Litchi chinensis seed prevents obesity and modulates the gut microbiota and mycobiota compositions in high-fat diet-induced obese zebrafish

Jun-Yan Xiang [1,3], Yan-Yu Chi [1,3], Jin-Xin Han [1,3], Ping Kong [1,3], Zehua Liang [1,3], Deli Wang [3*], Hongyu Xiang [1,2,3,4*], Qiuhong Xie [1,2,3,4*]

[1] Key Laboratory for Molecular Enzymology and Engineering of Ministry of Education, School of Life Sciences, Jilin University, Changchun 130012, People's Republic of China

[2] National Engineering Laboratory for AIDS Vaccine, School of Life Sciences, Jilin University, Changchun 130012, People's Republic of China

[3] School of Life Sciences, Jilin University, Changchun 130012, People's Republic of China

[4] Institute of Changbai Mountain Resource and Health, Jilin University, Fusong 134504, People's Republic of China

* Corresponding authors:

Deli Wang, E-mail: wangdl@jlu.edu.cn

Hongyu Xiang, Professor; E-mail: hyxiang@jlu.edu.cn

Qiuhong Xie, Professor; E-mail: qhxie@jlu.edu.cn

Tel & Fax: +86-431-85153832

ORCID ID: 0000-0002-3902-8436

Figures

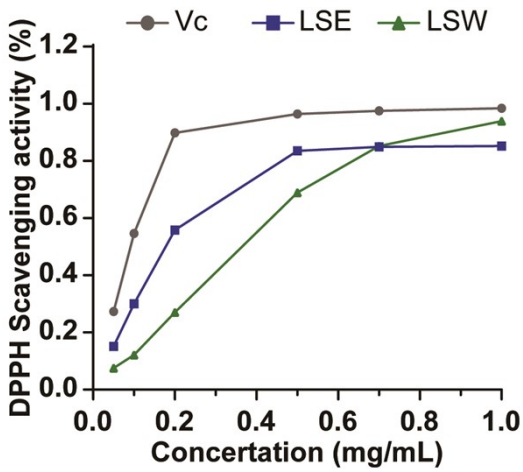

Fig. S1 Antioxidant activities of *L. chinensis* seed extracts

Scavenging effect on DPPH.

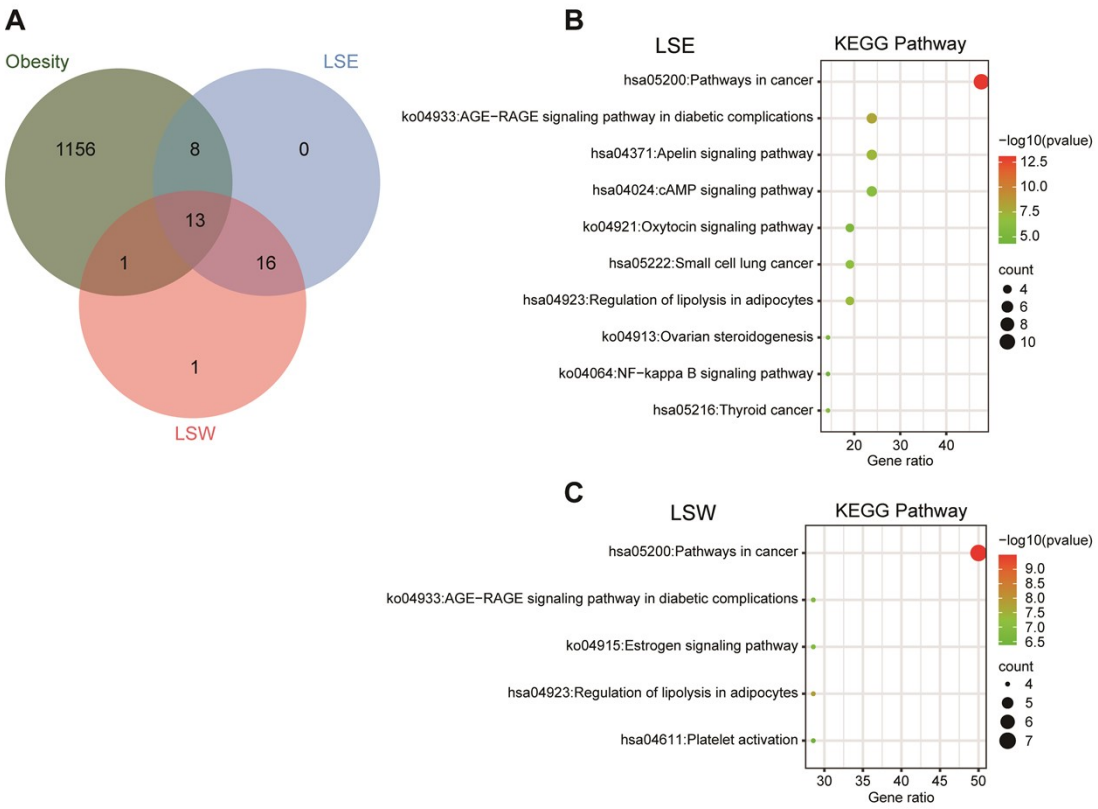

Fig. S2 Network pharmacology analysis of *L. chinensis* seed extracts in the treatment of obesity (A) Venn diagram for *L. chinensis* seed extracts and obesity targets. (B–C) KEGG pathway enrichment of *L. chinensis* seed ethanol and water extract in obesity treatment. LSE, *L. chinensis* seed ethanol extract; LSW, *L. chinensis* seed water extract.

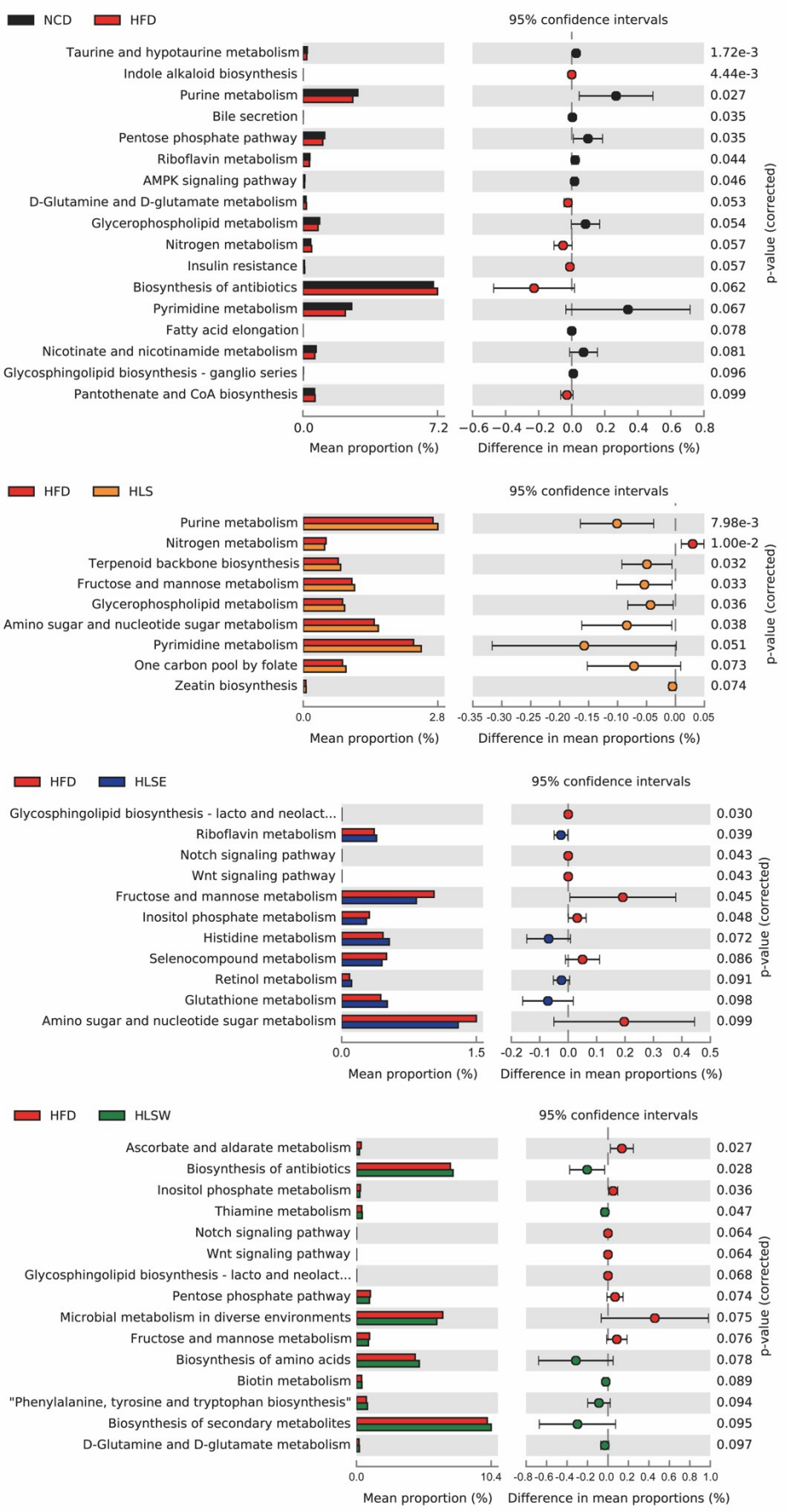

Fig. S3 The difference analysis of microbial functional prediction

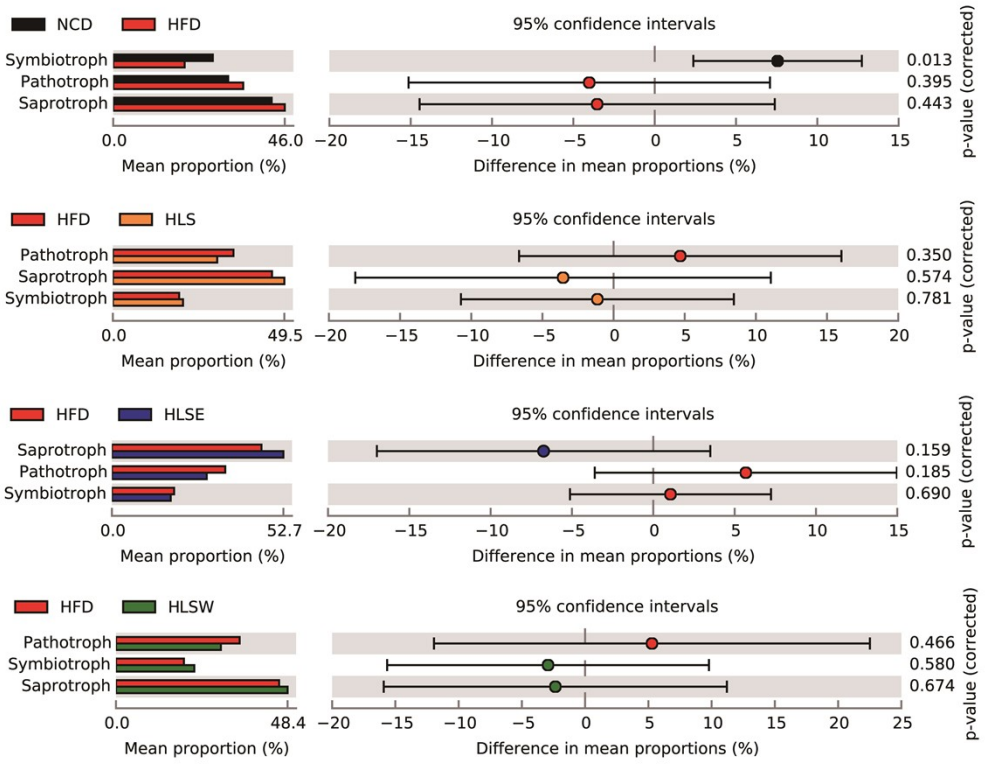

Fig. S4 The difference analysis of mycobial functional prediction

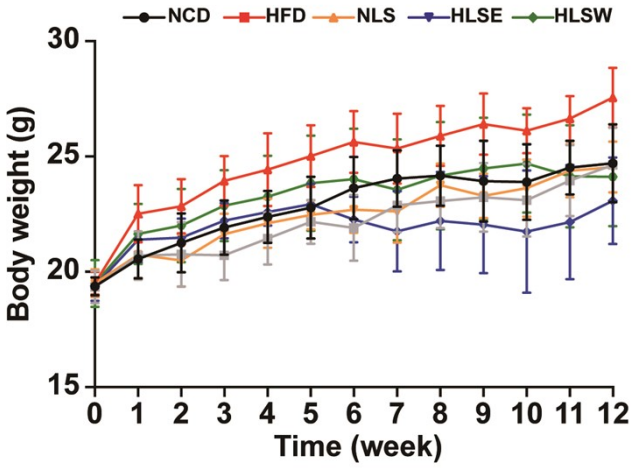

Fig. S5 Effect of *L. chinensis* seed on body weight in mice

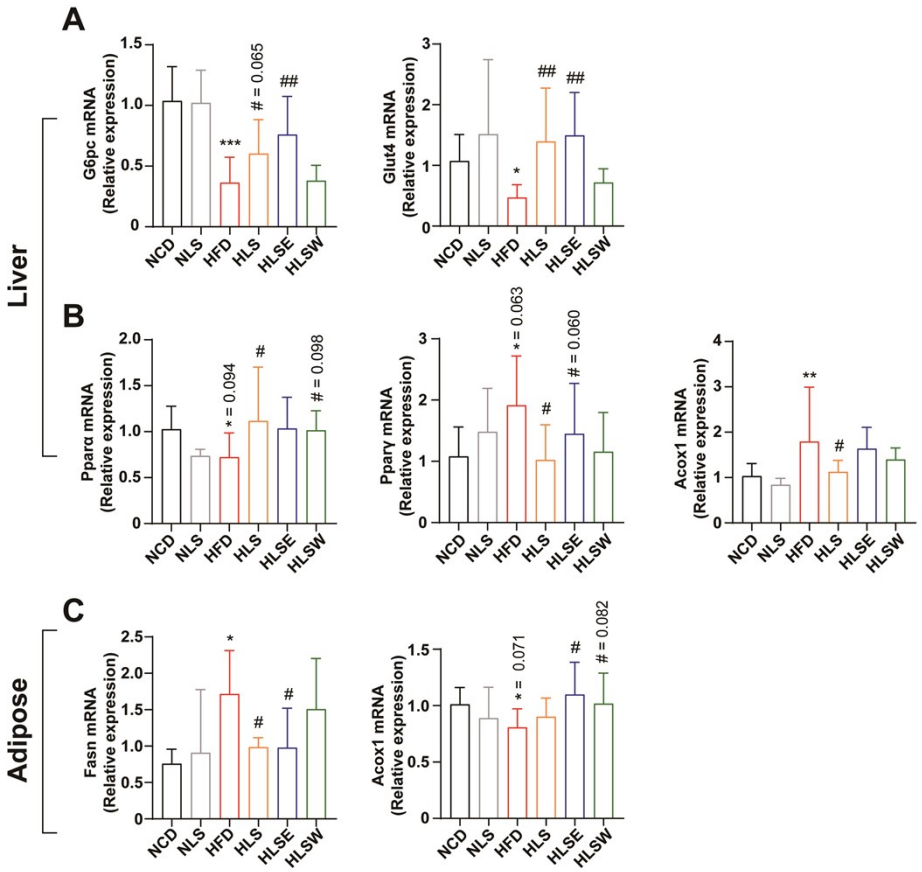

Fig. S6 Effects of *L. chinensis* seed on glucolipid metabolism in mice

(A) The expression of genes related to glucose metabolism in the liver. (B) Expression of genes related to lipid metabolism in the liver and (C) adipose. Results were shown as the mean ± SD. * $p<0.05$, *** $p<0.001$ compared with NCD group, and # $p<0.05$, ## $p<0.01$ compared with HFD group by ANOVA one-way statistical analysis.

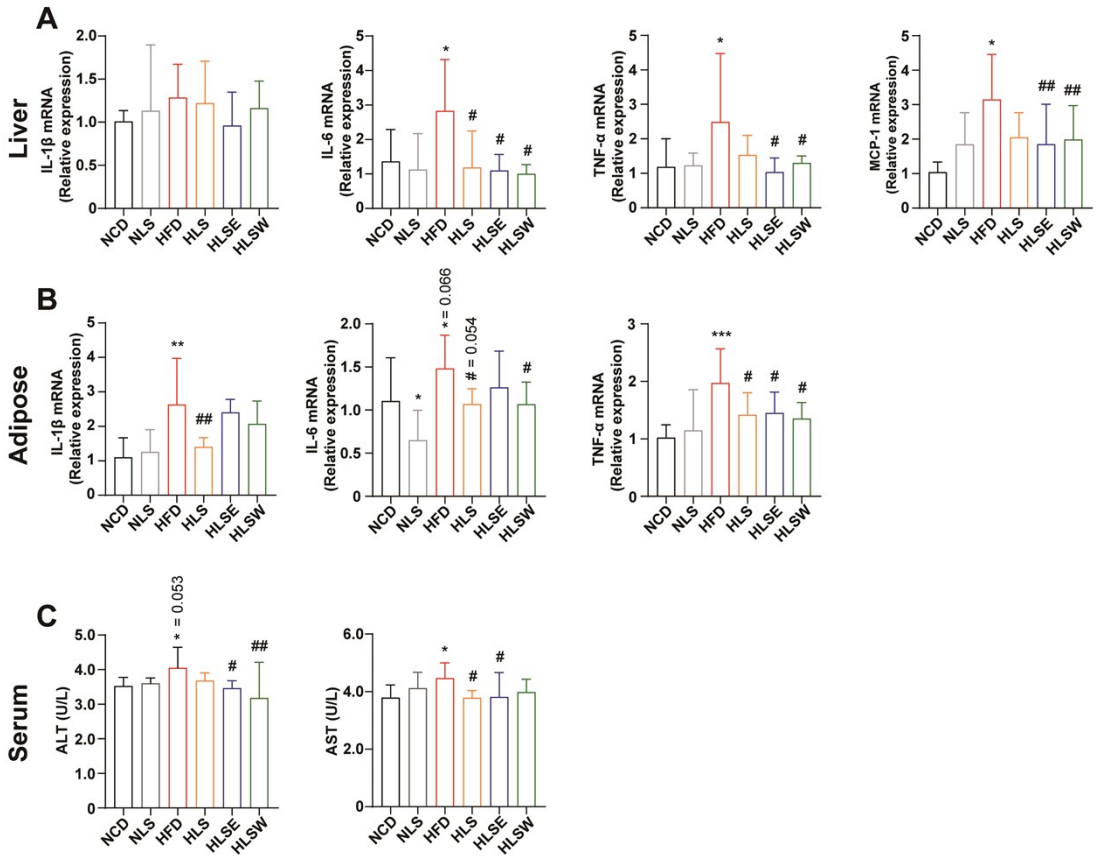

Fig. S7 Effects of *L. chinensis* seed on inflammation and liver function in mice

(A) The expression of inflammatory cytokines in the liver and (B) adipose. (C) Serum ALT and AST levels. Results were shown as the mean ± SD. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared with NCD group, and # $p<0.05$, ## $p<0.01$ compared with HFD group by ANOVA one-way statistical analysis.

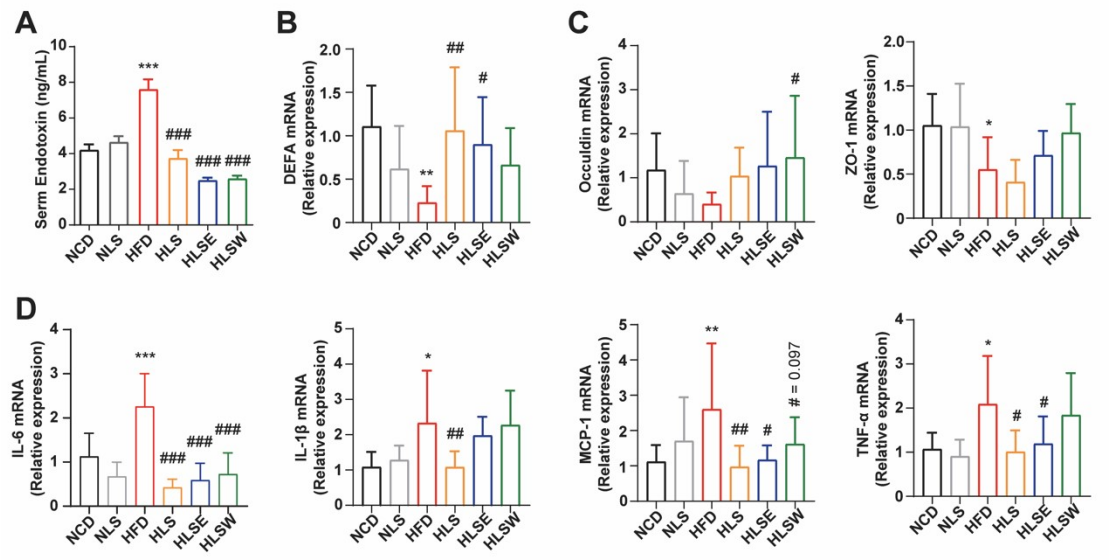

Fig. S8 Effects of *L. chinensis* seed on intestinal functions in mice

(A) Serum endotoxin levels. The expression of (B) *DefA*, (C) *Occludin*, *ZO-1*, and (D) inflammatory cytokines in the intestine. Results were shown as the mean ± SD. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared with NCD group, and # $p<0.05$, ## $p<0.01$, ### $p<0.001$ compared with HFD group by ANOVA one-way statistical analysis.

Table S1. Primer sequences for real-time reverse transcription polymerase chain reaction

Primer	Animal	Forward sequence (5'-3')	Reverse sequence (5'-3')
B2m	Mice	TTGTCTCACTGACCGGCCT	TATGTTCGGCTTCCCATTCTCC
Rpl-19	Mice	GAAGGTCAAAGGGAATGTGTTCA	CCTTGTCTGCCTTCAGCTTGT
Gapdh	Mice	GTGTTCCTACCCCCAATGTGT	ATTGTCATACCAGGAAATGAGCTT
Glut4	Mice	ACGACGGACACTCCATCTGTTG	GGAGACATAGCTCATGGCTGGAA
G6pc	Mice	AGGAAGGATGGAGGAAGGAA	TGGAACCAGATGGGAAAGAG
Fasn	Mice	TTCCAAGACGAAAATGATGC	AATTGTGGGATCAGGAGAGC
Acox1	Mice	CTATGGGATCAGCCAGAAAGG	AGTCAAAGGCATCCACCAAAG
Pparα	Mice	CAACGGCGTCGAAGACAAA	TGACGGTCTCCACGGACAT
Pparγ	Mice	TCGCTGATGCACTGCCTATG	GAGAGGTCCACAGAGCTGATT
Tnf-α	Mice	AGACCCTCACACTCAGATCA	TCTTTGAGATCCATGCCGTTG
Il-6	Mice	GTTCTCTGGGAAATCGTGGA	TGTACTCCAGGTAGCTA
Il-1β	Mice	TCCATGAGCTTTGTACAAGGA	AGCCCATACTTTAGGAAGACA
Mcp-1	Mice	TTAAAAACCTGGATCGGAACCAA	GCATTAGCTTCAGATTTACGGGT
ZO-1	Mice	TTTTTGACAGGGGGAGTGG	TGCTGCAGAGGTCAAAGTTCAAG
Occludin	Mice	ATGTCCGGCCGATGCTCTC	TTTGGCTGCTCTTGGGTCTGTAT
DefA	Mice	GGTGATCATCAGACCCCAGCATCAGT	AAGAGACTAAAACTGAGGAGCAGC
bax	Zebrafish	GATACGGGCAGTGGCAATGA	ACTCCGGGTCACTTCAGCAT
bcl-2	Zebrafish	CGAGTGTGTGGAGAAGGAGATG	TGGTTGTCTAGGTAGACGGTCAT
il-1β	Zebrafish	ATCCAAACGGATACGACCAG	TCGGTGTCTTTCCTGTCCAT
il-6	Zebrafish	TCAACTTCTCCAGCGTGATG	TCTTTCCCTCTTTTCCTCCTG
tnf-α	Zebrafish	AGGCAATTTCACTTCCAAGG	AGGTCTTTGATTCAGAGTTGTATCC
ifn-γ	Zebrafish	GAGAGGCTGGCACATGTTCAA	CTCTGCACTCTTGCCTGGAAA
ampkα	Zebrafish	AGTTATCAGCACACCGACAG	AGTAATCCACCCCTGAGATG
leptin A	Zebrafish	CATCATCGTCAGAATCAGGG	ATCTCGGCGTATCTGGTCAA
pparα	Zebrafish	ACGGGAAAGACAAGCACGC	GTTCAGCAGACCTCCGCAAGA
nr3c1	Zebrafish	TCTCCTCCCAACAGCAGGAC	GTTGCATACAGTCGCAGCC
orexin	Zebrafish	GCTCCTTGCAAACTACGAG	GCTCCTTGCAAACTACGAG
ghrelin	Zebrafish	CAAGAGTGGGCAGAAGAGAA	ATGTAGTTGTAGTGGATGGT
β-actin	Zebrafish	ACTGTATTGTCTGGTGGTAC	TACTCCTGCTTGCTAATCC
fabp11α	Zebrafish	AGTAGAGGAGCATCATTATTCGGG	CAAAACCCACACCTATAGCCTTC

Table S2. Content of effective components in *L. chinensis* seed extracts using chemical methods

Samples	Components (%)		
	Polysaccharide	Triterpenoid	Polyphenol
L. chinensis seed water extract	73.43 ± 1.77	10.65 ± 0.13	12.05 ± 2.15
L. chinensis seed ethanol extract	57.90 ± 1.99	20.24 ± 0.22	21.65 ± 2.89

Data were shown as the mean ± SD.

Comment [X]: Format was changed

Table S3. Effects of *L. chinensis* seed on the weight of adipose tissues and liver in mice

Comment [X]: Format was changed

Groups	Adipose tissues and liver weight (mg/g)				
	Inguinal fat	Epididymal fat	Mesenteric fat	Perinephric fat	Liver
NCD	4.61 ± 0.95	12.16 ± 2.27	9.69 ± 2.25	2.59 ± 0.58	44.21 ± 2.74
NLS	3.24 ± 0.43	11.52 ± 2.28	8.50 ± 3.82	2.28 ± 0.63	46.77 ± 6.05
HFD	8.36 ± 3.07***	18.27 ± 3.20***	16.00 ± 2.49***	4.36 ± 1.29***	48.87 ± 4.64*
HLS	6.90 ± 1.66	8.09 ± 2.04$^{\#\#\#}$	15.24 ± 2.25	3.39 ± 1.02$^{\#}$	45.26 ± 2.43
HLSE	7.28 ± 1.74	9.12 ± 2.36$^{\#\#\#}$	14.07 ± 1.90	3.06 ± 1.05$^{\#\#}$	42.32 ± 6.29$^{\#\#}$
HLSW	6.24 ± 1.59$^{\#}$	12.55 ± 2.01$^{\#\#\#}$	14.32 ± 3.50	4.19 ± 1.24	42.38 ± 5.84$^{\#\#}$

Data were shown as the mean ± SD.

Table S4. Effects of *L. chinensis* seed on serum lipids in mice

Groups	Serum lipids (mmol/L)			
	T-CHO	TG	HDL-C	LDL-C
NCD	2.50 ± 0.28	0.86 ± 0.19	0.90 ± 0.47	0.74 ± 0.09
NLS	2.56 ± 0.54	0.73 ± 0.28	1.03 ± 0.37	0.63 ± 0.14
HFD	3.49 ± 0.36***	1.08 ± 0.35	0.73 ± 0.15	1.08 ± 0.23***
HLS	2.89 ± 0.30$^{\#}$	0.80 ± 0.31	0.77 ± 0.09	0.74 ± 0.06$^{\#\#}$
HLSE	3.02 ± 0.22	0.61 ± 0.21$^{\#\#}$	1.09 ± 0.17$^{\#}$	0.63 ± 0.14$^{\#\#\#}$
HLSW	2.98 ± 1.05	0.65 ± 0.23$^{\#\#}$	0.87 ± 0.21	0.85 ± 0.30*

Data were shown as the mean ± SD.

Comment [X]: Format was changed