Non-estrogenic Xanthohumol Derivatives Mitigate Insulin Resistance and Cognitive Impairment in High-Fat Diet-induced Obese Mice

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Supplementary Information: Effect of xanthohumol (XN), α,β-dihydroxanthohumol (DXN), or tetrahydroxanthohumol (TXN) on the viability of C2C12 cells (Figure S1a) and of HepG2 cells (Figure S1b); Structural characterization of DXN by ^{1}H-NMR (Figure S2) and ^{13}C-NMR spectroscopy (Figure S3) and by electrospray mass spectrometry (Figure S4); Structural characterization of TXN by ^{1}H-NMR (Figure S5) and ^{13}C-NMR spectroscopy (Figure S6) and by electrospray mass spectrometry (Figure S7); The MS/MS spectra provided in Figures S4 and S7 were recorded on a Sciex 4000 QTrap mass spectrometer (see Methods). Supplementary Figure S8 contains full-length Western blots of liver and muscle biopsies from untreated mice (control, lanes 1-2) and treated with XN (lanes 3-4), DXN (lanes 5-6) and TXN (lanes 7-8). Tissue extracts were incubated with specific antibodies against pAMPK, AMPK and ß-actin. These original images were used to generate Figure 7. No exposure modifications were recorded.

Figure S1a. Exposure of C2C12 cells to XN, DXN, or TXN for one hour at concentrations 1-50 µM did not affect viability ($p > 0.05$ by ANOVA). Cell viability of treated cells is displayed as a percentage of vehicle treated cells (controls). Data are represented as mean ± SE of 4 replicate wells.

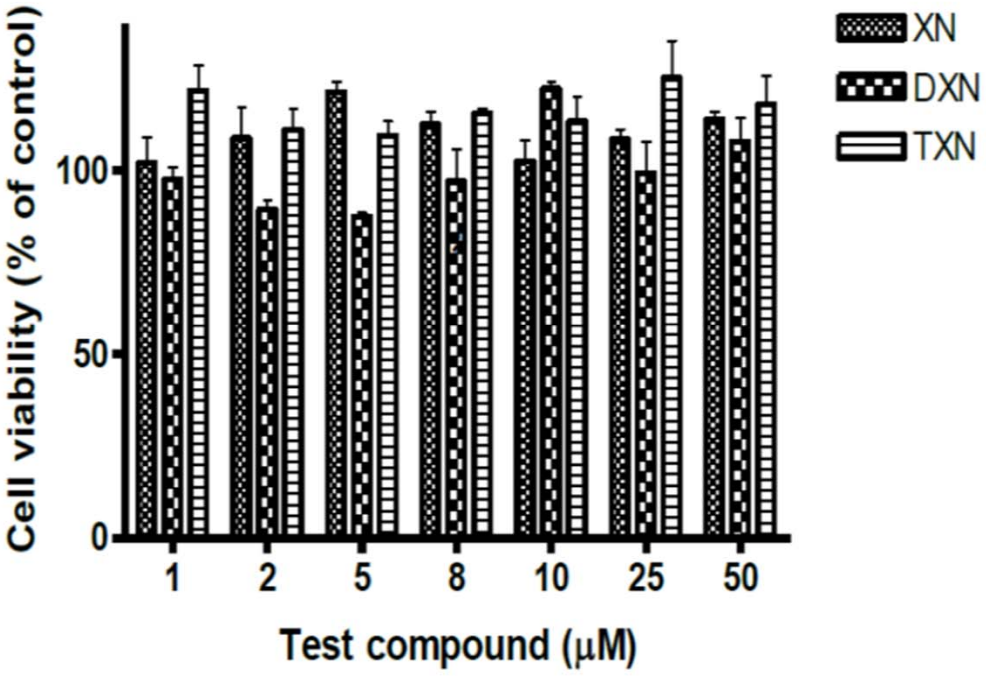

Figure S1b. Exposure of HepG2 cells to XN, DXN, or TXN for 24 h did not affect viability at concentrations up to 25 μM ($p > 0.05$ by ANOVA). However, at 50 μM, all the test compounds were cytotoxic. Cell viability of treated cells is displayed as a percentage of vehicle treated cells (controls). Data are represented as mean ± SE of 4 replicate wells.

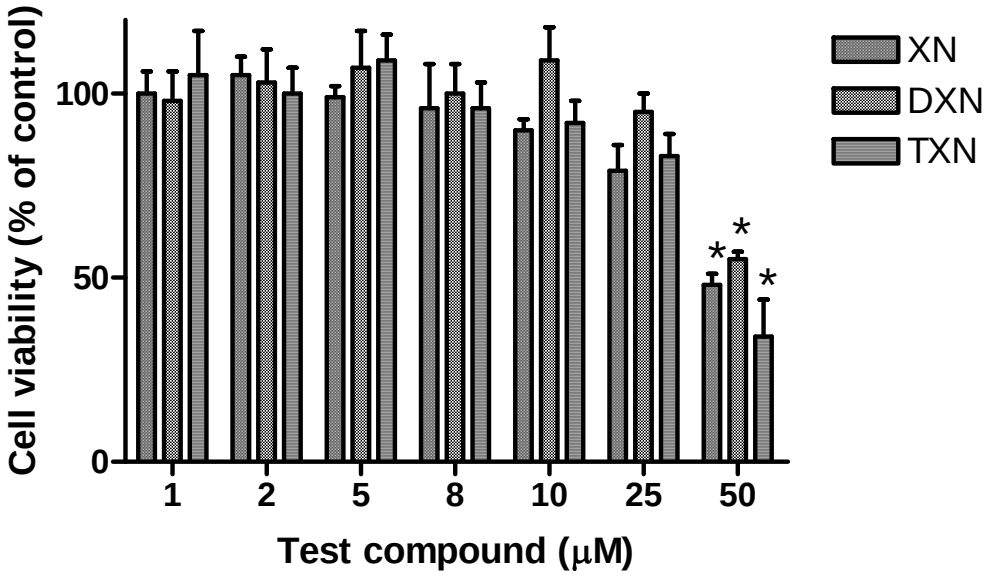

Figure S2. ^{1}H-NMR (700 MHz) spectrum of α,β-dihydroxanthohumol (DXN) recorded in methanol-d_4.

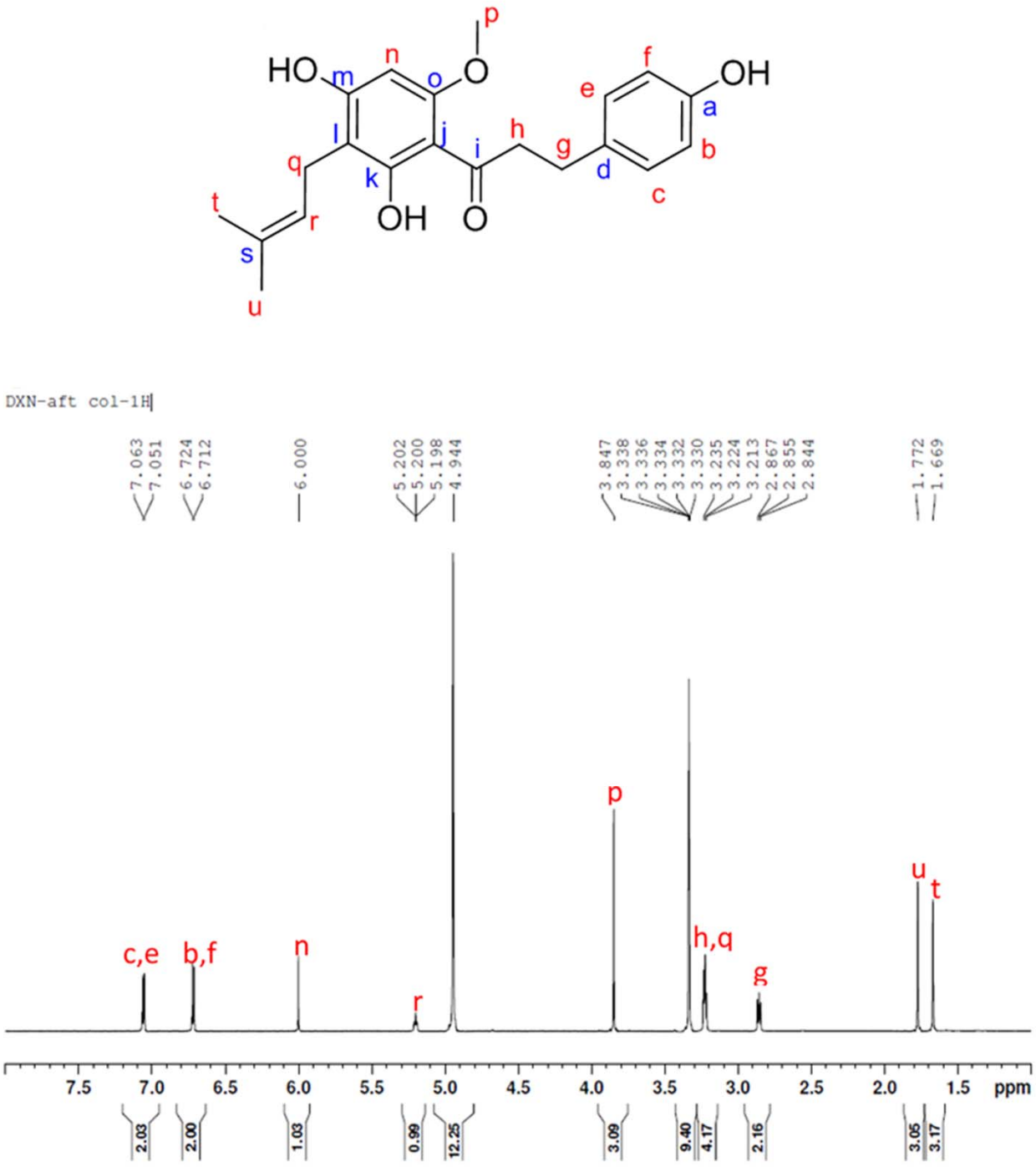

Figure S3. ^{13}C-NMR (175 MHz) spectrum of α,β-dihydroxanthohumol (DXN) recorded in methanol-*d*$_4$. For atom labels, see Figure S2. ^{13}C signals were assigned on the basis of HSQC and HMBC experiments.

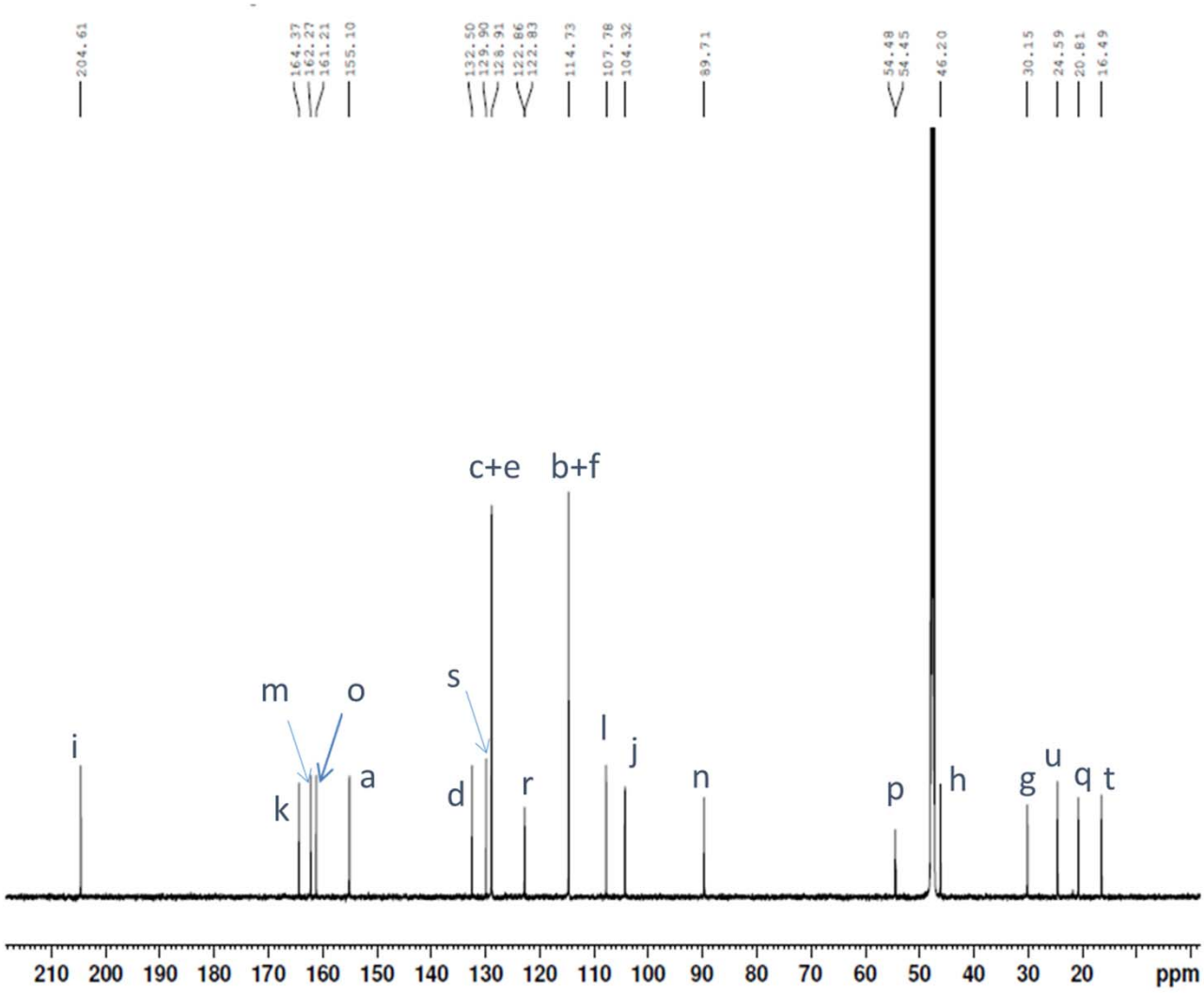

Figure S4. Electrospray MS/MS spectrum of α,β-dihydroxanthohumol (DXN) and its proposed fragmentation pathway.

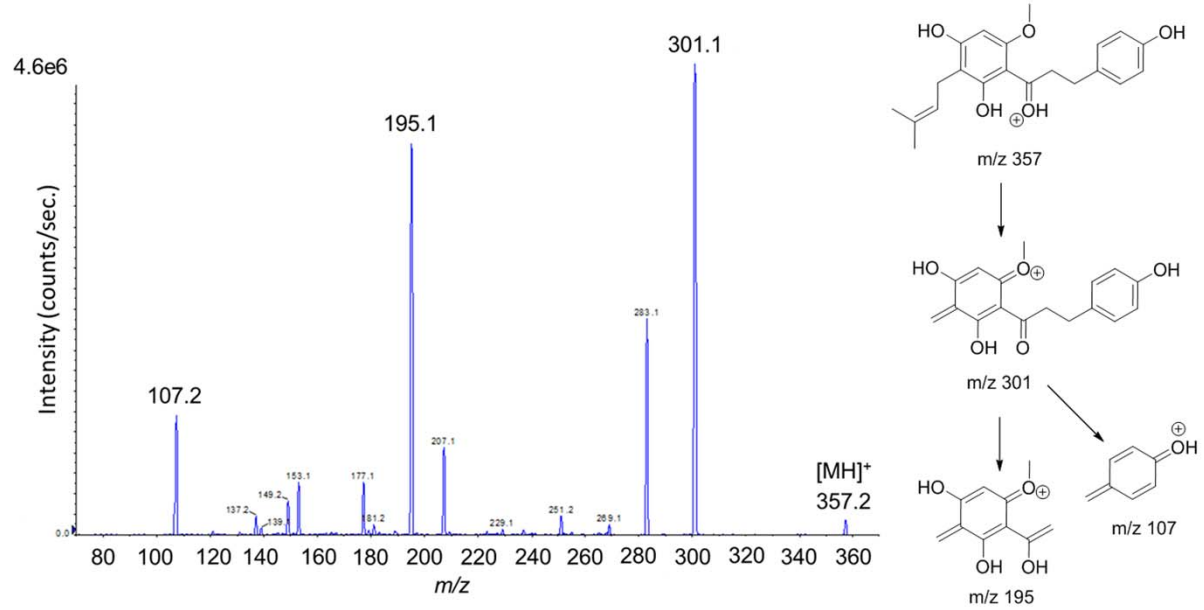

Figure S5. ^{1}H-NMR (700 MHz) spectrum of tetrahydroxanthohumol (TXN) recorded in methanol-d_4.

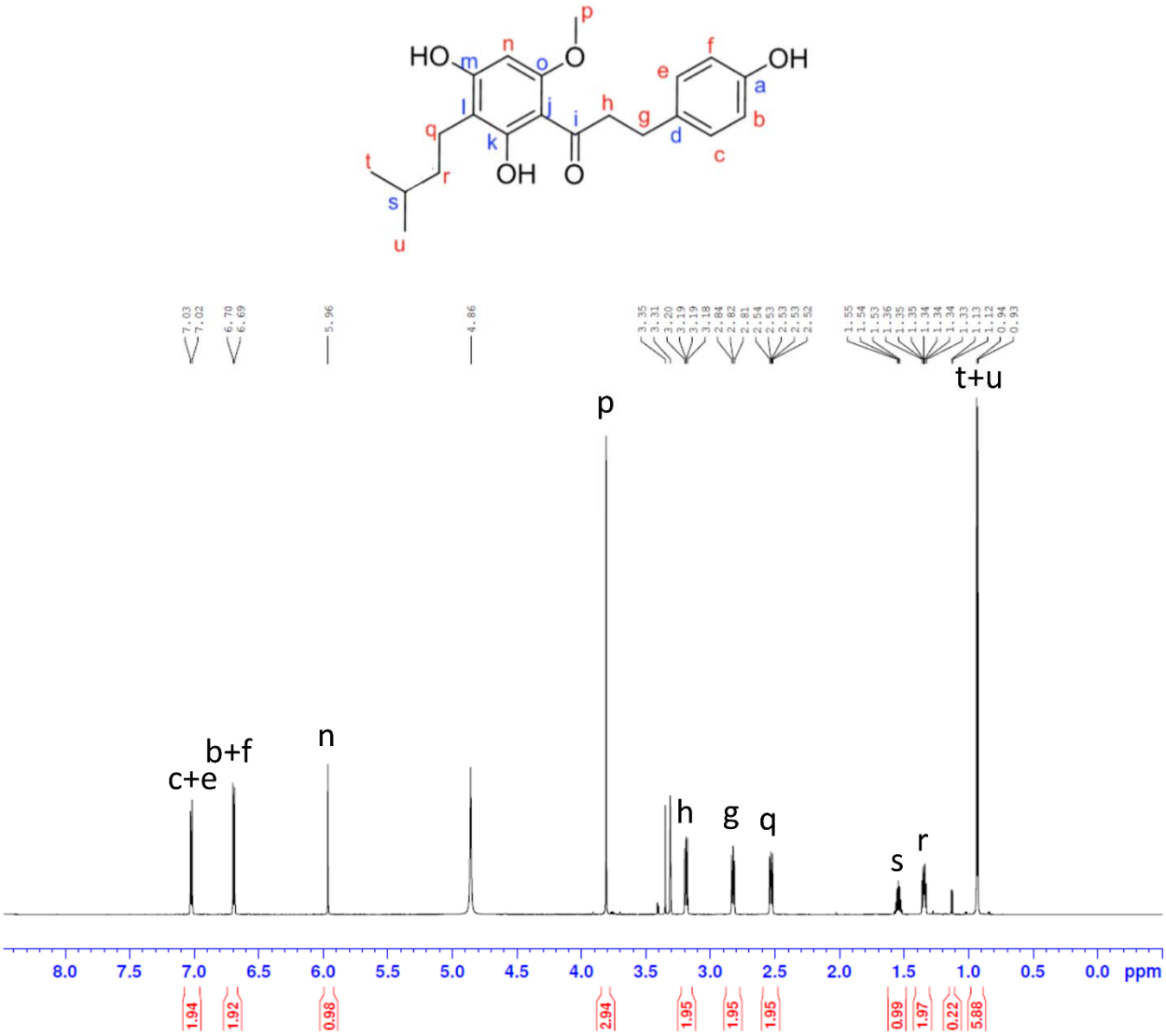

Figure S6. ^{13}C-NMR (175 MHz) spectrum of tetrahydroxanthohumol (TXN) recorded in methanol-d_4. For atom labels, see Figure S5. ^{13}C signals were assigned on the basis of HSQC and HMBC experiments.

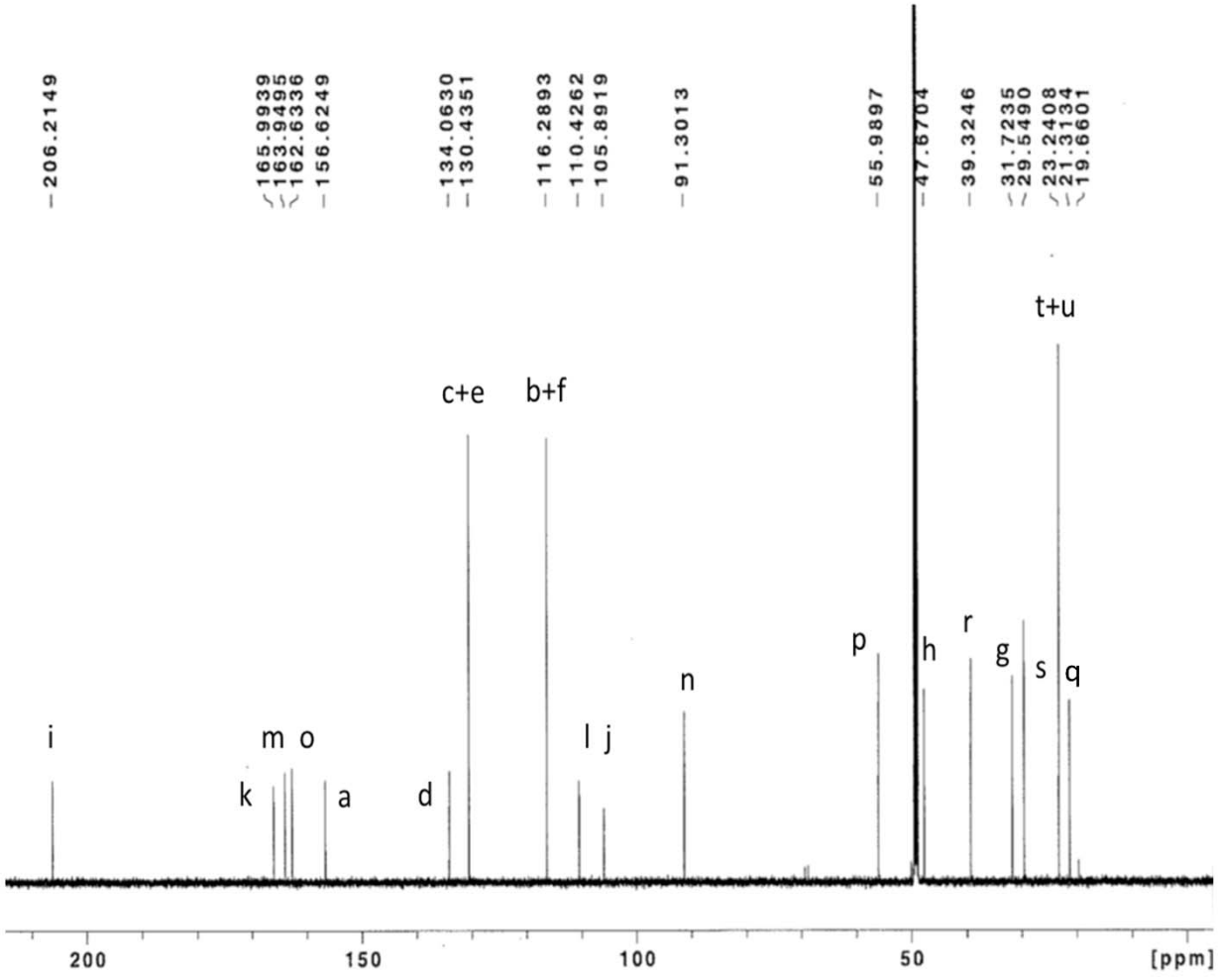

Figure S7. Electrospray MS/MS spectrum of tetrahydroxanthohumol (TXN) and its proposed fragmentation pathway.

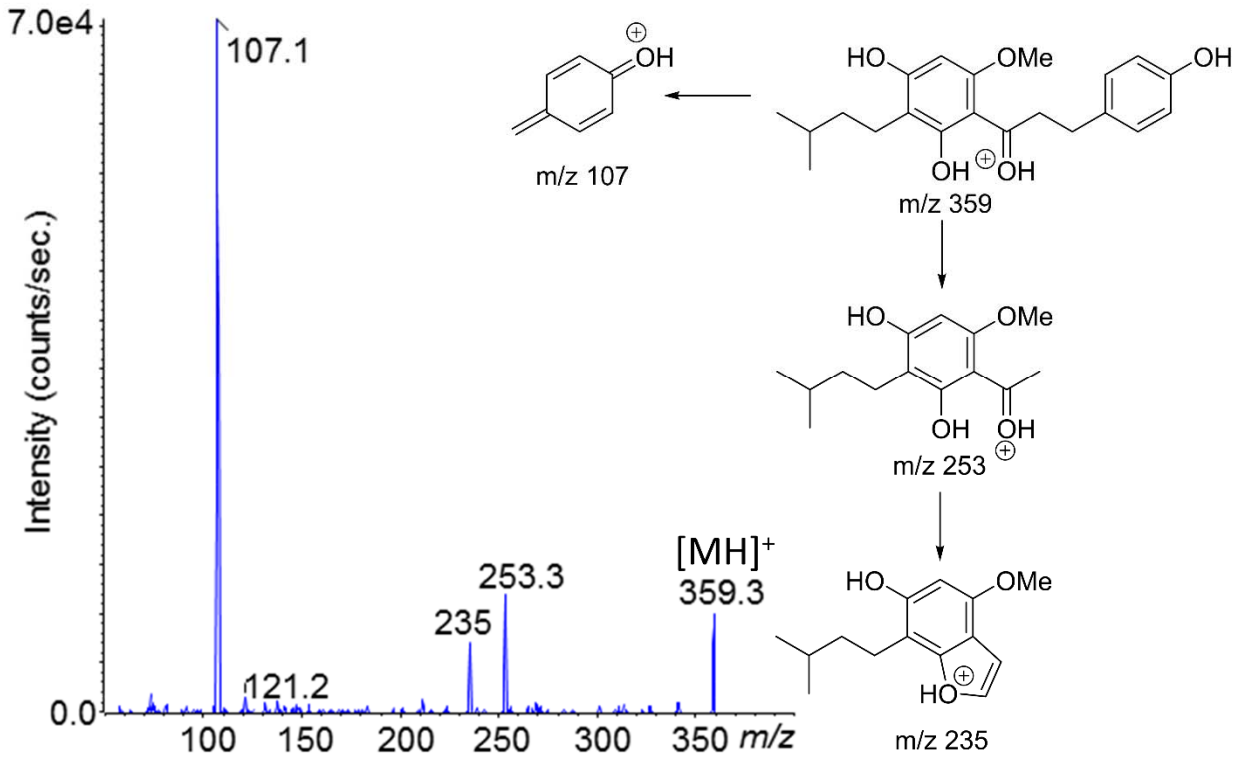

Figure S8. Full-length Western blots of liver and muscle biopsies from untreated mice (control, lanes 1-2) and treated with XN (lanes 3-4), DXN (lanes 5-6) and TXN (lanes 7-8). Tissue extracts were incubated with specific antibodies against pAMPK, AMPK and ß-actin. These original images were used to generate Figure 7. No exposure modifications were recorded.