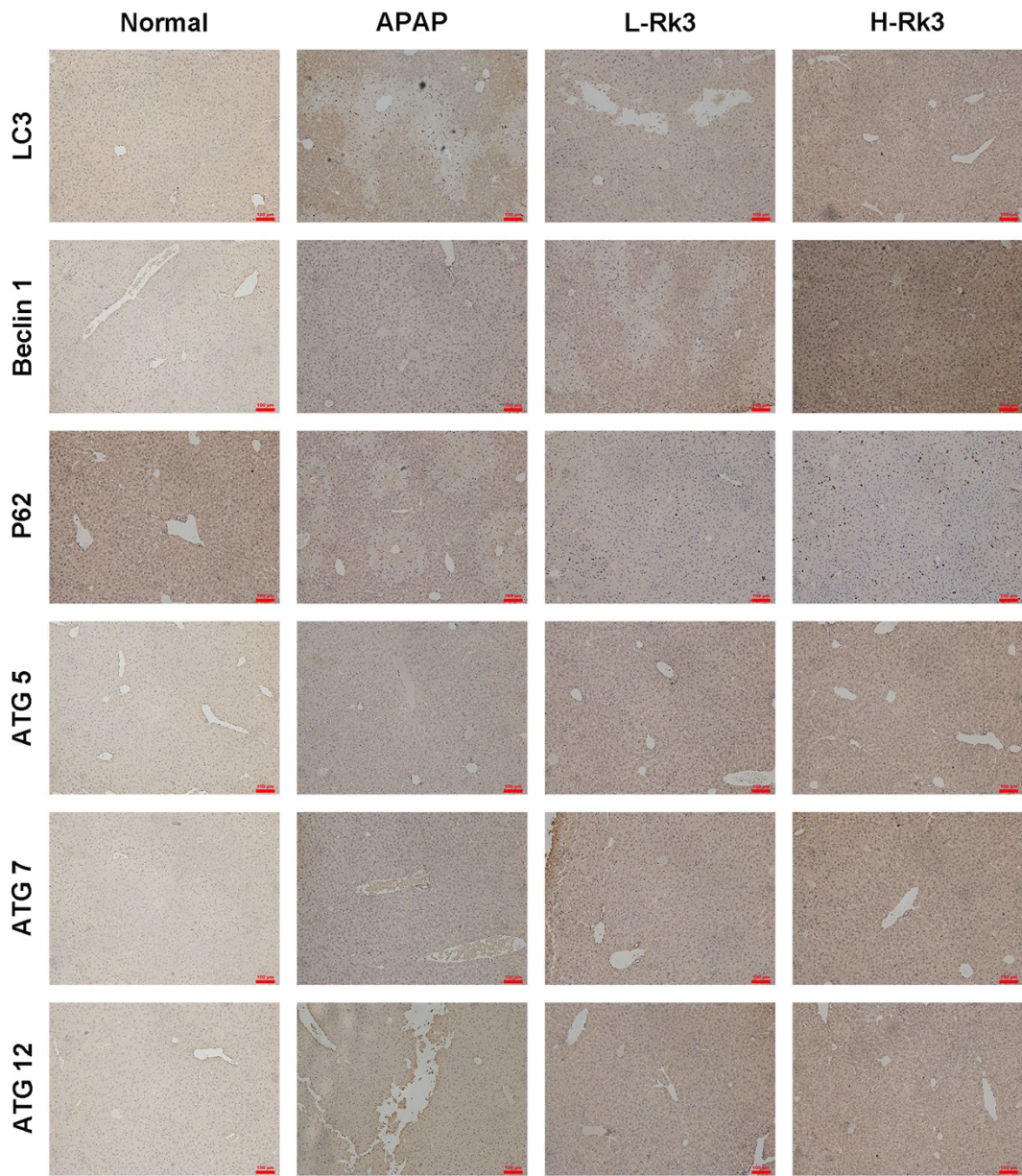


Figure S3 Immunohistochemical staining of LC3, Beclin 1, P62, ATG 5, ATG 7, and ATG 12 in liver.

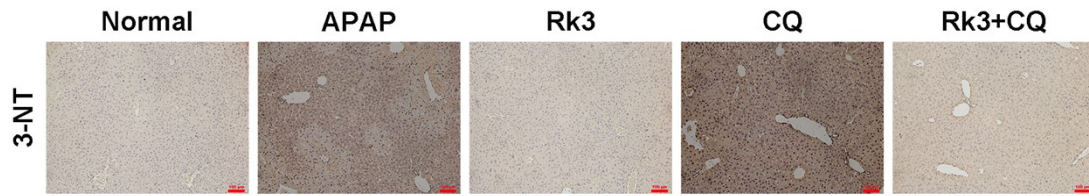


Figure S4 Immunohistochemical staining of 3-NT using liver sections.

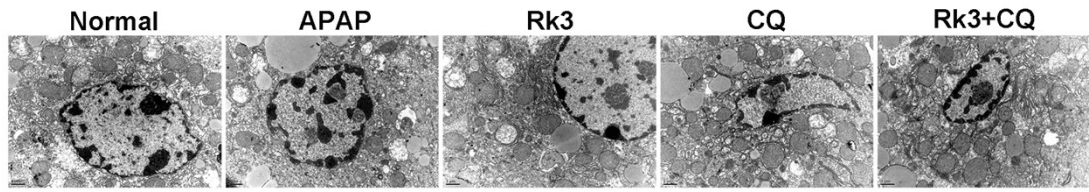


Figure S5 TEM analyses of liver sections at 12000 × magnification.

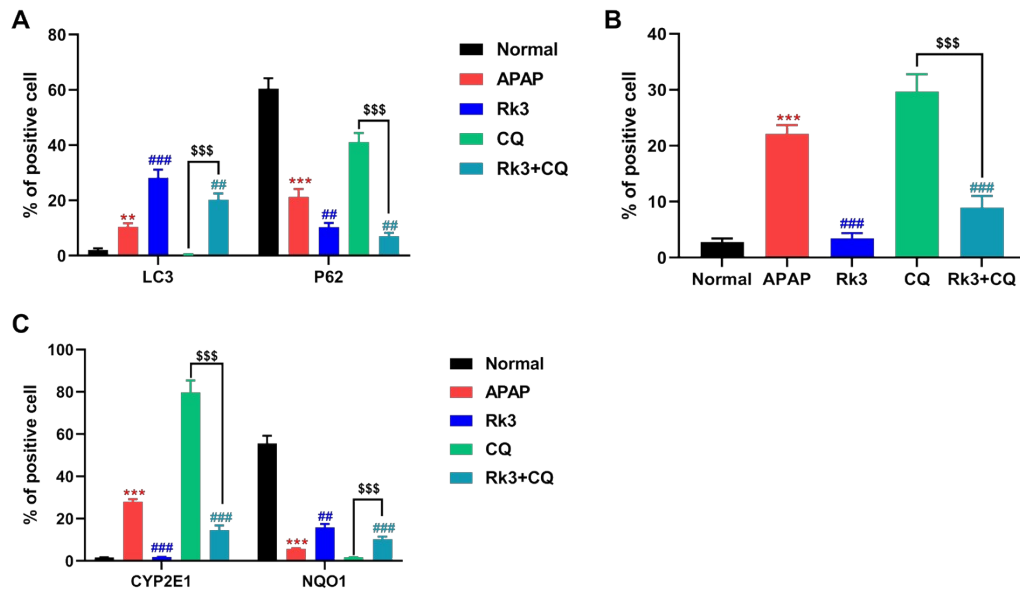


Figure S6 Immunofluorescence quantitative analysis.

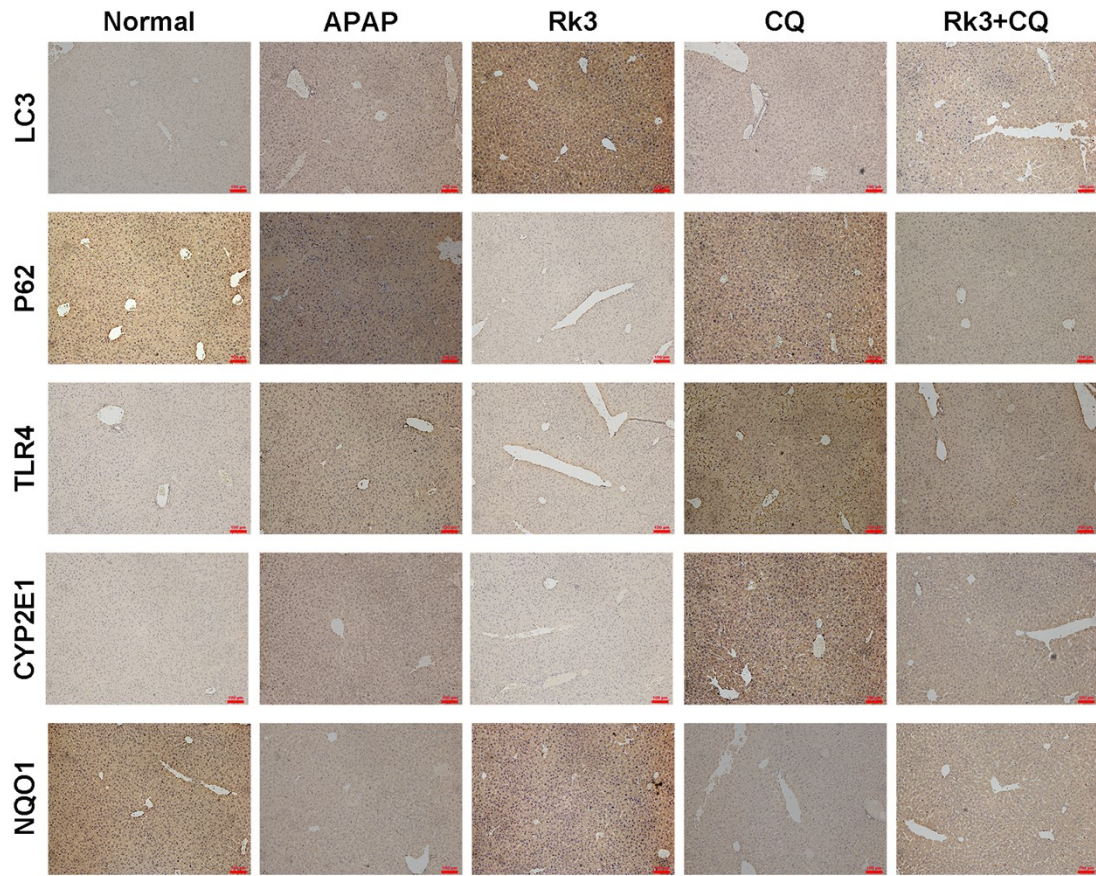


Figure S7 Immunohistochemical staining of LC3, P62, TLR4, CYP2E1, and NQO1 in liver.