Supplementary Information

Effects of *Momordica* saponins extract on alleviating fat accumulation in *Caenorhabditis elegans*

Chunxiu Lin[ab], Yizi Lin[ab], Yue Chen[ab], Jiena Xu[ab], Jun Li[ab], Yong Cao[ab], Zuanxian Su[c*], Yunjiao Chen[ab*]

1 LC-Q-TOF-MS/MS analysis

MSE were qualitatively analyzed using Uplc 1290-6540B Q-TOF (Agilent Technologies, Palo Alto, CA, USA). Chromatographic separation was carried out using an Agilent Eclips plus C18 column (100 × 2.1 mm, 1.8 μm) with a binary pump, degasser, column oven and autosampler. The analyses employed a 40-min linear gradient of acetonitrile (A) and 0.2% formic acid in ultrapure water (B), increasing from 10 to 90% B, with a 5-min hold at 90 % B, and a 5-min post-run at 10% B. The injection volume was 5 μL. The flow rate was set at 0.4 mL/min and oven temperature was 40°C. For the online TOF-MS and TOF-MS/MS analysis, experimental operation parameters were set as follows: nozzle voltage 1000 V; skimmer, 65 V; gas flow, 8 L/min; gas temp, 300°C; nebulizer, 45 psi. The collision energy (CE) was set at 0 eV for negative and positive ion mode. The mass scan was over the range of m/z 100–1700 for both modes. Data analysis was carried out using the Agilent Mass Hunter Qualitative software (version B.07.00). Accurate mass scan data were mined using the find by molecular feature (FMF), find by formula (FbF), find by targeted MS/MS and molecular formula generator (MFG) algorithms.

2 Determination of total saponins

The colorimetric method with vanillin–acetic acid system was performed for the quantification of total saponins.[1] Briefly, MSE was dried and reconstituted with a methanol solution at 1:1. After the solution(0.2 mL) was evaporated to dryness in a water-bath, a fresh solution of vanillin-acetic acid (5% w/v, 0.2 mL) solution was prepared and perchloric acid (0.8 mL) was added and kept at 70°C for 15 min. The solution was cooled in running water for 10 min before adding glacial acetic acid (5 mL). The solution was measured at 555 nm using a UV spectrophotometer (Agilent 8453, Agilent technologies, CA, USA), with a blank solution as reference. Quantification was based on the standard curve of ginsenoside Rg1 (0–160 μg), which was dissolved in methanol ($A = 3.7293C$, $R^2 = 0.9994$).

3 Primer sequences for qRT-PCR analysis

Table S1 Primer sequences for qRT-PCR analysis

Gene	Primer
sbp-1	CTACTCGCACCATTCTTCTCG (F)
	CCAAATCTCAACTGCTTCTGC (R)
nhr-49	CTTCCATCGAAAGATCCATGA (F)
	GGATTCTCTGCTCTCCGAGTT (R)
mdt-15	AACATCAGCTCAGGCAAGAAA (F)
	TCTGTCCACCTGGACGAATAC (R)
fat-5	GGGCTACAGTTGGATGGGTAT (F)
	ATCTCTGGCCCAGTCGATAAT (R)
fat-6	CTTGTGCTGCTTCATTCTTCC (F)
	GAAGTTGTGACCTCCCTCTCC (R)
fat-7	ACCCGTGGATTCTTCTTCACT (F)
	TAACGGAATGTTCCAGCTACG (R)
fasn-1	ACTGTCGGATCAGCTGAGAAA (F)
	GACGAGCCAAACATCTGAGAG (R)
pod-2	AACACCTTCGTCATCATCCTG (F)
	CCAGTGTACGGAGACTTGAGC (R)
acs-2	GGCTGAACAACAACGCATATT (F)
	GACTTTGATGGGAAGACCACA (R)
daf-2	GGATAAAGGCGAATCAAAGTGTC (F)
	CGATACACTTTCCCTTGTGATAGAC (R)
age-1	TTGTTCGTTTCCTTGTTCACC (F)
	ATCCATTGAAGGCTTCTTCGT (R)
daf-16	CTTCAAGCCAATGCCACTACC (F)
	GGAGATGAGTTGGATGTTGATAGC (R)
act-1	TCCAAGAGAGGTATCCTTAC (F)
	CGGTTAGCCTTTGGATTGAG (R)

4 Relative quantitative data using ImageJ software

Table S2 Relative quantitative data using ImageJ software

genotype	treatment	mean ± SD	*p*-value
normal feeding N2	CK	1.00 ± 0.02	0.000
	MSE	0.79 ± 0.02	
glucose feeding N2	CK	1.28 ± 0.04	0.000
	MSE	0.97± 0.00	
high-fat N2	CK	1.00 ± 0.02	0.004
	MSE	0.91 ± 0.01	
N2 (age pigment, day 3)	CK	1.00 ± 0.04	0.024
	MSE	0.89 ± 0.03	
N2 (age pigment, day 7)	CK	1.00 ± 0.04	0.039
	MSE	0.93 ± 0.01	
N2 (age pigment, day 11)	CK	1.00 ± 0.02	0.001
	MSE	0.76 ± 0.03	
N2 (body area)	CK	1.00 ± 0.14	0.271
	MSE	0.88 ± 0.04	
SBP-1::GFP	CK	1.00 ± 0.04	0.000
	MSE	0.79 ± 0.02	
daf-2	CK	1.27 ± 0.03	0.113
	MSE	1.31 ± 0.02	
age-1	CK	1.18 ± 0.06	0.365
	MSE	1.14 ± 0.04	
daf-16	CK	0.91 ± 0.00	0.306
	MSE	0.88 ± 0.04	
sbp-1	CK	0.75 ± 0.01	0.509
	MSE	0.76 ± 0.02	
mdt-15	CK	0.81 ± 0.04	0.598
	MSE	0.78 ± 0.07	
nhr-49	CK	0.89 ± 0.06	0.426
	MSE	0.93 ± 0.03	
fat-5	CK	0.67 ± 0.01	0.383
	MSE	0.68 ± 0.01	
fat-6	CK	0.57 ± 0.00	0.383
	MSE	0.58 ± 0.01	
fat-7	CK	0.63 ± 0.02	0.630
	MSE	0.64 ± 0.01	
fat-5/fat-6	CK	0.60 ± 0.01	0.136
	MSE	0.58 ± 0.01	
fat-5/fat-7	CK	0.58 ± 0.00	0.148
	MSE	0.59 ± 0.01	

5 Rrepresentative total ion chromatograms

Figure S1. Representative TIC of MSE. (A) TIC of MSE sample in positive ion mode. (B) TIC of MSE sample in negative ion mode.

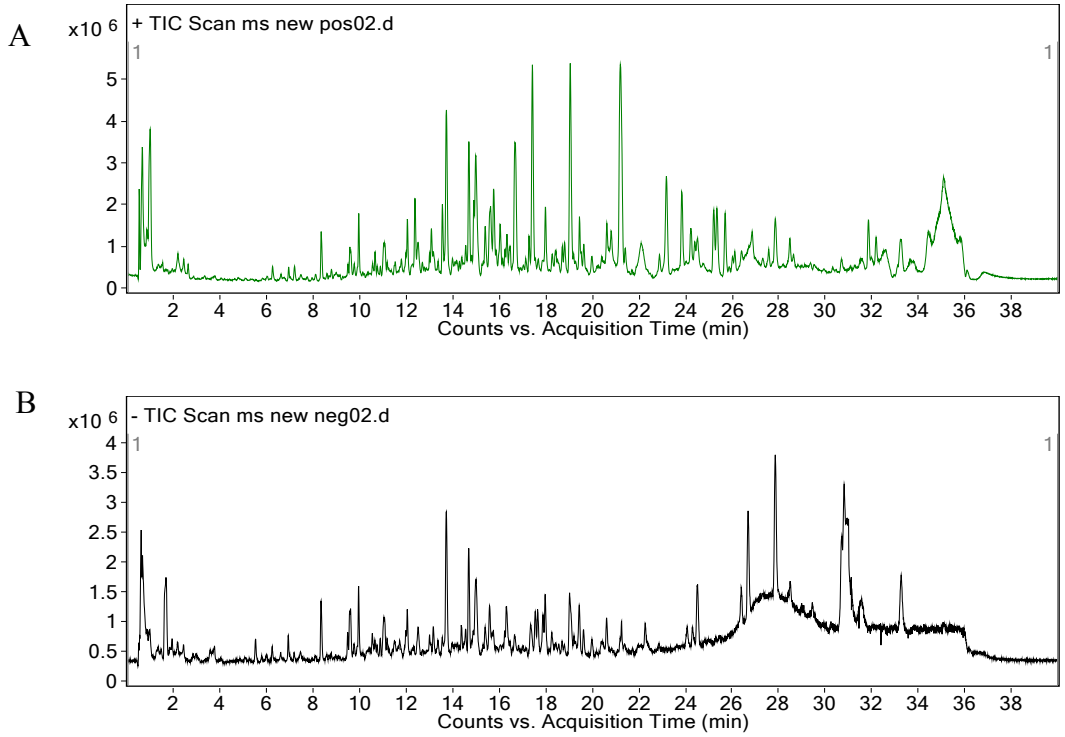

Fig. S1

6 The process of fat accumulation assay

Figure S2. The illustrative diagram indicating the treatment method of MSE in N2. (A) The treatment of normal feeding and glucose feeding. (B) The treatment mode of the high fat model.

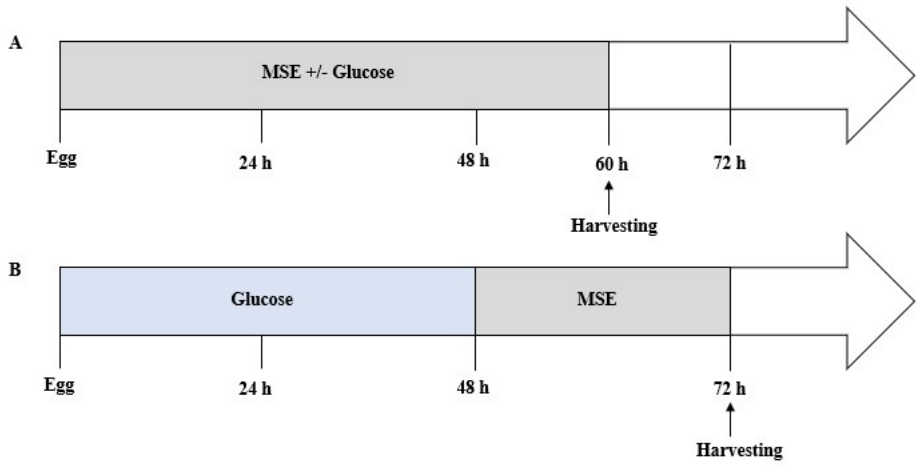

Fig. S2

7 Construction of glucose-induced high-fat N2

Figure S3. Construction of glucose-induced high-fat *C. elegans*. (A) Representative images of body fat after 48 hours treatment with 10 mM glucose in N2 by ORO staining. (B) Relative quantification of the lipid content using Image J software. High-glucose diet significantly increased the fat content, confirming the successful construction of high-fat *C. elegans.*

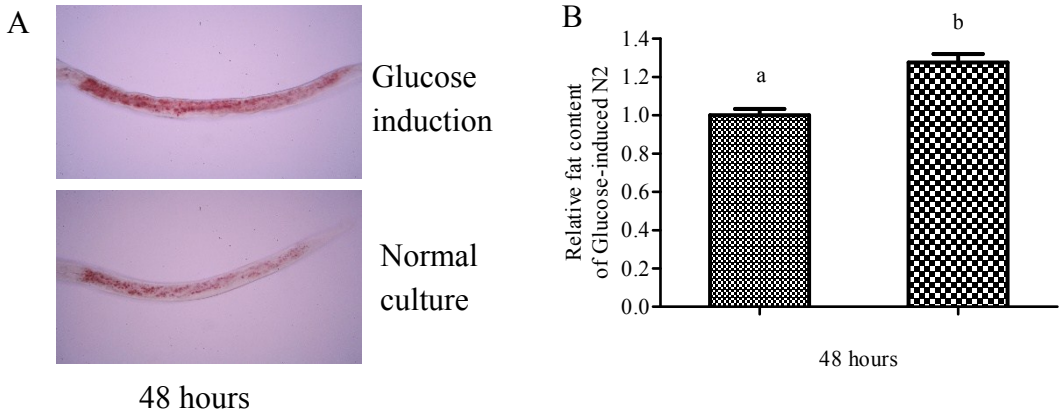

Fig. S3

References

1. Y. Y. Lu, Z. H. Ao, Z. M. Lu, H. Y. Xu, X. M. Zhang, W. F. Dou and Z. H. Xu, Analgesic and anti-inflammatory effects of the dry matter of culture broth of Termitomyces albuminosus and its extracts, *Journal of ethnopharmacology*, 2008, **120**, 432-436.