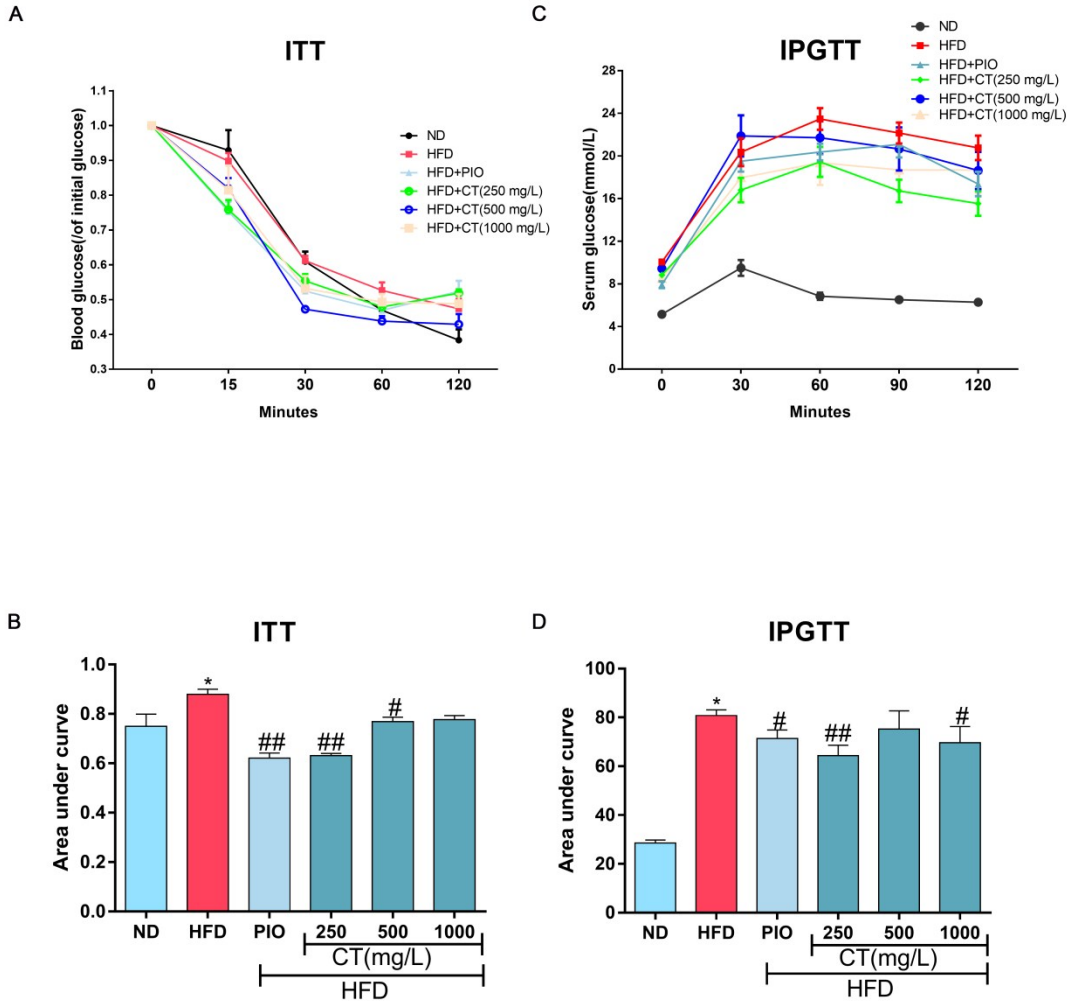
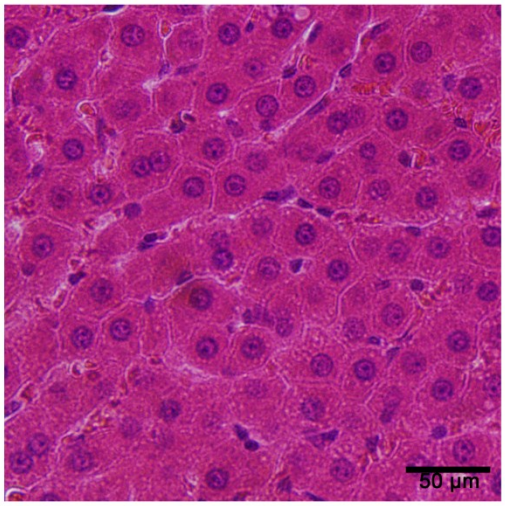
Electronic Supplementary Material (ESI) for Food & Function.

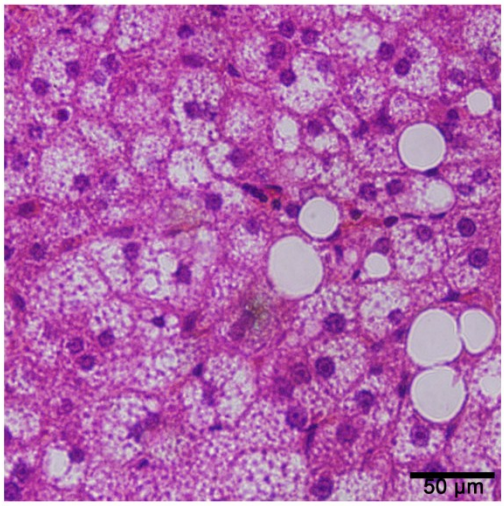


Supplementary Figure 1. Effects of CT on Insulin tolerance test (ITT) and Intraperitoneal glucose tolerance tests (IPGTT) in healthy ND rats, HFD-fed rats and HFD-fed rats with CT supplementation. (A) ITT; (B) Incremental area under the ITT curve; (C) IPGTT; (D) Area under the IPGTT curve. ND group vs the HFD group: *p<0.05, **p<0.01; HFD group vs the CT-supplemented groups: #p<0.05, ##p<0.01.

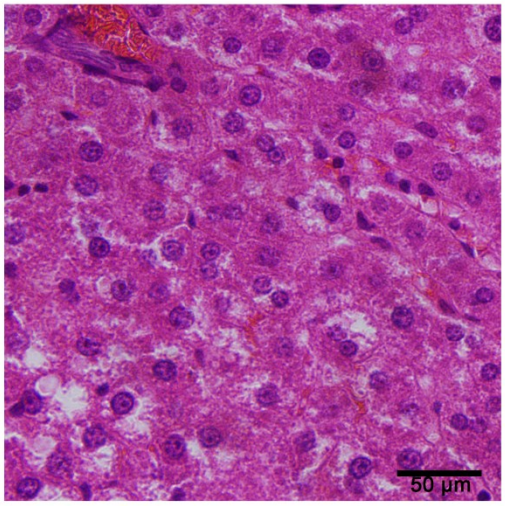
ND



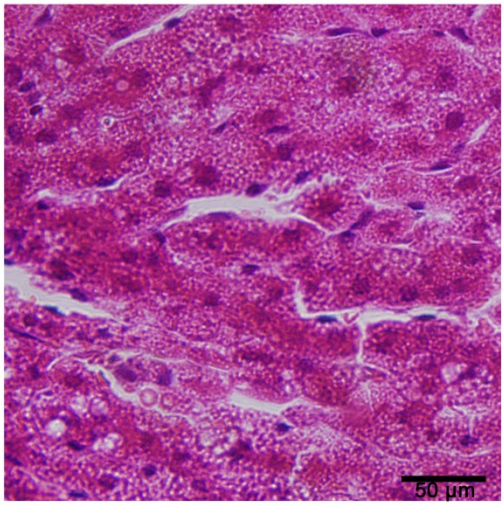
HFD



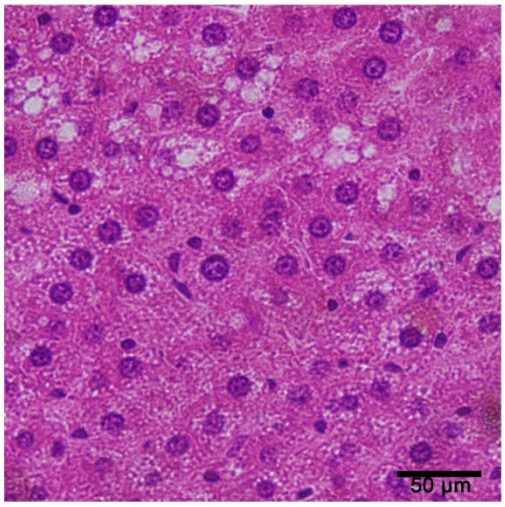
PIO



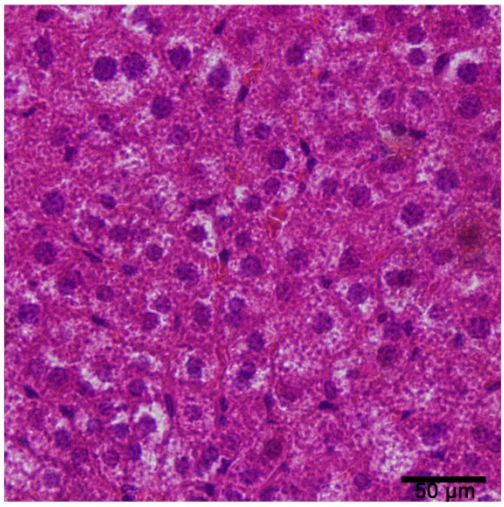
CT (250 mg/L)



CT (500 mg/L)



CT (1000 mg/L)



Supplementary Figure 2. Effects of CT on hepatic steatosis induced by HFD.

Representative haematoxylin and eosin staining of liver sections (Bar, 50 μm).

Methods:

DNA extraction

Specimens: Specimens (20 mg each) were powdered using a Mixer Mill MM400 (Retsch GmbH, Haan, Germany) at 30 r/s for 2 min. Total genomic DNA was extracted using a Plant Genomic DNA kit (Tiangen Biotech Co., Ltd.).

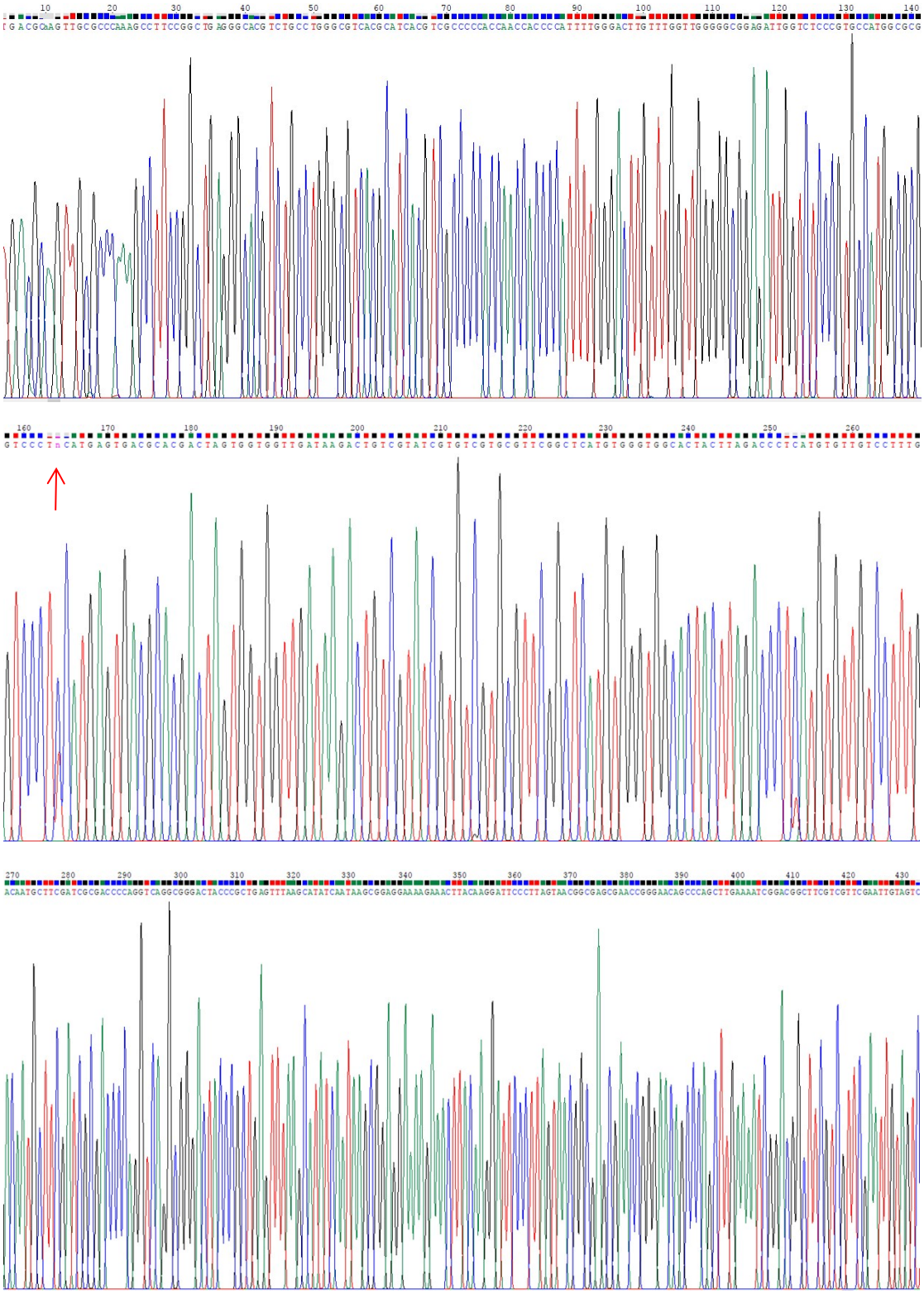
PCR amplification and sequencing

ITS2was amplified following DNA barcoding standard operating procedures (SOPs) developed by Chen et al.[1,2]. Then, agarose gel electrophoresis was applied to detect the preliminary results. All the PCR products were sent to Ruibio BioTech Company for sequencing. Samples were amplified using LA Taq polymerase (Takara). The 25μL reaction contained 0.25 μL of TaKaRa LA Taq®, 2.5 μL of 10× LA Taq Buffer II (Mg^{2+} plus/free), 2 μL of dNTP mix, 1μL of primers (2.5 μmol/L, ITS2F/3R), 1 μL of genomic DNA template and 17.25 μL of ddH_2O. The PCR procedure was the same as above. Gel electrophoresis with a 1× TAE agarose gel was used for gel extraction, and each well contained the entire 25μLPCR product together with 3 μL of 6× loading buffer.

DNA was sent for sequencing.

Results:

The PCR amplification success rate for the ITS2 region was 100%. Our samples' sequences were compared to the sequences of Prof. Jia's specimens. We analysed the DNA sequences using the DNAMAN program. As the figure below shows, the DNA sequencing results for our samples were consistent with those for Prof. Jia's specimens; we found only one single-nucleotide polymorphism (SNP) site among them. Taking the above results into account, we felt confident in the conclusion that the plants we purchased were indeed CT flowers.



Our ITS2 SEQUENCE:

TTTTGACGCaAGTTGCGCCCAAAGCCTTCCGGCTGAGGGCACGTCTGCCTGGGCGT
CACGCATCACGTCGCCCCCACCAACCACCCCATTTTGGGACTTGTTTGGTTGGGGG
CGGAGATTGGTCTCCCGTGCCATGGCGCGGTTGGCCTAAATAGAAGTCCCTnCATG
AGTGACGCACGACTAGTGGTGGTTGATAAGACTGTCGTATCGTGTCGTGCGTTCGG

CTCATGTCCCTGGCACTACTTAGACCCTCATGTGTTGTCCTTTGACAATGCTTCGAT
CGCGACCCCAGGTCAGGCGGGACTACCCGCTGAGTTTAAGCATATCAATAAGCGGA
GGAAAAGAAACTTACAAGGATTCCCTTAGTAACGGCGAGCGAACCGGGAACAGCCC
AGCTTGAAAATCGGACGGCTTCGTCGTTCGAATTGTAGTCTGAAAAAGCGTCAA

Upper line: our sample, from 1 to 441
Lower line: Jia's sample, from 1 to 441

2-HAO.ITS2-F.5174364.H04.seq:1-HAO.ITS2-F.5174363.H02.seq identity=
98.21%(438/446) gap=0.00%(0/446)

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1   TTTTGACGCAAGTTGCGCCCAAAGCCTTCCGGCTGAGGGCACGTCTGCCTGGGCGTCACG
    ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
1   TTTTGACGCAAGTTGCGCCCAAAGCCTTCCGGCTGAGGGCACGTCTGCCTGGGCGTCACG

61  CATCACGTCGCCCCCACCAACCACCCCATTTTGGGACTTGTTTGGTTGGGGGCGGAGATT
    ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
61  CATCACGTCGCCCCCACCAACCACCCCATTTTGGGACTTGTTTGGTTGGGGGCGGAGATT
                                                  N=(T/C)
121 GGTCTCCCGTGCCATGGCGCGGTTGGCCTAAATAGAAGTCCCTNCATGAGTGACGCACGA
    |||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||
121 GGTCTCCCGTGCCATGGCGCGGTTGGCCTAAATAGAAGTCCCTTCATGAGTGACGCACGA

181 CTAGTGGTGGTTGATAAGACTGTCGTATCGTGTCGTGCGTTCGGCTCATGTCCCTGGCAC
    ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
181 CTAGTGGTGGTTGATAAGACTGTCGTATCGTGTCGTGCGTTCGGCTCATGTCCCTGGCAC

241 TACTTAGACCCTCATGTGTTGTCCTTTGACAATGCTTCGATCGCGACCCCAGGTCAGGCG
    |||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||
241 TACTTAGACCCTNATGTGTTGTCCTTTGACAATGCTTCGATCGCGACCCCAGGTCAGGCG

301 GGACTACCCGCTGAGTTTAAGCATATCAATAAGCGGAGGAAAAGAAACTTACAAGGATTC
    ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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361 CCTTAGTAACGGCGAGCGAACCGGGAACAGCCCAGCTTGAAAATCGGACGGCTTCGTCGT
    ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
361 CCTTAGTAACGGCGAGCGAACCGGGAACAGCCCAGCTTGAAAATCGGACGGCTTCGTCGT

421 TCGAATTGTAGTCTGAAAAAGCGTCA
    |||||||||||||||||||||||||
421 TCGAATTGTAGTCTGAAAAAAGCGTC
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