

Supplementary methods

1. DPPH scavenging assay

The free radical scavenging activity of CAFB was tested using the DPPH assay. Different concentrations of CAFB (0, 1, 10, 50, 100, 500, 1000 µg/mL, and 100 µL) were mixed with 100 µL of 0.3 mM ethanolic DPPH in a 96-well plate. After 15 minutes of incubation at room temperature, the absorbance was detected using a spectrophotometer at 517 nm (Molecular Devices, Sunnyvale, CA, USA). An identical concentration of kojic acid was used as a positive control for comparison. The inhibition rate (%) was calculated using the following equation:

$$\text{DPPH free radical inhibition rate (\%)} = 1 - (\text{OD}_{\text{control}} - \text{OD}_{\text{sample}}) / \text{OD}_{\text{control}} \times 100\%$$

2. Nitric oxide assay in RAW 264.7 cell

RAW 264.7 cells were adjusted to 1×10^5 /well in a 24-well plate. After 24 hours, different concentrations of CAFB (0, 0.1, 0.5, and 1 µg/mL) were added to the wells and incubated for 4 hours. Next, lipopolysaccharide (LPS) (0.2 µg/mL) was added to all wells except the normal control and incubated for 24 hours. Finally, the supernatant was collected and used to assess the nitric oxide content according to the previous protocol [S1].

S1. Wang, J.H.; Shin, J.W.; Son, J.Y.; Cho, J.H.; Son, C.G. Antifibrotic effects of CGX, a traditional herbal formula, and its mechanisms in rats. *J Ethnopharmacol* 2010, 127, 534-542, doi:10.1016/j.jep.2009.10.001.

Figure S1 CAFA did not inhibit the melanin synthesis

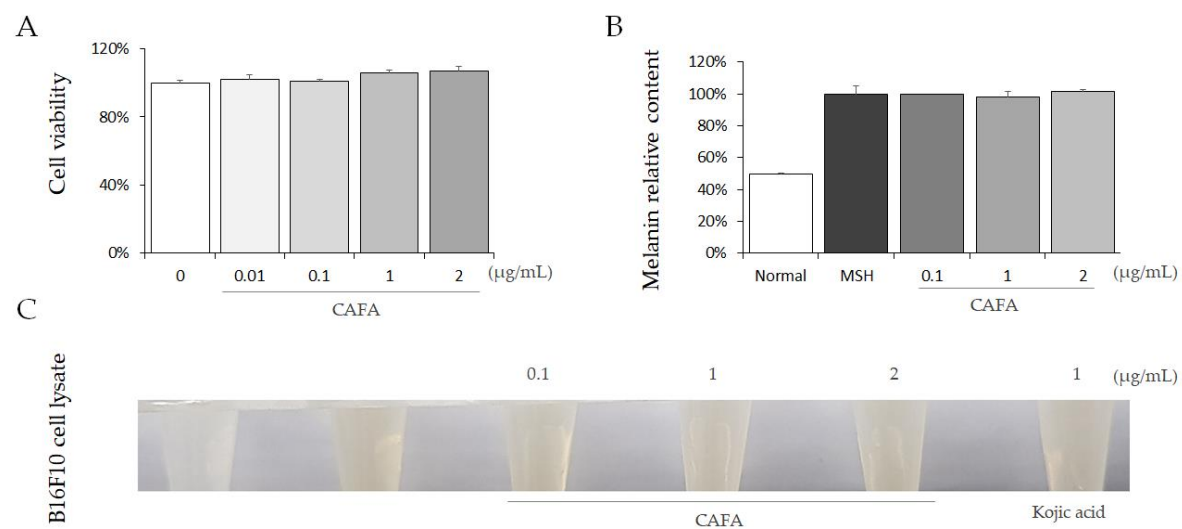


Figure S2 DPPH assay

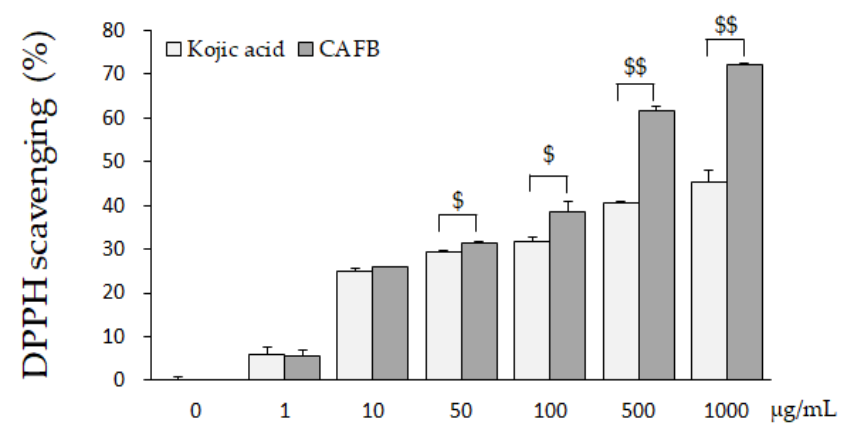


Figure S3 Nitric oxide assay

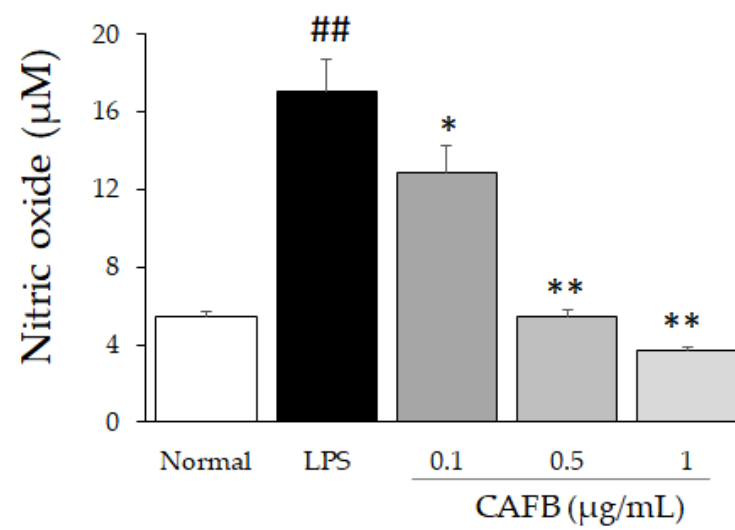


Table S1. qPCR primer information

Gene name	Primer sequence	OAT	Ref.
GAPDH	5'-CGT CCC GTA GAC AAA ATG GT-3'	60°C	[S2]
	5'-TTG ATG GCA ACA ATC TCC AC-3'		
TYR	5'-TTG CCA CTT CAT GTC ATC ATA GAA TAT T-3'	60°C	[S2]
	5'-TTT ATC AAA GGT GTG ACT GCT ATA CAA AT-3'		
MiTF	5'-CGC CTG ATC TGG TGA ATC G-3	60°C	[S2]
	5'-CCT GGC TGC AGT TCT CAA GAA-3'		
TRP1	5'-ATG CGG TCT TTG ACG AAT GG-3'	60°C	[S2]
	5'-CGT TTT CCA ACG GGA AGG T-3'		

Abbreviations: OAT, optimized annealing temperature; ACTIN, β -actin; TYR, Tyrosinase; MiTF, Microphthalmia-associated transcription factor; TRP1, tyrosinase related protein 1

S2. Sato, K.; Takei, M.; Iyota, R.; Muraoka, Y.; Nagashima, M.; Yoshimura, Y. Indomethacin inhibits melanogenesis via down-regulation of Mitf mRNA transcription. *Biosci Biotechnol Biochem* 2017, 81, 2307-2313, doi:10.1080/09168451.2017.1394812.

Table S2. Western blot antibodies information

Peptide/Protein target	Manufacturer	Catalog	Dilution ratio
Actin	Thermo Fisher scientific	MA 5-11869	1:1,000
TYR	abcam	ab170905	1:1,000
TRP1	abcam	ab235447	1:1,000
TRP2	abcam	ab221144	1:1,000
MiTF	abcam	ab12039	1:1,000
Goat Anti-Rabbit IgG (HRP)	GeneTex	GTX213110-01	1:5,000
Goat Anti-Mouse IgG (HRP)	GeneTex	GTX213111-01	1:5,000

Abbreviations: Actin, β -actin; TYR, Tyrosinase; MiTF, Microphthalmia-associated transcription factor; TRP1, tyrosinase related protein 1; TRP2, tyrosinase related protein 2; HRP, horseradish peroxidase; IgG, Immunoglobulin G.

Table S3. Body mass, food intake, serum biochemistry, and complete blood count.

Contents	Normal		UVB		0.25%	CAFB		1%	Kojic acid	
						0.5%			1%	
Body mass (g)	23.0	± 1.2	21.1	± 1.5 [#]	20.9	± 1.0	22.2	± 1.0	21.3	± 0.8
Food intake (g/mice/day)	6.57	± 0.13	5.40	± 0.55	5.60	± 0.89	5.87	± 0.59	6.04	± 0.87
AST (IU/L)	41.5	± 5.7	42.9	± 5.1	42.2	± 5.0	41.7	± 5.1	45.8	± 2.6
ALT(IU/L)	19.1	± 3.3	20.5	± 3.2	21.7	± 2.0	20.7	± 1.8	23.5	± 1.9
AST/ALT ratio	2.19	± 0.23	2.10	± 0.14	1.94	± 0.09	2.02	± 0.26	1.97	± 0.22
LDH (IU/L)	189.3	± 66.9	187.3	± 80.8	145.3	± 31.6	185.2	± 31.6	183.9	± 21.9
BUN (mg/dL)	21.1	± 2.2	20.9	± 0.9	20.6	± 2.1	21.6	± 2.2	20.9	± 2.5
CRE (mg/dL)	0.33	± 0.05	0.29	± 0.06	0.32	± 0.06	0.32	± 0.10	0.32	± 0.06
RBC (10 ⁹ /mL)	7.8	± 1.1	7.8	± 0.5	8.0	± 0.5	8.1	± 0.6	7.9	± 0.6
HGB (g/dL)	12.4	± 1.5	12.1	± 0.6	12.5	± 0.8	12.7	± 1.0	12.6	± 0.9
WBC (10 ⁶ /mL)	9.2	± 3.2	11.5	± 3.4	10.5	± 2.4	11.8	± 2.5	11.8	± 3.4
PLT (10 ⁶ /mL)	608	± 223	474	± 207	539	± 184	579	± 247	492	± 132

AST, aspartate transaminase; ALT, alanine transferase; TBIL, total bilirubin; LDH, lactate dehydrogenase; BUN, blood urea nitrogen; CRE, creatinine; RBC, red blood cell; HGB, hemoglobin; WBC, white blood cell; PLT, platelets; UVB, ultraviolet B; CAFB, fraction B of *Cynanchum atratum*; [#]*p* < 0.05 as compared to the normal; ^{**}*p* < 0.01 as compared to the UVB.