Ulva lactuca Extracellular Vesicles Protect Against High-Fat Diet-Induced Intestinal Damage via Nrf2/Keap1/HO-1 Activation

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Antioxidant capacity analysis

DPPH assay: Various concentrations of UEVs samples (0, 50, 100, 200, 400, 600, 800, and 1000 μg protein/mL) were mixed with an equal volume of DPPH ethanol solution (0.2 mM ethanol solution, 200 μL) and incubated in the dark at room temperature for 40 min. The absorbance was spectrophotometrically monitored every 5 min in quartz cuvettes by UV-Vis spectrophotometer, and the percentage of DPPH scavenging activity was calculated.

ABTS assay: ABTS stock solution was generated by reacting 2.6 mM $K_2S_2O_8$ solution with 7.4 mM ABTS solution in equal volumes, which was then incubated in the dark for 12 h. Prior to analysis, the stock solution was diluted with 10 mM PBS (pH 7.4) to an absorbance of 0.7 ± 0.02 at 734 nm. Subsequently, various concentrations of UEVs samples were added to the diluted ABTS solution at a 1:3 (v/v) ratio, and the percentage of ABTS scavenging was calculated by measuring UV absorbance every 5 min over a 40 min period.

$•O_2^-$ scavenging activity: In this system, $•O_2^-$ was generated from xanthine under the catalytic action of XOD, forming a blue precipitate upon reaction with NBT. Briefly, 50 μL of UEVs samples were incubated with a superoxide anion-generating system consisting of 10 mM xanthine, 33 U/mL XOD, and 6 mM NBT (50 μL each). The reduction of NBT was spectrophotometrically monitored at 560 nm every 10 min over 40 min, during which the full UV-vis spectra were scanned from 400 to 800 nm.

Figure S1. (A-C) Visualize change in color and UV-vis absorbance spectra of (A) DPPH•, (B) ABTS+•, and (C) •O2-.

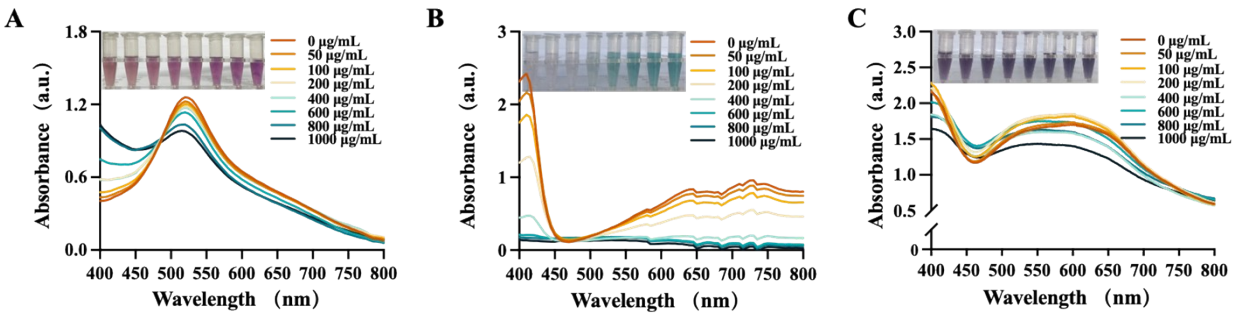

Figure S2. Western Blot detection of changes in apoptosis-related protein and expression of Bcl-2