

Supplemental Material

Carnosic acid protects against Doxorubicin-induced cardiotoxicity through enhancing the Nrf2/HO-1 pathway

SUPPLEMENTARY INFORMATION

Materials and reagents

Carnosic acid(CA) was purchased from MedChemExpress (#HY-N0644, USA). The purity of Carnosic acid is $\geq 99\%$. Doxorubicin was also obtained from MedChemExpress (HY-15142A, USA). CA and DOX was dissolved in DMSO (Sigma, #D2650, USA.) at a concentration of 50 mmol L⁻¹ as a stock solution and the final DMSO concentration in the cell media was less than 0.1%.

Cell Counting Kit 8 (CCK-8) was purchased from Shanghai Beyotime Biotechnology (#C0037, China). Lactate dehydrogenase (LDH); creatine kinase isoenzymes (CK-MB); cardiac isoform of troponin T(cTnT) assay Kits were obtained from Nanjing Jiancheng Bioengineering Institute (#A020-2-2, #H197-1-1, #H149-2, China). DAPI was purchased from Servicebio(#GDP1024, China). Dihydroethidium (DHE), 2',7' -dichlorodihydro fluorescein diacetate (DCFH-DA) were purchased from Shanghai Beyotime Biotechnology (#S0033S, #S0063, China); Superoxide dismutase (SOD) detecting kits, Malondialdehyde (MDA) assay kit, glutathione peroxidase (GSH-Px) activity detecting kits were purchased from Nanjing Jiancheng Bioengineering Institute (#A001-1-1, #A003-1-1, #A005-1-1, China). Primary antibodies against Anti-Lamin B1, Anti-Histone H3 were obtained from Servicebio(#GB111802, #GB11102, China); Anti-B cell lymphoma 2 (BCL-2, #26593-1), anti-BCL-2-associated X protein (BAX, #50599-2), caspase3(#19677-1) and Caspase1(#22915-1) anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH, #10494-1) were purchased from Proteintech Group(Rosemont, USA). Heme oxygenase-1 (HO-1, #ab13243), NF-E2-related factor 2 (Nrf2, #ab92946), nucleotide-binding oligomerization domain-like receptor containing pyrin domain 3 (NLRP3, #ab264468), IL-1 β (#ab283818), IL-18(#ab191860), α -actinin (#ab108198) were all obtained from Abcam (Cambridge, UK). Nrf2 small interfering RNA (siRNA) and control siRNA were purchased from GenePharma (Shanghai, China). siRNA-Nrf2 sequence: Sense5'→3': GCAAGAAGCCAGAUACAAATT; anti-sense5'→3': UUUGUAUCUGGCUUCUUGCTT.

Table.1 Primer information for QPCR analysis of expression of target genes

Gene	Species	Forward primer (5'→3')	Reverse primer (5'→3')
Nrf2	mouse	ATGCTTGTGGCTTGACCT	ACCTGTTCTCTGTTCTGCTG
Nrf2	rat	CTGGGGAGCTTGGAGAGA	GGGCAGTATGTTGGAGGTG

NLRP3	mouse	GACTGGCAAAAGGCTGTG	AGTTTCTCCAAGGCTACCG
NLRP3	rat	TGTTGTCAGGATCTCGCA	AGTGAAGTAAGGCCGGAAT
GAPDH	mouse	TGTTTCCTCGTCCCGTAGA	ATCTCCACTTTGCCACTGC
GAPDH	rat	ATGGCTACAGCAACAGGGT	TTATGGGGTCTGGGATGG

Table 2 List of all abbreviations

Full name	Abbreviations
Doxorubicin	DOX
reactive oxygen species	ROS
Food and Drug Administration	FDA
Nuclear transcription factor erythroid 2-related factor 2	Nrf2
heme oxygenase-1	HO-1
nucleotide-binding oligomerization domain-like receptor containing pyrin domain 3	NLRP3
interleukin1 β	IL-1 β
interleukin18	IL-18
Carnosic acid (CA)	
left ventricle fractional shortening	LVFS
left ventricle ejection fraction	LVEF
LV end diastolic diameter	LVIDd
LV systolic diameter	LVIDs
LV end systolic volume	LVESV
LV end diastolic volume	LVEDV
Neonatal rat ventricular myocytes	NRVMs
lactate dehydrogenase	LDH
creatine kinase isoenzymes	CK-MB
cardiac isoform of troponin T	cTnT
superoxide dismutase	SOD
malondialdehyde	MDA
glutathione peroxidase	GSH-px