

Supplementary material for:

Antioxidant Cuttlefish Collagen Hydrolysate Against Ethyl Carbamate-induced Oxidative Damage

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Table S1 Experimental values and coded levels of variables for Box-Behnken designs (BBD)

Coded	variables	Low level (-1)	Medium level (0)	High level (+1)
X ₁	Temperature (°C)	40	45	50
X ₂	pH	7	8	9
X ₃	Enzyme (U/g)	6500	7000	7500

Table S2 Box-Benhnken designs (BBD) and response

Run	Variables ^a			Y: DPPH radical scavenging rate (%)
	X ₁ : Temperature	X ₂ : pH	X ₃ : Enzyme	
1	-1	1	0	67.58
2	0	1	-1	62.41
3	-1	-1	0	70.93
4	0	1	1	68.49
5	1	-1	0	69.61
6	-1	0	-1	64.44
7	0	-1	1	73.42
8	1	0	1	69.23
9	1	1	0	64.92
10	-1	0	1	72.61
11	0	-1	-1	66.65
12	1	0	-1	64.96
13	0	0	0	78.82
14	0	0	0	78.19
15	0	0	0	78.62
16	0	0	0	78.46
17	0	0	0	79.09

^aLow level (-1) Medium level (0) High level (+1)

Table S3 Up- and down-regulated genes in HepG2 cells treated with CCH or EC

Gene symbol	Gene description	Log ₂ fold change (compared to control group) ^a	
		EC	CCH-EC
AKR1C2	Aldo-keto reductase family 1, member C2	+5.47	+4.21
APOE	Apolipoprotein E	+0.47	+1.21
ATOX1	ATX1 antioxidant protein 1 homolog (yeast)	+1.74	+0.48
BAG2	BCL2-associated athanogene 2	+1.48	+0.22
BNIP3	BCL2/adenovirus E1B 19 kDa interacting protein 3	-0.52	+0.21
CAT	Catalase	+2.48	+2.22
CCL5	Chemokine (C-C motif) ligand 5	-0.62	-0.91
CCS	Copper chaperone for superoxide dismutase	+3.49	+2.54
DHCR24	24-dehydrocholesterol reductase	+2.48	+1.22
DUSP1	Dual specificity phosphatase 1	+2.78	+1.52
EPX	Eosinophil peroxidase	+4.47	+4.16
FHL2	Four and a half LIM domains 2	+0.48	+0.22
FOXM1	Forkhead box M1	+1.48	+0.22
FTH1	Ferritin, heavy polypeptide 1	+0.07	+0.47
GCLC	Glutamate-cysteine ligase, catalytic subunit	+0.48	+0.22
GCLM	Glutamate-cysteine ligase, modifier subunit	+2.64	+1.48
GLA	Galactosidase, alpha	+1.48	-0.14
GPX1	Glutathione peroxidase 1	+0.47	-0.78
GPX3	Glutathione peroxidase 3	+4.48	+4.22
GPX4	Glutathione peroxidase 4	+1.48	+1.21
GSR	Glutathione reductase	+0.48	+1.21
GSS	Glutathione synthetase	-0.08	+0.65
GSTP1	Glutathione S-transferase pi 1	-0.53	-0.78
GSTZ1	Glutathione transferase zeta 1	+0.48	+0.23
HMOX1	Heme oxygenase (decycling) 1	-2.53	-0.78
HSP90AA1	Heat shock protein 90 kDa α , class A member 1	+1.48	+0.23
HSPA1A	Heat shock 70 kDa protein 1A	+1.47	+0.98
LHPP	Phospholysine phosphohistidine inorganic pyrophosphate phosphatase	-0.53	-0.78

MB	Myoglobin	-4.55	-1.78
MBL2	Mannose-binding lectin (protein C) 2, soluble	+4.07	+3.21
MPV17	MpV17 mitochondrial inner membrane protein	+0.48	+0.22
MSRA	Methionine sulfoxide reductase A	+5.48	+4.22
MT3	Metallothionein 3	+1.53	+1.22
NCF2	Neutrophil cytosolic factor 2	-0.52	-1.78
NCOA7	Nuclear receptor coactivator 7	+0.48	-0.78
NOX5	NADPH oxidase, EF-hand calcium binding domain 5	+4.47	-5.84
NQO1	NAD(P)H dehydrogenase, quinone 1	+0.47	-0.80
NUDT1	Nucleoside diphosphate linked moiety X-type motif 1	+0.48	+0.22
PDLIM1	PDZ and LIM domain 1	-1.53	-0.78
PRDX1	Peroxiredoxin 1	+0.71	+0.45
PRDX2	Peroxiredoxin 2	-0.53	-0.79
PRDX4	Peroxiredoxin 4	+0.46	+0.21
PRDX6	Peroxiredoxin 6	+0.47	+0.20
PRNP	Prion protein	+2.48	+1.22
PTGR1	Prostaglandin reductase 1	+1.48	+1.22
PTGS1	Prostaglandin-endoperoxide synthase 1	+1.48	+0.22
PTGS2	Prostaglandin-endoperoxide synthase 2	-0.99	-1.25
RNF7	Ring finger protein 7	+2.70	+1.46
SEPP1	Selenoprotein P, plasma, 1	+0.07	-0.19
SIRT2	Sirtuin 2	+3.48	+2.22
SLC7A11	Solute carrier family 7, member 11	+3.48	+2.21
SOD1	Superoxide dismutase 1, soluble	+0.74	+0.50
SOD2	Superoxide dismutase 2, mitochondrial	-0.52	-0.78
SOD3	Superoxide dismutase 3, extracellular	+5.47	+4.22
SPINK1	Serine peptidase inhibitor, Kazal type 1	+1.46	+3.21
SQSTM1	Sequestosome 1	+0.97	+0.70
SRXN1	Sulfiredoxin 1	+2.48	+2.22
TPO	Thyroid peroxidase	+5.48	+4.22
TRAPPC6A	Trafficking protein particle complex 6A	+2.48	+1.22
TXN	Thioredoxin	+0.47	+0.22
TXNRD1	Thioredoxin reductase 1	+0.49	+0.21

TXNRD2	Thioredoxin reductase 2	-1.51	-1.77
UCP2	Uncoupling protein 2 (mitochondrial, proton carrier)	+3.47	+2.22
VIMP	VCP-interacting membrane protein	+1.49	+1.22

^a A gene was defined as differentially expressed gene only when it was up- or down-regulated more than 1.5-fold changes compared to control.

Table S4 Gene Ontology (GO) biological processes (BP) of enriched genes that were differentially expressed in HepG2 cells treated with CCH or EC

GO biological process	Enriched genes	
	EC	CCH-EC
response to oxidative stress [GO:0006979]	ATOX1/CAT/CCS/DHCR24/DUSP1/EPX/GCLM/GPX3/HMOX1/MB/MBL2/MSRA/MT3/NOX5/PDLIM1/PRNP/SIRT2/SLC7A11/SOD3/SRXN1/TPO/TXNRD2/UCP2 (23/26)	CAT/CCS/DUSP1/EPX/GPX3/MB/MBL2/MSRA/NCF2/NOX5/SIRT2/SLC7A11/SOD3/SRXN1/TPO/TXNRD2/UCP2 (17/19)
cellular oxidant detoxification [GO:0098869]	CAT/CCS/EPX/GPX3/MT3/SOD3/SRXN1/TPO/TXNRD2 (9/26)	CAT/CCS/EPX/GPX3/SOD3/SRXN1/TPO/TXNRD2 (8/19)
cellular detoxification [GO:1990748]	CAT/CCS/EPX/GPX3/MT3/SOD3/SRXN1/TPO/TXNRD2 (9/26)	CAT/CCS/EPX/GPX3/SOD3/SRXN1/TPO/TXNRD2 (8/19)
Detoxification [GO:0098754]	CAT/CCS/EPX/GPX3/MT3/SOD3/SRXN1/TPO/TXNRD2 (9/26)	CAT/CCS/EPX/GPX3/SOD3/SRXN1/TPO/TXNRD2 (8/19)
cellular response to oxidative stress [GO:0034599]	CAT/CCS/GPX3/HMOX1/MT3/MSRA/NOX5/SIRT2/SLC7A11/SOD3/SRXN1/TXNRD2 (12/26)	CAT/CCS/GPX3/MSRA/NCF2/NOX5/SIRT2/SLC7A11/SOD3/SRXN1/TXNRD2 (11/19)
cellular response to toxic substance [GO:0097237]	CAT/CCS/EPX/GPX3/HMOX1/MT3/SOD3/SRXN1/TPO/TXNRD2 (10/26)	CAT/CCS/EPX/GPX3/SOD3/SRXN1/TPO/TXNRD2 (8/19)
response to reactive oxygen species [GO:0000302]	CAT/CCS/DUSP1/HMOX1/MB/MT3/SOD3/TXNRD2/UCP2 (9/26)	CAT/CCS/DUSP1/MB/SOD3/TXNRD2/UCP2 (7/19)
reactive oxygen species metabolic process [GO:0072593]	CAT/CCS/EPX/GPX3/MT3/NOX5/SIRT2/SOD3/TPO (9/26)	CAT/CCS/EPX/GPX3/NCF2/NOX5/SIRT2/SOD3/TPO (9/19)
response to toxic substance [GO:0009636]	MT3/HMOX1/SOD3/TPO/DUSP1/EPX/CAT/SLC7A11/CCS/TXNRD2/MB/GPX3/SRXN1 (13/26)	SOD3/TPO/DUSP1/EPX/CAT/SLC7A11/CCS/TXNRD2/MB/GPX3/SRXN1 (11/19)

oxidation-
reduction process
[GO:0055114]

MT3/HMOX1/SOD3/TPO/DHCR24/EPX/C
AT/AKR1C2/CCS/MSRA/TXNRD2/GPX3/
NOX5/SRXN1 (14/26)

SOD3/TPO/NCF2EPX/CAT/AKR1C2/
CCS/MSRA/TXNRD2/GPX3/NOX5/SR
XN1 (12/19)

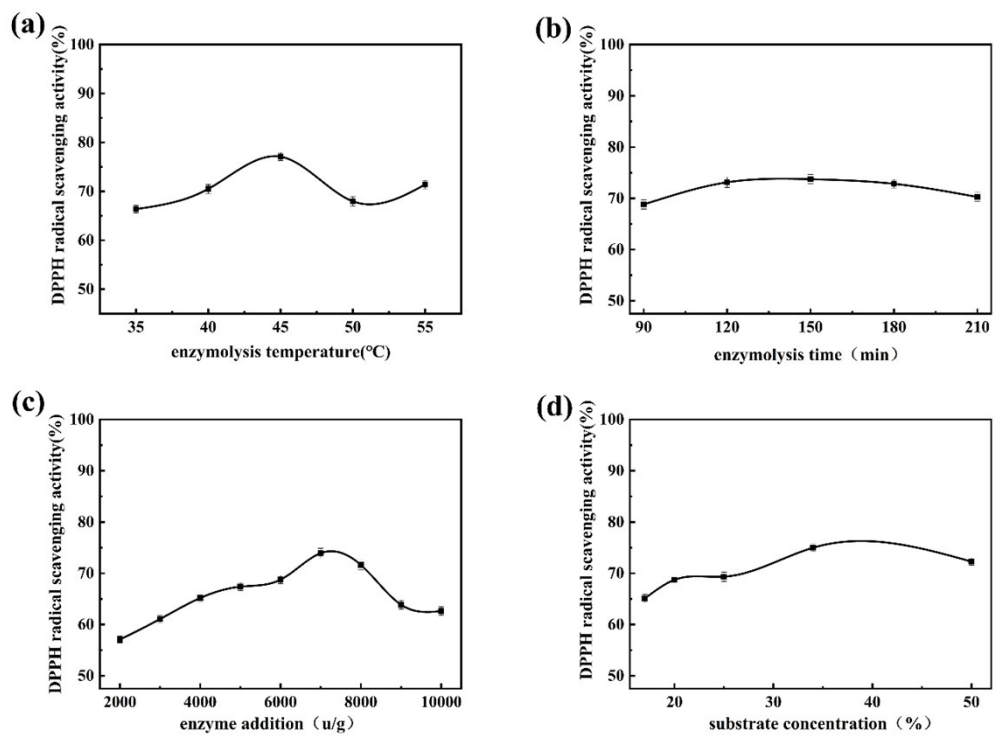


Fig S1 Effect of single-factor including (a) enzymolysis temperature, (b) enzymolysis time, (c) enzyme addition, and (d) substrate concentration on DPPH radical scavenging activity.

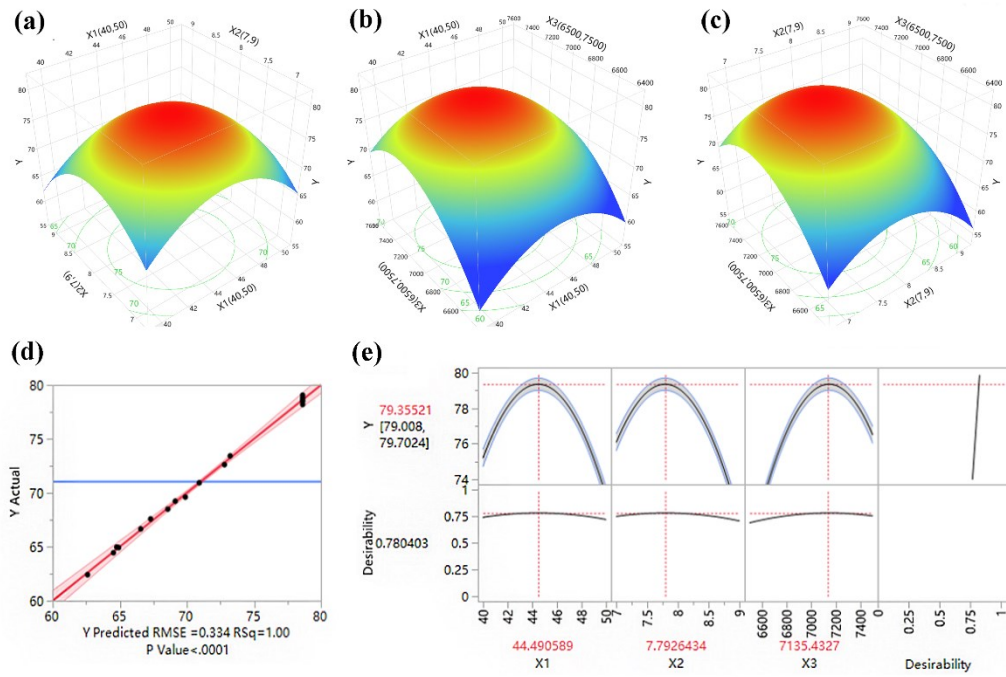


Fig S2 Surface plots for DPPH scavenging of CCH. (a) Effect of temperature and pH on DPPH Scavenging of CCH. (b) Effect of enzyme quantity and temperature on DPPH scavenging of CCH. (c) Effect of enzyme concentration and pH on DPPH scavenging of CCH. (d) Actual antioxidant capability versus model predicted the antioxidant capability of CCH. (e) Desirability plots of variables for maximum antioxidant capability.

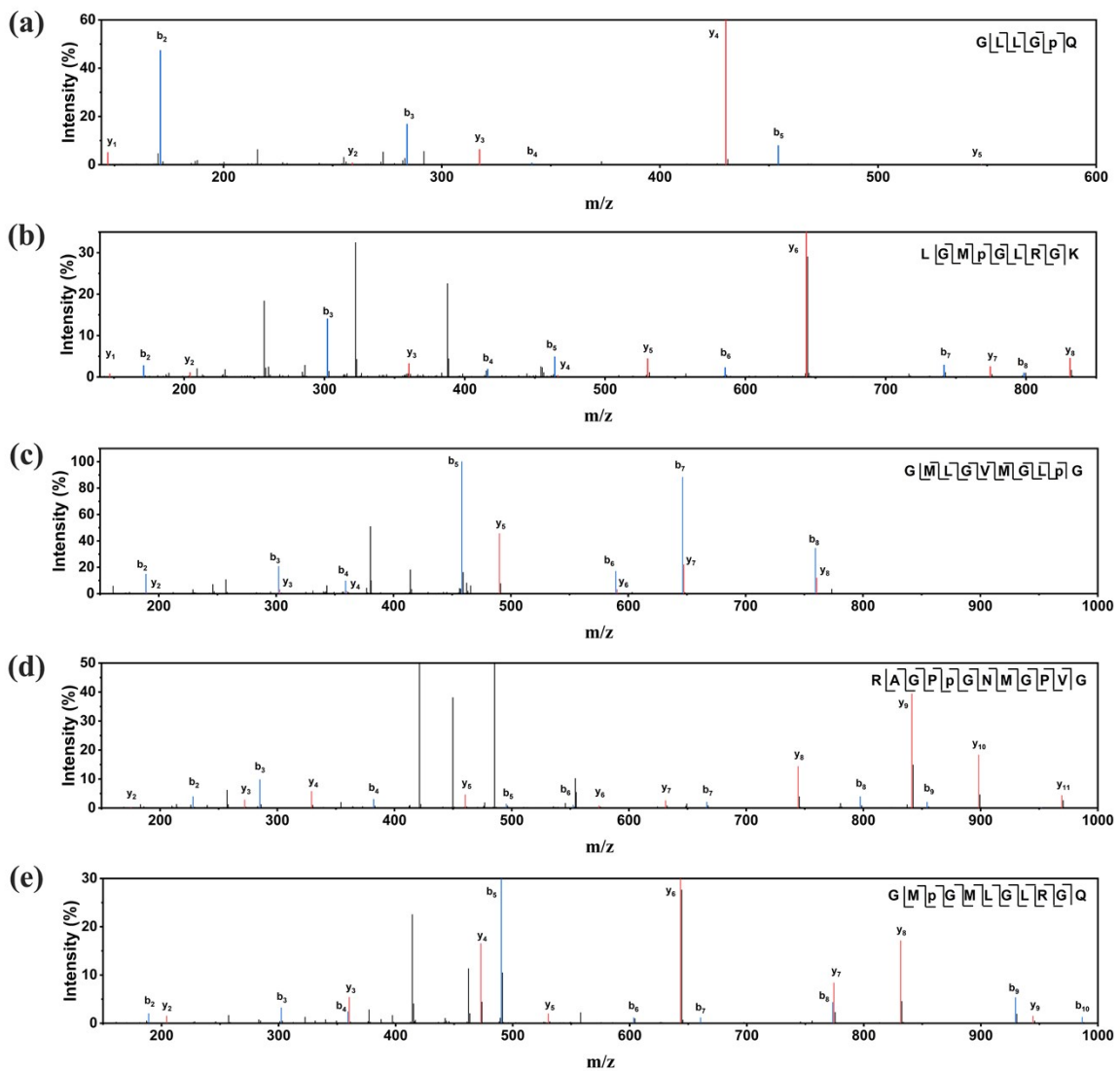


Fig S3 Results from MS/MS spectra for the five major peptides in CCH. (a) MS/MS with the parent ion m/z 300.7. (b) MS/MS with the parent ion m/z 472.7. (c) MS/MS with the parent ion m/z 474.2. (d) MS/MS with the parent ion m/z 563.3. (e) MS/MS with the parent ion m/z 566.8.

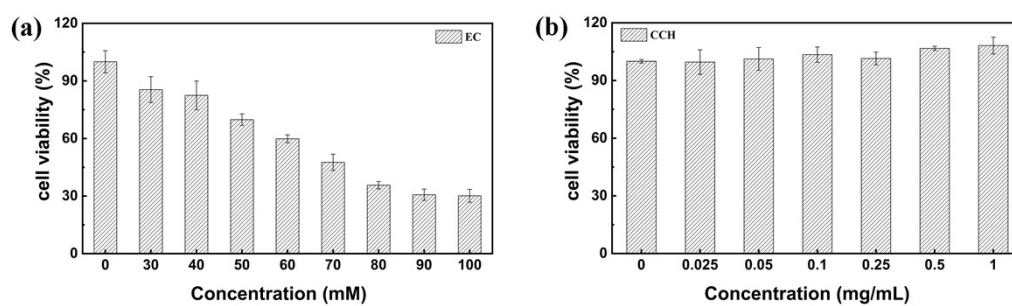


Fig S4 Cell cytotoxicity of HepG2 cells (a) treated with EC and (b) induced by CCH in different concentrations.

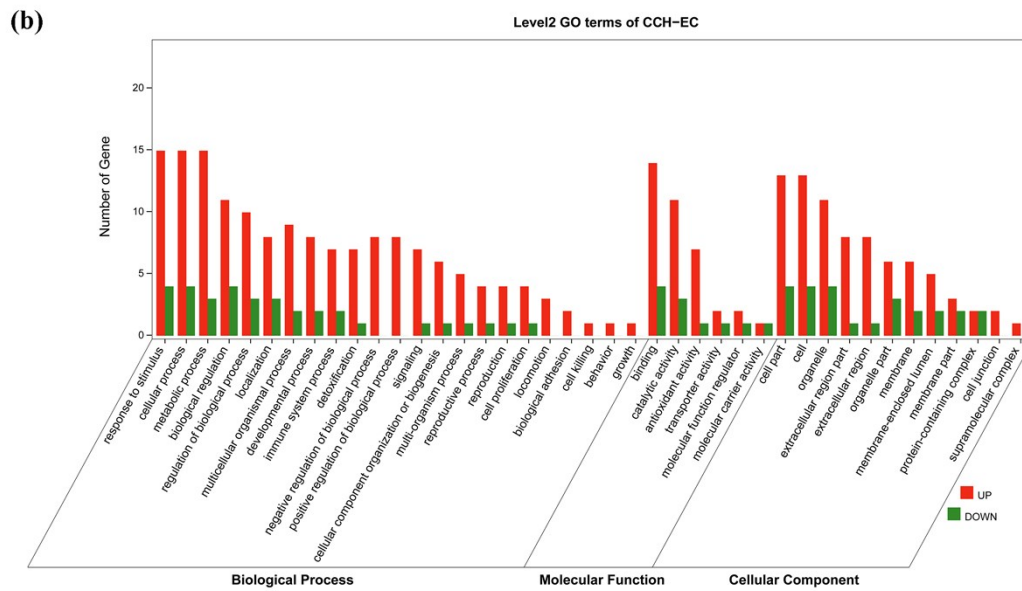
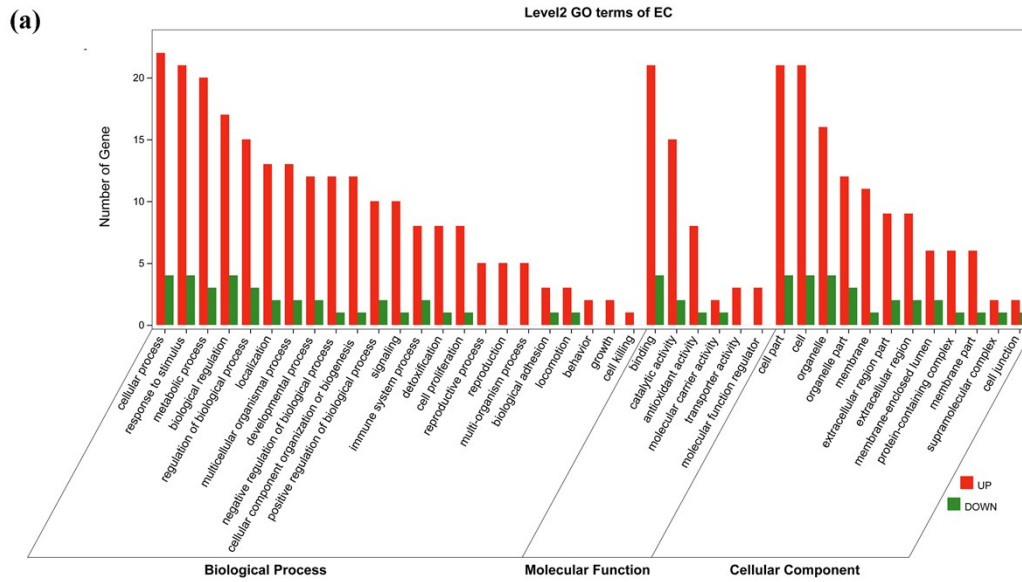


Fig S5 Gene Ontology (GO) classification map of cells treated with (a) EC and (b) CCH-EC.

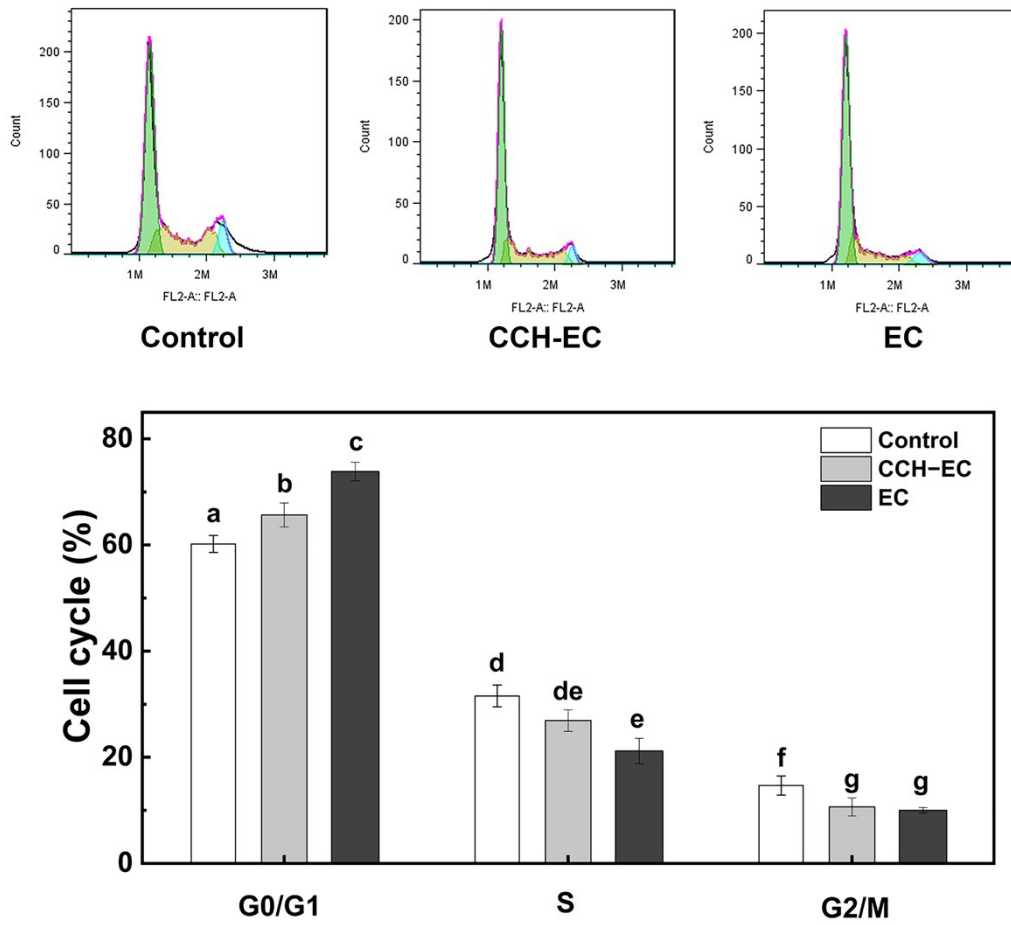


Fig S6 The cell cycle and apoptosis of HepG2 cells treated with 65 mM EC and CCH.