

Article

# *Garcinia cambogia* Ameliorates Non-Alcoholic Fatty Liver Disease by Inhibiting Oxidative Stress-Mediated Steatosis and Apoptosis through NRF2-ARE Activation

Joo-Hui Han <sup>1,\*</sup>, Min-Ho Park <sup>2</sup> and Chang-Seon Myung <sup>1,\*</sup>

<sup>1</sup> Department of Pharmacology, College of Pharmacy, Chungnam National University, Daejeon 34134, Korea

<sup>2</sup> Institute of Drug Research and Development, College of Pharmacy, Chungnam National University, Daejeon 34134, Republic of Korea

\* Correspondence: han5621@cnu.ac.kr (J.-H.H.); cm8r@cnu.ac.kr (C.-S.M.);  
Tel.: +82-42-821-7299 (J.-H.H.); +82-42-821-5923 (C.-S.M.)

## Supporting Information

**Citation:** Han, J.-H.; Park, M.-H.; Myung, C.-S. *Garcinia cambogia* Ameliorates Non-Alcoholic Fatty Liver Disease by Inhibiting Oxidative Stress-Mediated Steatosis and Apoptosis through NRF2-ARE Activation. *Antioxidants* **2021**, *10*, x. <https://doi.org/10.3390/antiox10081226>

Academic Editor: Greg Barritt

Received: 13 July 2021

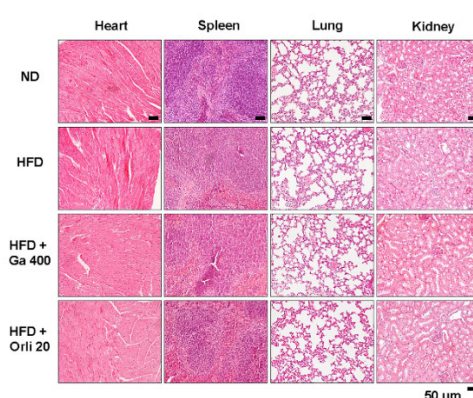
Accepted: 28 July 2021

Published: 29 July 2021

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

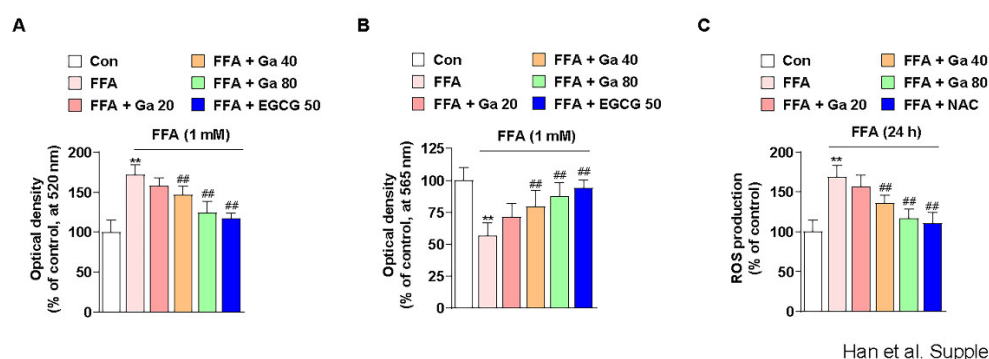


**Copyright:** © 2021 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).



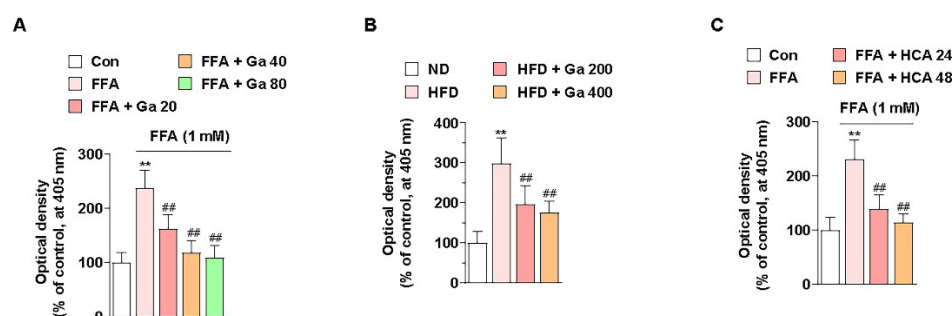
Han et al. *Supple* 1

**Figure S1.** *G. cambogia* attenuates the HFD-induced NAFLD without organ toxicity. Histological images of the heart, spleen, lung and kidney using H&E staining. Scale bars: 50  $\mu$ m.



Han et al. Supple 2

**Figure S2.** Effect of *Garcinia cambogia* on FFA-induced hepatic steatosis, cell viability and ROS production in HepG2 cells for 48 h. (a) Oil red O assay showing the effect of *G. cambogia* (20–80  $\mu$ g/mL) on free fatty acid (1 mM FFA)-induced lipid accumulation in HepG2 cells. FFA-induced HepG2 cells were treated with *G. cambogia* and EGCG (50  $\mu$ M, positive control) for 48 h ( $n=5$  per group). (b) MTT assay showing the effect of *G. cambogia* on FFA-induced cell viability. Cells were treated with *G. cambogia* (20–80  $\mu$ g/mL) and EGCG (50  $\mu$ M) for 48 h ( $n=5$  per group). (c) Effect of *G. cambogia* on ROS level in FFA-treated HepG2 cells. Cells were treated with *G. cambogia* (20–80  $\mu$ g/mL) or NAC (5 mM, positive control of antioxidant) for 48 h. ROS production was measured using H<sub>2</sub>DCFDA ( $n=5$  per group). \*\* $p < 0.01$  vs. Con, ## $p < 0.01$  vs. FFA.



Han et al. Supple 3

**Figure S3.** Effect of *Garcinia cambogia* and HCA on HFD- and FFA-induced caspase 3 activation. (a) Caspase 3 assay showing the effect of *G. cambogia* (20–80  $\mu$ g/mL) on FFA (1 mM)-induced caspase 3 activation in HepG2 cells. FFA-induced HepG2 cells were treated with *G. cambogia* for 24 h ( $n=5$  per group). (b) Effect of *G. cambogia* on caspase 3 activity of liver tissues in HFD-fed mice ( $n=6$  per group). (c) Caspase 3 assay showing the effect of HCA (24 and 48  $\mu$ g/mL) on FFA (1 mM)-induced caspase 3 activation in HepG2 cells. FFA-induced HepG2 cells were treated with HCA for 24 h ( $n=5$  per group).

**Table S1.** Primers for real-time PCR

Genes	Accession number	Sense primers (5'-3')	Antisense primers (5'-3')
Mouse/Human $\beta$ -actin	NM_001101.5/ NM_031144.3	TCCATCATGAAGTGTGAC GT	GCTCAGGAGGAG- CAATGAT
Human <i>FASN</i>	NM_004104.5	CCCCTGATGAA- GAAGGATCA	ACTCCACAGGTGG- GAACAAG

Human <i>FABP4</i>	NM_001442.3	ACAGGAAAGTCAAGAG- CACC	AACTCTCGTG- GAAGTGACGC
Human <i>SCD</i>	NM_005063.5	TGATGTTCCAGAG- GAGGTACT	AGCACCACAGCATATCG- CAA
Human <i>HMOX1</i>	NM_002133.3	CTGCTCAACATCCAGCTCT TTG	ATCTTGCACTTTGTT- GCTGGC
Human <i>SOD1</i>	NM_000454.5	GTGAAGGTGTGGGGAA- GCAT	AAGTCTCCAACATGCCTCT CTT
Mouse <i>Fasn</i>	NM_007988.3	CGGTG- TATCCTGCTGTCCAA	TGGGCTT- GTCCTGCTCTAAC
Mouse <i>Fabp4</i>	NM_024406.2	AAATCACCGCAGAC- GACAGG	CATAACACATTCCACCAC- CAGC
Mouse <i>Scd</i>	NM_009127.4	GCCCACATGCTCCAAGAG	GAGGGGCACCGTCTTCAC
Mouse <i>Hmox1</i>	NM_010442.2	CCCCAC- CAAGTTCAAACAGC	GCTCCTCAAACAGCTCAA TGT
Mouse <i>Sod1</i>	NM_011434.2	CGATGAAAGCGGTGTGCG	TGCACTGGTACAGCCTT- GTGT