Supporting Information

Identification of a novel oligopeptide from defatted walnut meal hydrolysate as a potential neuroprotective agent

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Method

Stability *in vitro*

Using HCL and NaOH, a buffer solution with pH = 2, 7, 9, and 12 was prepared. Then, 200 μL of a 15 mg·mL^{-1} AQ aqueous solution was added to 1.8 mL of the acid-base solution. The mixture was thoroughly mixed and incubated at room temperature for 1h. After adjusting the pH value to 7, the volume was adjusted to a fixed value of 4 mL using a neutral solution. After centrifugation at 13000 rpm, the acid-base stability of AQ was analyzed by HPLC.

The 1.5 mg·mL^{-1} AQ solution was configured and incubated in a water bath at 25℃, 37℃ and 60℃ for 1h, followed by centrifugation at 13000 rpm, and the temperature stability of the AQ was analyzed by HPLC.

Column temperature was set to 25℃. A volume of 10 μL of each sample was injected into the column and the pump flow-rate was 1.0 ml/min. Water containing 0.1 % (v/v) formic acid was mobile phase A and ACN was mobile phase B. The gradient elution method was performed as follows: 0 ~ 8 min: 4 % B; 8 ~ 15 min:4 - 30 % B; 15 ~ 25 min: 30 - 90% B; 25 ~ 30 min: 90 - 4 % B; 30 ~ 35 min: 4 % B. The elution was monitored at 220 nm.

Assessment of toxicity *in vivo*

The mice were adaptively housed for 3 days with unrestricted access to standard rodent chow and sterile water. The physiological condition of the mice was assessed following oral administration of 5g·kg^{-1} AQ. After 7 days of administration, the mice were anesthetized using isoflurane, blood samples and major organs (heart, liver, spleen, lungs, and kidneys) were collected. Serum biochemical parameters (AST, ALT, CREA, UREA) were measured and organs were stained with H&E.

Results

2D NMR of AQ

The 2D NMR spectrum of AQ is presented in Figure S1, while Table S1 displays the chemical shift analysis and assignment results for various amino acid fragments C and H.

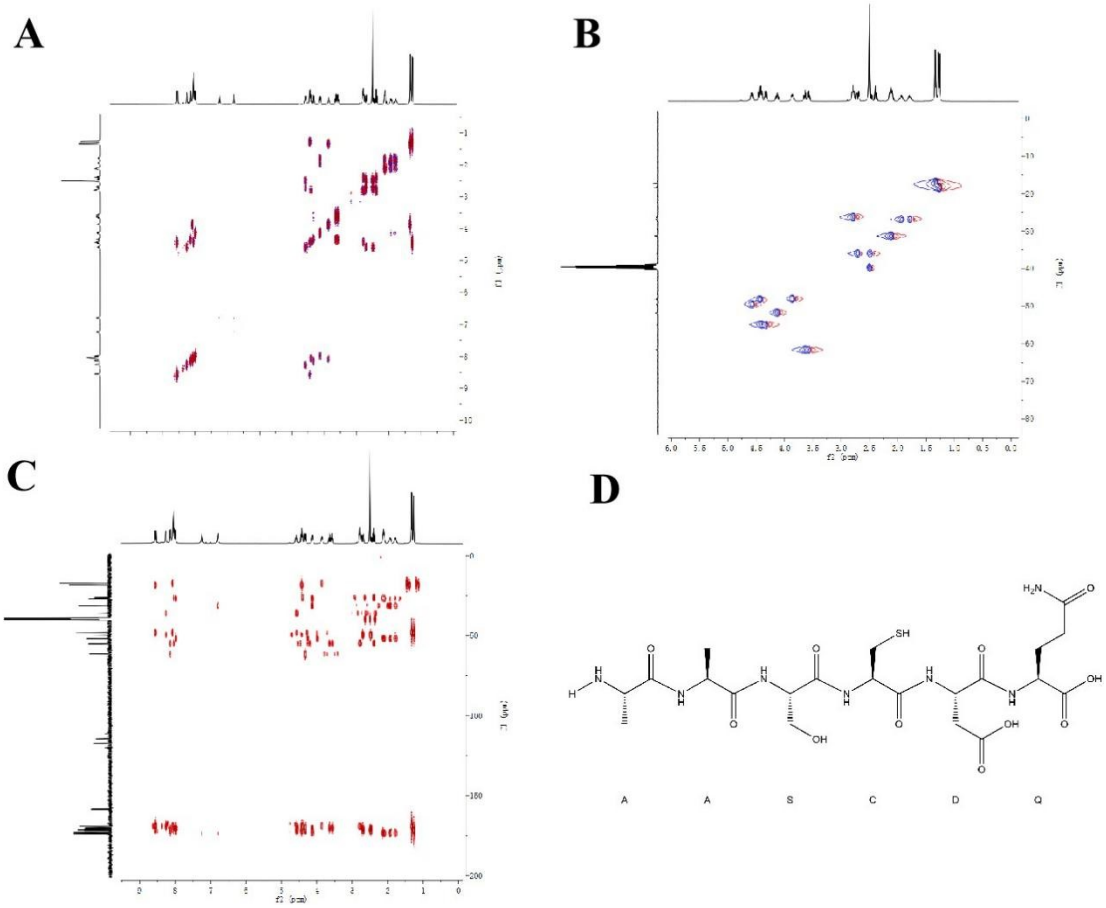


Figure S1. (A) ^{1}H-^{1}H COSY (B) HSQS (C) HMBC (D) Structure of AQ

Table S1 ^{13}C/^{1}H signal attribution results of NMR spectra

Amino acid fragments	^{13}C/^{1}H Chemical shift (δ_C/δ_H)

	C1	C2	C3	C4	C5
Ala-	17.2/1.33	48.2/3.85	169.1		
-Ala-	18.4/1.27	48.1/4.45	171.6		
-Ser-	61.6/3.6	55.0/4.33	170.5		
-Cys-	26.3/2.78	54.9/4.40	169.4		
-Asp-	171.9	36.0/2.70	49.6/4.57		
-Gln	173.6	31.3/2.12	26.9/1.80	51.8/4.13	170.2

Stability *in vitro*

The chromatographic peaks of AQ were integrated to determine the peak areas at different pH and temperature levels under identical liquid phase conditions. Analysis of the peak area indicated that AQ exhibited stability in acidic, alkaline, and neutral environments. Moreover, it remained stable at temperatures of 25°C, 37°C, and 60°C.

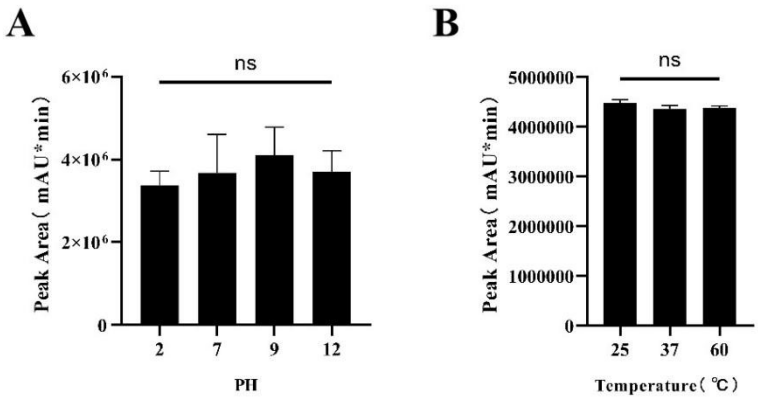


Figure S2. (A) The analysis of acid-base stability. (B) The analysis of temperature stability.

Assessment of toxicity *in vivo*

The treated group and the normal group exhibited no significant difference in AST, ALT, CREA and UREA levels. H&E staining analysis revealed that the cells in the organs displayed normal morphology without obvious pathological changes. The experimental results demonstrated that AQ exhibited negligible toxicity when administered at a high dose of $5g \cdot kg^{-1}$.

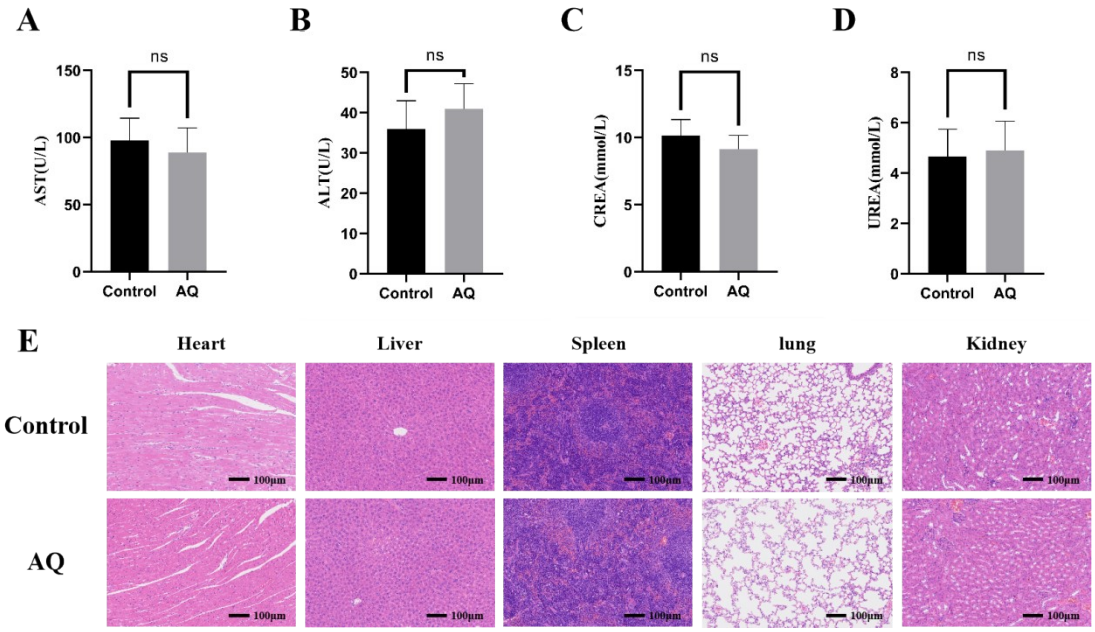
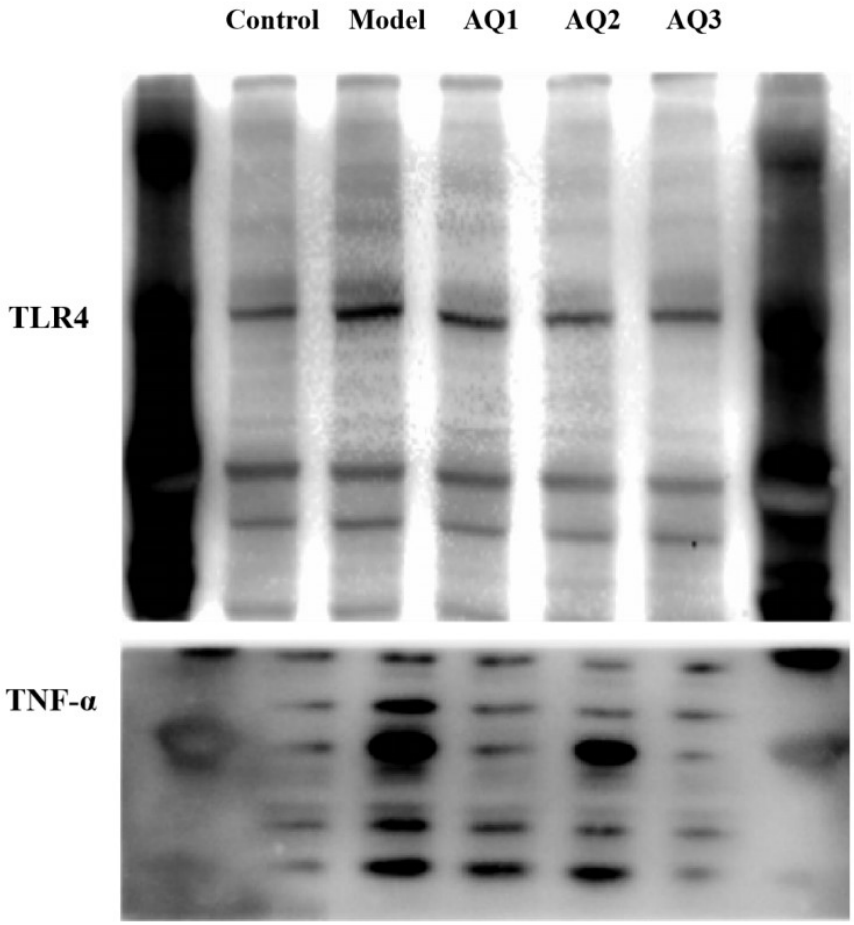
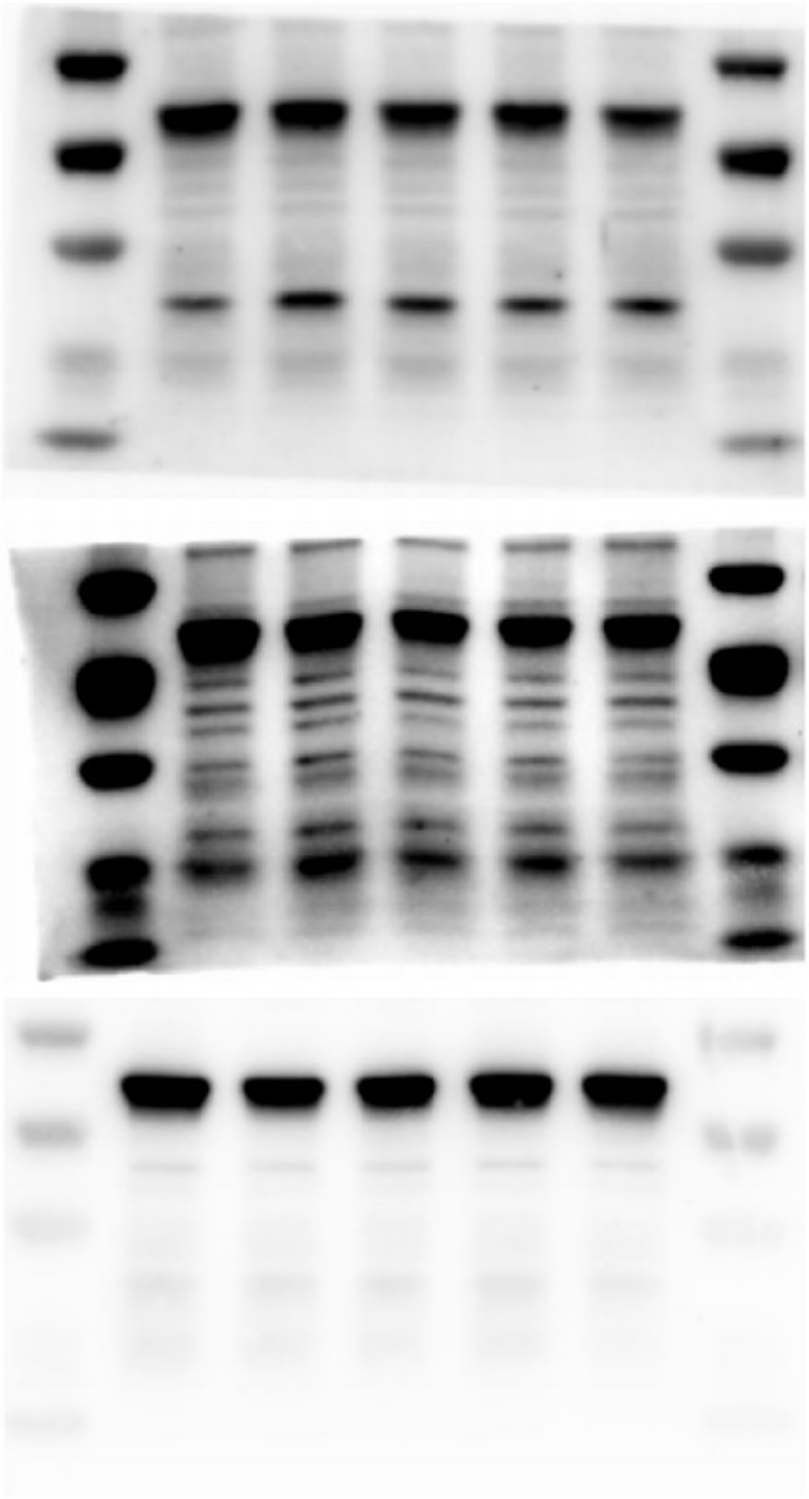


Figure S3. (A) Serum biochemical test results associated with toxicity. (B) Histological analysis of organ sections stained with H&E.


Control
Model
AQ1
AQ2
AQ3
TLR4
TNF-α


IL-6
IL-17A
GAPDH