Dieckol Attenuated Glucocorticoid-Induced Muscle Atrophy by Decreasing NLRP3 Inflammasome and Pyroptosis

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Supplementary Tables

Table S1. Results on the effects of ECE and DK in Dexa-induced muscle atrophy.

Results		Control	Dexa/ Saline	Dexa/ ECE50	Dexa/ ECE100	Dexa/ ECE150	Dexa/ DK
Binding ratio	**AGE-RAGE**	+	++++	+++	++	++	+++
	AGE-TLR4	+	++++	+++	++	++	+++
	HMGB1-RAGE	+	+++++	++++	++	++	+++
	HMGB1-TLR4	+	+++++	++++	+++	++	+++
NF-κB activation related molecules	**pJNK**	+	++++	+++	++	++	++
	pP38	+	+++++	+++	++	++	++++
	NF-κB	+	+++++	++++	+++	++	++
Pyroptosis related molecules	**NLRP3**	+	+++++	++++	+++	++	+++
	ASC	+	+++++	+++++	+++	++	+++
	c-Cas1	+	+++++	++++	+++	+	+
	IL-1β	+	+++++	++++	+++	++	++
	c-GSDMD	+	++++++	+++++	+++	++	++++

	Murf1	+	+++++	++++	++	++	+++
	Atrogin-1	+	++++	+++	++	++	+++
Muscle fiber size and function	**CSA**	++++	+	++	+++	+++	+++
	Grip strength	++++	+	++	+++	+++	+++

AGE, advanced glycation end products; ASC, adaptor molecule apoptosis-associated speck-like protein containing a CARD; Cas1, caspase 1; c-Cas1, cleaved-caspase 1; CSA, cross-sectional area; c-GSDMD, cleaved-gasdermin D; Dexa, dexamethasone; DK, dieckol; ECE, ecklonia cava extract; GSDMD, gasdermin D; HMGB1, high mobility group box 1; IL-1β, interleukin-1beta; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells;NLRP3, NOD-like receptor pyrin domain-containning protein 3; RAGE, receptor of AGE; pP38, phopho-P38; p-SAPK/JNK, phospho-SAPK/JNK; SAPK/JNK, stress-activated protein kinase/Jun-amino-terminal kinase; TLR4, toll like receptor 4.

Table S2. List of antibodies for ELISA, Western blotting and immunohistochemistry used in this study.

Antibody name	Company	Dilution rate		
		ELISA	Western blotting	Immunohistochemistry
AGE	abcam	1:1,000		
HMGB1	abcam	1:1,000		
RAGE	Santa cruz Biotechnology	1:200		
TLR4	Novos Biological	1:500		
SAPK/JNK	Cell signaling		1:1,000	
p-SAPK/JNK	Cell signaling		1:1,000	
P38	Cell signaling		1:1,000	
pP38	Cell signaling		1:1,000	
NF-κB	Cell signaling			1:400
NLRP3	Abca,		1:500	
ASC	Santa cruz Biotechnology		1:1,000	
Cas1	Santa cruz Biotechnology		1:1,000	
c-Cas1	Cell signaling		1:1,000	
IL-1β	Santa cruz Biotechnology		1:500	
GSDMD	Cell signaling		1:1,000	
c-GSDMD	Cell signaling		1:1,000	
β-actin	Cell signaling		1:1,000	1:200

Table S3. List of primer for qRT-PCR used in this study.

Gene		Primers
actb	Forward	5'-ACA AAG CTG TTC AGT GTC TCC A-3′
	Reverse	5'-CTC CGT TTC CAG AAT ACA CAC A-3′
MuRF1	Forward	5'-ATC TAG CCT GAT TCC TGA TGG A-3′
	Reverse	5'-ACC ACA GGC TTG GTA AAC ATC T-3′
Atrogin-1	Forward	5'-GAC ATT CAG AAC AGC AAA ACC A-3′
	Reverse	5'-GCT CCT TCG TAC TTC CTT TGT G-3′

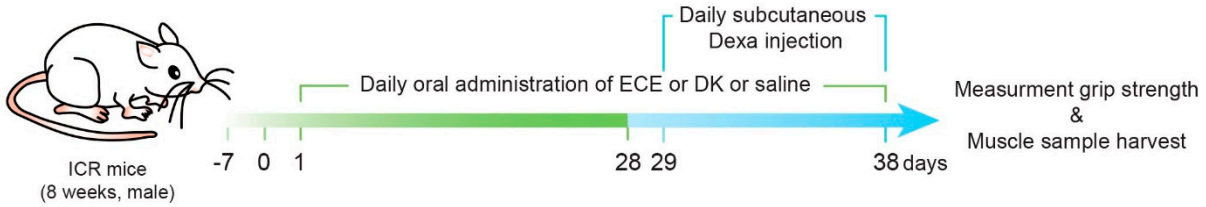

Figure S1. A schematic diagram of the animal model to confirm the effect of ECE or DK in Dexa-induced muscle atrophy. After 1 week of adaption period, ECE (50, 100 or 150 mg/kg) or DK (2.5 mg/kg) or saline were administrated by gavage once a day for 28 days prior to Dexa injection and maintained throughout the experimental period of 38 days. The muscle atrophy was induced by subcutaneous injection of Dexa (1mg/kg) once a day for 10 days. ECE, *Ecklonia cava* extract; DK, dieckol; Dexa; dexamethasone.