

Supporting Information

**Cytochrome P450-dependent reactive oxygen species (ROS)
production contributes to Mn₃O₄ nanoparticle-caused liver
injury**

Zongkai Yue^a, Xiao Zhang^a, Qilin Yu^b, Lu Liu^c, Xiaomeng Zhou^{a, *}

^aCenter for Aircraft Fire and Emergency, Civil Aviation University of China, Tianjin 300300, PR China

^bMinistry of Education Key Laboratory of Molecular Microbiology and Technology, College of Life Science, Nankai University, Tianjin 300071, PR China

^c Tianjin Key Laboratory of Environmental Remediation and Pollution Control, College of Environmental Science and Engineering, Nankai University, Tianjin 300071, P.R. China

*Corresponding author at: Center for Aircraft Fire and Emergency, Civil Aviation University of China, Tianjin 300300, PR China

E-mail address: zhouxm@nankai.edu.cn (X. Zhou).

Evaluation of CYP1A2 activity assays

CYP1A2 activity assays were detected by the formation rate of acetaminophen which is the production of phenacetin.¹ Microsomal proteins were incubated in a mixture (total volume of 0.2 ml) containing 0.1 mol/L PBS buffer (pH 7.4), 12 μ L NADPH, and increasing concentrations of phenacetin. Reactions were started by the addition of microsomes, following thermal equilibration at 37°C of incubation mixtures. They were conducted in a shaking water bath at 37°C in aerobic conditions and stopped after 5 min by adding 0.2 ml of ice-cold acetonitrile. Denatured proteins were then removed by centrifugation for 10 min at 12,000 r/nin and an aliquot (0.1ml) of the supernatant was analyzed by HPLC with UV detection,

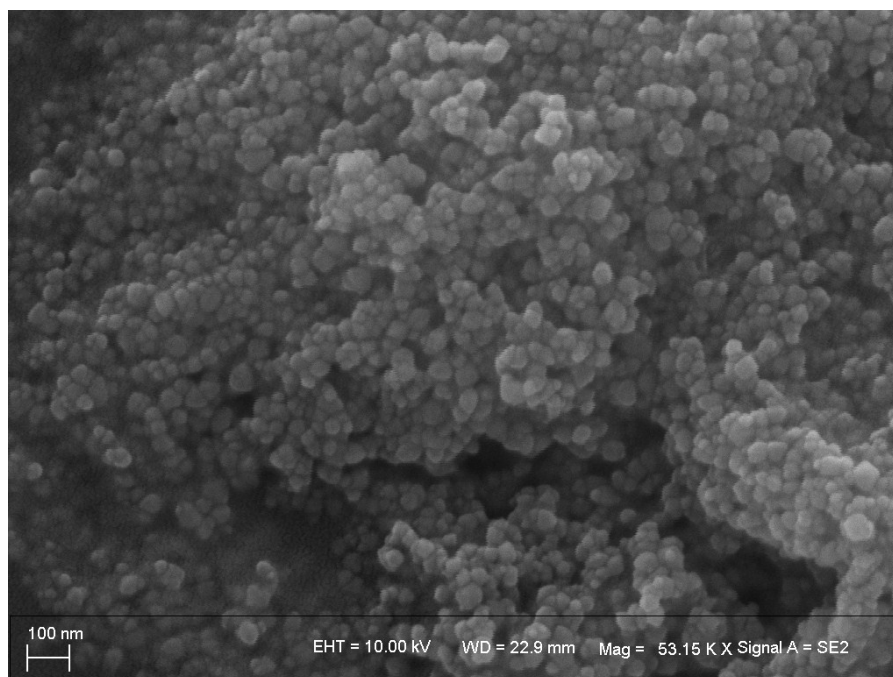


Fig. S1 SEM observation of the synthesized Mn₃O₄ NPs.

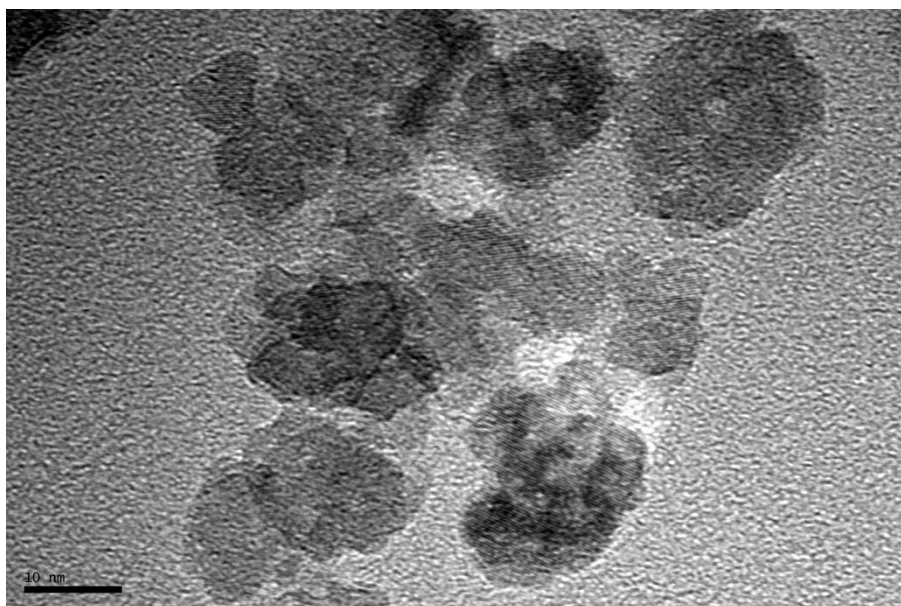


Fig. S2 TEM observation of the synthesized Mn₃O₄ NPs.

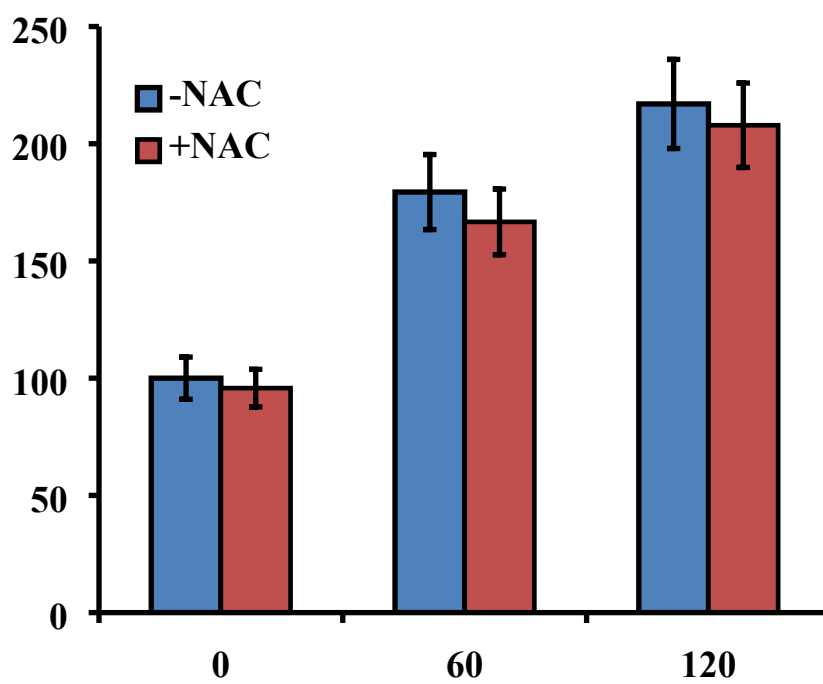


Fig. S3 Contents of CYP1A2 activity assays in the livers.

[1] L. Quintieri, P. Palatini, A. Nassi, Flavonoids diosmetin and luteolin inhibit midazolam metabolism by human liver microsomes and recombinant CYP3A4 and CYP3A5 enzymes [J]. *Biochem. Pharmacol.*, 2008, **75**, 1426-37.