

Two polysaccharides in *Zanthoxylum bungeanum* Maxim with protective effects against Parkinson's disease

Jiahui Huang

Dalian Medical University

Haochen Hui

Dalian Medical University

Meng Gao

Dalian Medical University

Xuerong Zhao

Dalian Medical University

Kunjie Li

Dalian Medical University

Ziyi Luo

Dalian Medical University

Bo Song

Weifang Medical University

Lian-hong Yin (✉ yinlianhong1015@163.com)

Dalian Medical University

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Abstract

Background

Zanthoxylum bungeanum Maxim can alleviate cognitive dysfunction, improve learning and memory impairment in mice. However, there are no reports on structure identification of polysaccharides from *Z. bungeanum*, and no data on the effects and possible mechanisms of polysaccharides from the plant against Parkinson's disease (PD).

Methods

In the present study, crude polysaccharides were obtained by hydroextraction and alcoholic precipitation, DEAE-52 and dextran G-75 chromatographic columns were used for the separation and purification. high-performance gel-permeation chromatography, ion chromatography, GC-MS and NMR were used for structure identification. Activity evaluation was performed by CCK-8, ROS, RT-PCR and Western Blotting experiments.

Results

Two degraded polysaccharides (WZBP-1-2 and WZBP-2) from *Z. bungeanum* were purified and identified, and their biological activities were evaluated. WZBP-1-2 and WZBP-2 with average molecular weight (M_w) of 10.89 and 18 kDa mainly contained galacturonic acid, and they were homogalacturonans with the main chain of $\rightarrow 4$)- α -D-GalAp-(1 $\rightarrow$. Biological investigation showed that WZBP-1-2 and WZBP-2 significantly increased the viabilities of MN9D cells induced by N-methyl-4-phenylpyridinium (MPP^+) and reduced ROS levels compared with model groups. Mechanism study showed that WZBP-1-2 and WZBP-2 markedly increased the expression levels of SIRT1 and Nrf2, and inhibited the protein or mRNA levels of NF- κ B, TNF- α and IL-6.

Conclusion

WZBP-1-2 and WZBP-2 from *Z. bungeanum* may exhibit protective effects against MPP^+ -induced neuroinflammation and oxidative stress through activation of SIRT1 signaling and should be considered as candidates for the treatment of PD.

Background

Parkinson's disease (PD), a chronic degenerative disease of the central nervous system, is characterized by selective loss of dopaminergic neurons in the substantia nigra pars compacta (SNPC) [1]. The loss of dopaminergic neurons can lead to dopamine (DA) deficiency and cause various of dyskinesia. Due to

aging population, natural environmental pollution and some bad living habits, the numbers of PD patients are keeping increasing [2–4]. Numerous studies have confirmed that oxidative stress and neuroinflammation are common pathological mechanisms in neurodegenerative diseases [5]. Damaged neurons can activate glial cells that release a large number of proinflammatory substances, and produce amounts of reactive oxygen species (ROS). Excessive ROS can lead to imbalance of antioxidant system in the body, then promote neurons to generate oxidative stress responses, and cause neuronal damage and other pathological changes [6, 7]. Drugs are still the main treatment for PD.

Plant extracts have attracted much attention for their natural properties, and many of them have favorable preventive and therapeutic effects on central nervous system diseases [8]. Plant polysaccharides have various pharmacological activities including anti-oxidant, anti-inflammatory, anti-tumor and immunoregulating effects [9, 10]. For example, the polysaccharides from *Agaricus bisporus* can reduce homocysteine and neurotransmitter content and enhance body's antioxidant capacity to improve brain function [11]. *Cordyceps cicada* polysaccharides can protect PC12 cells from glutamate excitatory toxicity [12]. *Ganoderma lucidum* polysaccharides have neuroprotective effect on experimental spinal cord injury [13]. The polysaccharides from *Lycium barbarum*, *Gynostemma pentaphyllum*, *Polygonatum sibiricum* and *Hericium hercyrhiza* have neuroprotective effects [14–17]. Moreover, some studies have confirmed that structural modification such as phosphorylation or selenide modification on polysaccharides can improve self-biological activities [18–20].

Zanthoxylum bungeanum Maxim, a medicinal and edible plant, belongs to *Rutaceae* family with long history of cultivation. Its medicinal and economic value in China can be traced back to more than 2000 years ago [21]. *Z. bungeanum* with analgesic, anti-diarrheal, anti-pruritic effects has been used to treat abdominal pain, vomiting and other diseases caused by cold and humidity [22]. In daily life, due to its unique fragrance, *Z. bungeanum* is often used for food flavor. Data have shown that *Z. bungeanum* is rich in flavonoids, unsaturated fatty acids, volatile oil and other components, and then has good therapeutic effects on many diseases [21]. In order to promote the development and application of *Z. bungeanum*, expand the application fields, this study conducted further research on the ingredients and functions of *Z. bungeanum*.

Some chemical extracts from *Z. bungeanum* can alleviate cognitive dysfunction in mice with scopolamine-induced dementia [23], decrease the accumulation of A β oligomers in cerebral cortex, inhibit A β -induced neuronal apoptosis [24], and improve D-galactose-induced learning and memory impairment in mice via activating PI3K/Akt signaling pathway [25]. However, there are no reports on structure identification of polysaccharides from *Z. bungeanum*, and no data on the effects and possible mechanisms of polysaccharides from the plant against PD.

Therefore, in our work, water soluble polysaccharides from *Z. bungeanum* were extracted, purified and identified. Furthermore, the protective effects and the possible mechanisms of the isolated polysaccharides against PD on cell model were carried out.

Materials and methods

Materials

Dry *Z. bungeanum* was purchased from Xufang Traditional Chinese Medicine Co., Ltd. (Hebei, China), which was identified by Dr. Zhao (College of Pharmacy, Dalian Medical University). DEAE-52 cellulose and Sephadex G-75 were purchased from Beijing Solarbio Technology (Beijing, China). MN9D cells were purchased from the Institute of Biochemistry and Cell Biology (Shanghai, China). N-methyl-4-phenylpyridinium (MPP⁺) was supplied from MedChemExpress (New Jersey, USA). Dextran Standard 1.152 kDa was obtained from Yuanye Biotechnology Inc. (Shanghai, China). Dextran standards 5, 11.6, 23.8, 48.6, 273, 409.8 and 667.8 kDa were purchased from Sigma-Aldrich (St. Louis, MO, USA). Standard monosaccharides and methylation kit were obtained from Bo Rui Saccharide Biotech Co., Ltd (Jiangsu, China). All antibodies were obtained from ZEN-BIOSCIENCE Co., Ltd (Chengdu, China). ROS detection kit was supplied by Beyotime (Shanghai, China).

Acquisition of the crude polysaccharides

Crude polysaccharides were extracted according to the conventional method [26]. Total of 250 g crude *Z. bungeanum* powders (20–40 mesh) were weighed, and 2 L solvent (95% ethanol–petroleum ether = 1: 1, v/v) was added for extraction under reflux for 4 h at 70°C. The extract solution was filtered and the residues were collected and dried at 45°C in oven. Then, the herbs were processed with 2500 mL boiling water for 2.5 h. The extracts were concentrated to 250 mL under reduced pressure, and the supernatant was collected and added with 95% ethanol until the final concentration of ethanol reached to 80%, and the solutions were left to alcoholic sedimentation at 4°C for 24 h. The precipitate was obtained by vacuum filtration and washed with 95% ethanol, and dried in a vacuum drying oven. Then, the crude polysaccharide from *Z. bungeanum* (WZBP) was obtained.

In the study, the crude polysaccharides from *Z. bungeanum* were primary purified by AB-8 macroporous resin column chromatography. The crude polysaccharide solution (5 mg/mL) was prepared and loaded into the column, which was eluted with ultrapure water. The eluent was collected and concentrated.

Polysaccharide purification

The crude polysaccharide was further purified by DEAE-52 cellulose column chromatography. The crude polysaccharide aqueous solution (10 mg/mL) at 65°C was added to the DEAE-52 cellulose column (φ 45 mm × 30 cm) and equilibrated for 10 min. Then, the column was eluted with distilled water, 0.1 M NaCl solutions, 0.2 M NaCl solutions and 0.3 M NaCl solutions at the flow rate of 1 mL/min, successively. Each of the elution fraction were dialyzed (3500 Da) and freeze-dried. The content of polysaccharides were determined by phenol-sulfuric acid method [27]. The fractions eluted by 0.2 and 0.3 M NaCl solutions named WZBP-1 and WZBP-2 were obtained.

WZBP-1 and WZBP-2 were prepared into 10 mg/mL solutions, and loaded into Sephadex G-75 column (φ 20 mm × 50 cm) which were eluted with ultrapure water at the flow rate of 0.8 mL/min. In each tube, 10

mL of eluent was collected, and the absorbance values (490 nm) of the eluent were measured. Further, the elution fractions were dialyzed (3500 Da) and freeze-dried. The polysaccharide samples were obtained by weighing 1.0 g of sample precisely, adding it to 1 M TFA solution (100 mL), shaking vigorously at 25°C for 8 h, and freeze-drying after dialysis.

2.4. Homogeneity and molecular weight of WZBP-1-2 and WZBP-2

The homogeneity and molecular weights of WZBP-1-2 and WZBP-2 were analyzed by high-performance gel-permeation chromatography (HPGPC) [28]. High performance liquid chromatography (HPLC) equipped with BRT105-104-102 gel column (8 × 300 mm) and RI-10A refractive index detector were used. A series of standard substances and the polysaccharide samples were accurately weighed to prepare 5 mg/mL solution. 0.05 M NaCl solution was used to the mobile phase with the flow rate of 0.6 mL/min. The injection volume was 20 mL, the column temperature was 40°C. The solution of standard substance was injected into for the analysis, and the molecular weights of WZBP-1-2 and WZBP-2 were determined.

Monosaccharide composition

The monosaccharide composition were determined by Ion chromatography (ThermoFisher ICS5000, USA) with a DionexCarbopacTMPA20 column and an electrochemical detector [29, 30]. Polysaccharide (10 mg) was dissolved in 10 mL 3 M trifluoroacetic acid (TFA), and hydrolyzed at 120°C for 3 h. The acid hydrolysis solution was blow-dried with nitrogen to remove TFA. Then, 10 mL water was added, mixed, and then take 0.1 mL of the solution, diluted 10 times and centrifuged (12000 rpm, 5 min). The supernatant was taken for the analysis.

Methylation analysis

Methylated sugar alcohol acetate was evaluated by gas chromatography -mass spectrometry (GC-MS) [31, 32]. Reduction and complete acid hydrolysis of the glyoxylate were performed prior to methylation, and the compositions of the monosaccharide were determined using ion chromatography to confirm that all of the glyoxylate was reduced.

Methylation reaction was carried out according to the kit. The samples were determined by gas chromatography-mass spectrometry (Shimadzu, Japan). GC-MS analytical conditions were as follows: RXI-5-SIL-MS column (30 m × 0.25 mm × 0.25 µm), programmed temperature (starting temperature was 120°C, heating to 250°C/min at a rate of 3°C/min, maintain 5 min), injector temperature and detector temperature were 250°C, flow rate was 1 mL/min.

Nuclear magnetic resonance (NMR)

The polysaccharide sample (30 mg) was dissolved in 0.5 mL D₂O and freeze-dried. The 1D NMR (¹H, ¹C and Dept135) and 2D NMR (HSQC, COSY, HMBC and NOESY) were measured using 600 MHz Bruker NMR spectrometer at 25°C (Bruker, Canada). MestReNova v12.0.3 software was used to process the data.

Cytotoxicity assay

MN9D cells were cultured in DMEM medium supplemented with 1% penicillin-streptomycin and 10% fetal bovine serum (FBS) at 37°C in 5%CO₂ in a humidified incubator. The cells (1×10^5 cells/mL, 200 μ L) were inoculated into 96-well plates and then treated with the polysaccharide solutions (0, 5, 10, 20, 40, 80, 160 μ g/mL) in DMEM medium for 3, 6, 9 h, respectively. Total of 10 μ L CCK-8 solution was added, and cell viability was detected by microplate reader at 450 nm (TECAN, Switzerland).

Cell viability assessment

MN9D cells were inoculated into 96-well plates (1×10^5 cells/mL, 200 μ L). After growth for 18 h, polysaccharide solutions (10, 20, 40 μ g/mL) were added into the cells for 6 h. Next, MPP⁺ (1200 μ M) was added for 24 h, and CCK-8 method was used to measure the cell viability.

ROS assay

MN9D cells were inoculated into 24-well plates for 18 h (1×10^5 cells/mL, 500 μ L). Then, the cells were processed through the same way mentioned above. In the end, 300 μ L of DCFH-DA solution (10 μ M) was added to each well for 30 min, and the sample was observed by inverted fluorescence microscopy and photographed (ZEISS, Germany).

Real-time PCR assay

MN9D cells were inoculated into 6-well plates (1×10^5 cells/mL, 2mL). Then, the cells were treated. TransZol UP kit was used to extract the total RNA, which was operated to produce cDNA according to the TransScript® All-in-One First-Strand cDNA Synthesis SuperMix for qPCR kit instruction. Finally, the mRNA levels were measured according to the instruction of TransStart® Top Green qPCR SuperMix kit, and the results were calculated by the $2^{-\Delta\Delta C_t}$ method. All primers were designed by Real-time PCR primer design principles.

Western blot assay

MN9D cells were inoculated into 6-well plates (1×10^5 cells/mL, 2mL). Then, the cells were treated, and cellular protein was extracted. After determination of protein concentration by BCA protein quantitation kit (Seven-biotech, China), the samples were mixed with the solution (5 $\times$ loading buffer), boiled in water for 5 min and store at -20°C. The protein samples were transferred to a polyvinylidene fluoride membrane (Millipore, USA) by SDS-PAGE. After 5% skim milk was used to block at 25°C for 2 h, primary antibody was added to membranes overnight at 4°C. Then, the membranes were incubated with secondary antibody for at 25°C 2 h. Protein levels were visualized by enhanced chemiluminescence reagents (ECL) and imaged using Bio-Spectrum Gel Imaging System (UVP, USA). The bands were normalized and GAPDH was used as internal control.

Statistical analysis

The data of all experiments recorded as means $\pm$ standard deviations (SD) were analyzed with GraphPad Prism 7.0 analysis software. SPSS 20.0 software was used to analyze anti-inflammatory activity data by

one-way ANOVA. Differences at $p < 0.05$ or $p < 0.01$ were generally considered as significant.

Results

Isolation and purification of WZBP-1-2 and WZBP-2

In AB-8 macroporous resin column chromatography step, the retention rates, decolorization rates and protein removal rates of the crude polysaccharides from *Z. bungeanum* were 73%, 91% and 81%. In DEAE-52 cellulose column chromatography step, the main fractions of 0.2 M and 0.3 M NaCl elution fractions were named WZBP-1 and WZBP-2, respectively, and the elution curve is shown in Fig. 1A. WZBP-1 was further separated into two fractions on Sephadex G-75 column process, as shown in Fig. 1B, the polysaccharide fraction (WZBP-1-2) was collected for subsequent experiment. And elution curve of WZBP-2 was a single peak (Fig. 1C), it also was collected for the subsequent experiment.

Molecular weight and monosaccharide composition

Both of WZBP-1-2 and WZBP-2 showed a single peak on HPGPC assay (Fig. 1D–E), indicating good homogeneities of the isolated polysaccharides. According to the $\lg M_w$ -RT correction curve: $y = -0.1889x + 12.007$ ($R^2 = 0.99430$), the average molecular weights of WZBP-1-2 and WZBP-2 were 10.89 kDa and 18 kDa. The results of monosaccharide composition are presented in Table S1. WZBP-1-2 (Fig. 2A) mainly contained galacturonic acid (89%) and a small amount of rhamnose (2.16%), arabinose (5.55%), galactose (6.7%), glucose (1.96%) and xylose (1.09%). Nine types of monosaccharides were detected in WZBP-2 (Fig. 2B), mainly including arabinose (18%), galactose (18%), galacturonic acid (52%). The monosaccharide composition results indicated that WZBP-1-2 and WZBP-2 are high galacturonic acid polysaccharide.

Methylation analysis

The types and contents of glycosidic bonds are shown in Table S2. Reduced WZBP-1-2 (Fig. 2C) was composed of 12 different kinds of glycosidic bonds, among which Galp-1-4 (72.62%) was the main linkage. Based on monosaccharide composition and the reduction reaction before methylation analysis, most of the Gal in the reduction product almost came from GalA. It could be inferred that GlcA in WZBP-1-2 mainly existed in the form of Galp-1-4 linkage. Reduced WZBP-2 was consisted of 10 different types of glycoside bonds as shown in Fig. 2D, and the main linkage was 1, 4-Galp (79.06%). While the results of monosaccharide composition showed that WZBP-2 contained galactose (18%), galacturonic acid (52%), resulting in the inability to accurately attribute the Galp connection method.

NMR results of WZBP-1-2 and WABP-2

By analyzing the NMR spectra, the sugar residues configuration and connection sites in polysaccharides were further determined [33]. The proton signal of WZBP-1-2 was mainly concentrated between $\delta 3.0$ – 5.5 ppm and the carbon signal was mainly concentrated between $\delta 60$ – 120 ppm, which were typical proton

and carbon chemical shifts for polysaccharide. ^1H spectrum (Fig. 3A) exhibited five anomeric carbon signals at δ 4.97, 5.70, 4.85, 5.21 and 4.49 ppm. ^{13}C NMR spectrum (Fig. 3B) of WZBP-1-2 showed five anomeric carbon signals at δ 100.38, 108.18, 101.34, 93.62 and 97.48 ppm, labeled as A, B, C, D, and E. The Dept135 (Fig. 3C) showed no inverted peaks, indicating that alduronic acid was formed for C6. The two high-frequency signals that appear at δ 172.70 and 176.2 ppm can be attributed to the carboxyl C-6 resonance in GalpA [34]. According to HSQC, ^1H -NMR and ^{13}C -NMR data, the corresponding anomeric hydrogen and carbon chemical shifts of WZBP-1-2 were δ 4.97/100.38 (A), δ 5.70/108.18 (B), δ 4.85/101.34 (C), δ 5.21/93.62 (D) and δ 4.49/97.48 (E) ppm.

Residue A had an anomeric signal at δ 4.97/100.38 ppm. The chemical shift of H2 was δ 3.67 ppm which was corresponding to ^1H in COSY spectra (Fig. 3D). By searching HSQC spectra (Fig. 3E), the chemical shift of C2 (δ 69.40 ppm) associated with H2. δ 3.93/70.05 ppm was attributed to H-3/C-3 by COSY and HSQC spectra. H-4/C-4 at δ 4.36/79.15 ppm and H-5/C-5 at δ 4.68/72.65 ppm were also observed in HSQC and COSY spectra. Combined with methylation analysis, these chemical shifts were attributed to $\rightarrow$ 4)- α -D-GalAp-(1 $\rightarrow$ [35, 36].

For anomeric signals at residues B, C, D and E, H-2/C-2, H-3/C-3, H-4/C-4 and H-5/C-5 could be attributed by COSY and HSQC spectra as residue A. Finally, five main types of sugar residues in WZBP-1-2 were obtained: $\rightarrow$ 4)- α -D-GalAp-(1 $\rightarrow$ (A), α -D-GalAp-(1 $\rightarrow$ (B), $\rightarrow$ 3,4)- α -D-GalAp-(1 $\rightarrow$ (C), $\rightarrow$ 4)- α -D-GalAp (D), $\rightarrow$ 4)- β -D-GalAp (E). The signals of these residues are shown in Table S3. Carbon signaling at δ 54.13 ppm in ^{13}C spectrum and proton signal at δ 3.71 ppm in ^1H spectrum indicated the presence of methoxy esterification in WZBP-1-2, while δ 171.77 ppm signal in HMBC spectrum (Fig. 3F) further demonstrated the presence of methoxy ester groups [35, 37]. In NOESY spectrum (Fig. 3G), the anomeric hydrogen of $\rightarrow$ 4)- α -D-GalAp-(1 $\rightarrow$ had associated signal peaks with its own H4, indicating a glycosidic linkage of $\rightarrow$ 4)- α -D-GalAp-(1 $\rightarrow$ 4)- α -D-GalAp-(1 $\rightarrow$. The anomeric hydrogen of $\rightarrow$ 4)- α -D-GalAp-(1 $\rightarrow$ was associated with glycoside bond $\rightarrow$ 3,4)- α -D-GalAp-(1 $\rightarrow$, indicating the presence of glycoside bonds $\rightarrow$ 4)- α -D-GalAp-(1 $\rightarrow$ 3,4)- α -D-GalAp-(1 $\rightarrow$.

In summary, the possible structure of WZBP-1-2 was inferred that the main chain was make up of $\rightarrow$ 4)- α -D-GalAp-(1 $\rightarrow$. The terminal group α -D-GalAp-(1 $\rightarrow$ was attached to the backbone by the O-3 bond of $\rightarrow$ 3,4)- α -D-GalAp-(1 $\rightarrow$. And $\rightarrow$ 4)- α -D-GalAp-(1 $\rightarrow$ or $\rightarrow$ 3,4)- α -D-GalAp-(1 $\rightarrow$ residue with methylated substitution, there was a small amount of O-2 or O-3 acetylation substitution. The specific structure is shown in Fig. 3H.

For WZBP-2 NMR spectrum (Fig. 4A–G), in which ^1H spectrum exhibited five anomeric carbon signals at δ 4.97, 5.08, 4.83, 5.22 and 4.51 ppm. ^{13}C NMR spectrum also showed six anomeric carbon signals at δ 100.38, 100.148, 172.36, 172.16 and 174.70 ppm. The two high-frequency signals at δ 172.36, 172.16 and 174.70 ppm could be attributed to the carboxyl C-6 resonance in GalpA. Just like the data on WZBP-1-2, the carbon and proton chemical displacement attribution are shown in Table S4. We found the inverted peaks at δ 60.8 ppm in Dept135, indicating that C6 did not form alduronic acid. Five residues of $\rightarrow$ 4)- α -D-GalAp-(1 $\rightarrow$ (A), α -D-GalAp-(1 $\rightarrow$ (B), $\rightarrow$ 3,4)- α -D-GalAp-(1 $\rightarrow$ (C), $\rightarrow$ 4)- α -D-GalAp (D) and $\rightarrow$ 4)- β -D-

GalAp (E) were identified, which were similar to WZBP-1-2 (Fig. 4H). Combined with the results of structural characterization, it was speculated that the bone chain compositions of the WZBP-1-2 and WZBP-2 were homogalacturonans.

WZBP-1-2 and WZBP-2 prevented MPP⁺-induced MN9D cell damage

The cytotoxicity of WZBP-1-2 and WZBP-2 on MN9D cells was investigated, and the data were shown in Fig. 5A. WZBP-1-2 and WZBP-2 at the concentrations in the range of 5–40 µg/mL had no significant cytotoxic effects on the cells under 9 h treatment. Then, the protective effects of WZBP-1-2 and WZBP-2 on cell model induced by MPP⁺ were investigated. As shown in Fig. 5B, the cell viabilities in model groups were significantly decreased compared with control groups, WZBP-1-2 and WZBP-2 effectively improved cell viabilities. The results suggested that WZBP-1-2 and WZBP-2 prevented MN9D cell damage induced by MPP⁺ in vitro.

WZBP-1-2 and WZBP-2 inhibited MPP⁺-induced oxidative stress and inflammation

The results of ROS levels are shown in Fig. 5C. Compared with control groups, the ROS levels in model groups were significantly increased, which were significantly reduced after WZBP-1-2 and WZBP-2 treatment. Meanwhile, the mRNA expression levels of IL-6 and TNF-α in model groups were significantly increased compared with control groups. However, their expression in WZBP-1-2 and WZBP-2 pretreatment groups were obviously decreased compared with MPP⁺ model groups (Fig. 5D). Those data confirmed that WZBP-1-2 and WZBP-2 reduced MPP⁺-induced oxidative stress and inflammation.

WZBP-1-2 and WZBP-2 regulated SIRT1-mediated Nrf2/NF-κB pathway

As shown in Fig. 6A–B, compared with MPP⁺ model groups, the protein levels of SIRT1 were up-regulated by WZBP-1-2 and WZBP-2. As expected, the levels of Nrf2 and SOD2 were also slightly up-regulated by WZBP-1-2 and WZBP-2. Consistently, NF-κB protein levels were highly decreased in WZBP-1-2 and WZBP-2 pretreatment groups. Thus, WZBP-1-2 and WZBP-2 activated SIRT1-mediated Nrf2/NF-κB signal pathway to perform anti-oxidant and inflammation activity.

Discussion

Polysaccharides with complex structures and diverse biological activities are widely present in nature. *Z. bungeanum* is a medicinal and edible plant, and its dried and ripe peel is called peppercorn, which can not only be used in medicine, but also used as a condiment in daily life [22]. In this study, WZBP-1-2 and WZBP-2 were isolated and purified from *Z. bungeanum* for the first time, which were typical

homogalacturonans. WZBP-1-2 and WZBP-2, with average molecular weight of 10.89 and 18 kDa, mainly contained galacturonic acid. The main chains of WZBP-1-2 and WZBP-2 were made up of $\rightarrow 4$)- α -D-GalAp-(1 $\rightarrow$. The terminal group α -D-GalAp-(1 $\rightarrow$ was attached to the backbone by the O-3 bond of $\rightarrow 3,4$)- α -D-GalAp-(1 $\rightarrow$.

Increasing evidence supports the potential application of polysaccharides in prevention and treatment on PD. Oxidative stress has been shown to be strongly associated with the development of PD [38]. Excessive generation of ROS (endogenous and exogenous) can cause a state of redox imbalance leading to cellular and tissue damage. Furthermore, the effects of Nrf2 and NF- κ B signals on neurodegenerative and neuroinflammatory diseases have been confirmed [39]. We investigated the protective effects of WZBP-1-2 and WZBP-2 on cellular neurotoxicity by constructing a cellular PD model induced by MPP⁺ [40]. Silent information regulator 1 (SIRT1), a histone deacetylase that relies on nicotinamide adenine dinucleotides (NAD), is involved in regulating multiple physiological functions [41]. It had been shown that SIRT1 has regulatory effects on neurodegenerative diseases to inhibit oxidative stress damage, nerve cell apoptosis, and neuroinflammation [42]. The results showed that WZBP-1-2 and WZBP-2 significantly inhibited the mRNA levels of TNF- α and IL-6, and down-regulated intracellular ROS levels. Our data confirmed that WZBP-1-2 and WZBP-2 isolated from *Z. bungeanum* had inhibitory effects on MPP⁺-induced oxidative stress injury and neuroinflammation through regulating SIRT1-mediated Nrf2/NF- κ B pathway, which was worthy of in-depth research and development. Nowadays, many natural products have been used to treat various central nervous system dysfunctions [43, 44]. With the increasing incidences of PD, prevention and treatment of the disease is particularly important.

Two water-soluble polysaccharides from *Zanthoxylum bungeanum* Maxim were isolated and identified, WZBP-1-2 and WZBP-2 are mainly composed of galacturonic acid. It was confirmed that the two polysaccharides had significant protective effects on MPP⁺-induced oxidative stress injury and neuroinflammation through regulating the SIRT1-mediated Nrf2/NF- κ B pathway. The deeply mechanisms, drug targets and clinical applications of WZBP-1-2 and WZBP-2 from *Z. bungeanum* are needed further investigation, and the use of polysaccharides from *Z. bungeanum* are highly promising in terms of long-term drug safety.

Abbreviations

Parkinson's disease, PD; substantia nigra pars compacta, SNPC; dopamine, DA; N-methyl-4-phenylpyridinium, MPP⁺; reactive oxygen species, ROS; crude polysaccharide from *Zanthoxylum bungeanum* Maxim, WZBP; high-performance gel-permeation chromatography, HPGPC; high performance liquid chromatography, HPLC; nuclear magnetic resonance, NMR; gas chromatography-mass spectrometry, GC-MS; silent information regulator 1, SIRT1; nuclear erythroid factor 2-related factor 2, Nrf2; superoxide dismutase 2, SOD2; tumor necrosis factor- α , TNF- α ; interleukin-6; IL-6.

Declarations

Ethical Approval

Not applicable.

Competing interests

The authors declare no competing interests.

Author contributions

JH: Methodology; investigation; writing-original draft; visualization. HH: Investigation; writing-original draft; visualization. MG: Investigation; resources; supervision; writing-review & editing. XZ: Formal analysis; resources. KL: Supervision; writing-review & editing. ZL: Writing-original draft; resources. BS: Conceptualization; supervision; writing-review and editing. LY: Supervision; project administration; writing-review and editing. All authors read and approved the final manuscript.

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Availability of data and materials

All data to support the findings of this manuscript are presented in Supplementary materials.

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Figures

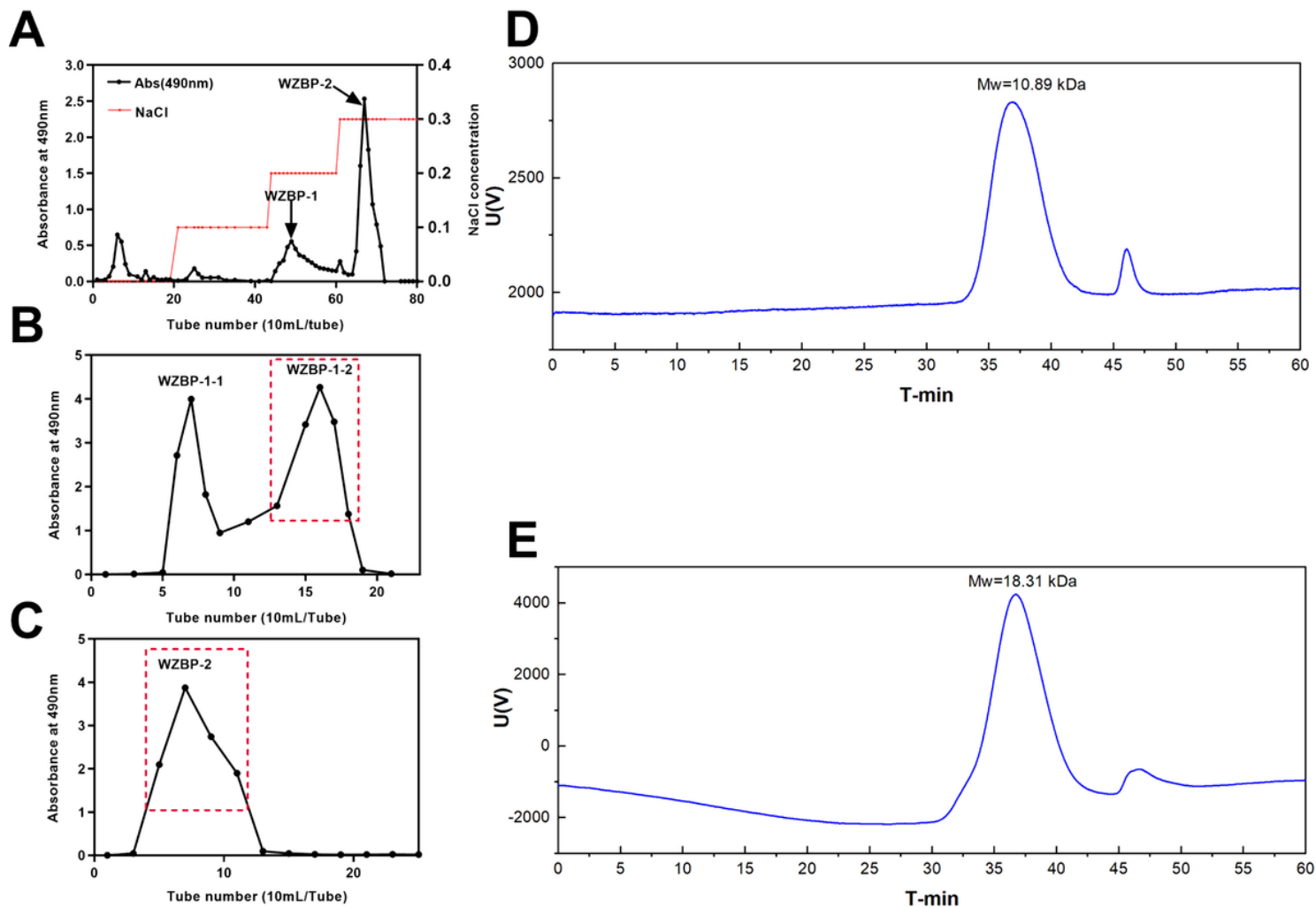


Figure 1

Separation and purification of polysaccharides from *Zanthoxylum bungeanum* Maxim. (A) The elution curve of crude polysaccharide on DEAE-52 cellulose column. (B) Elution curves of WZBP-1-2 on Sephadex G-75 column. (C) Elution curves of WZBP-2 on Sephadex G-75 column. (D) High-performance gel-permeation chromatography (HPGPC) chromatograms of WZBP-1-2. (E) HPGPC chromatograms of WZBP-2.

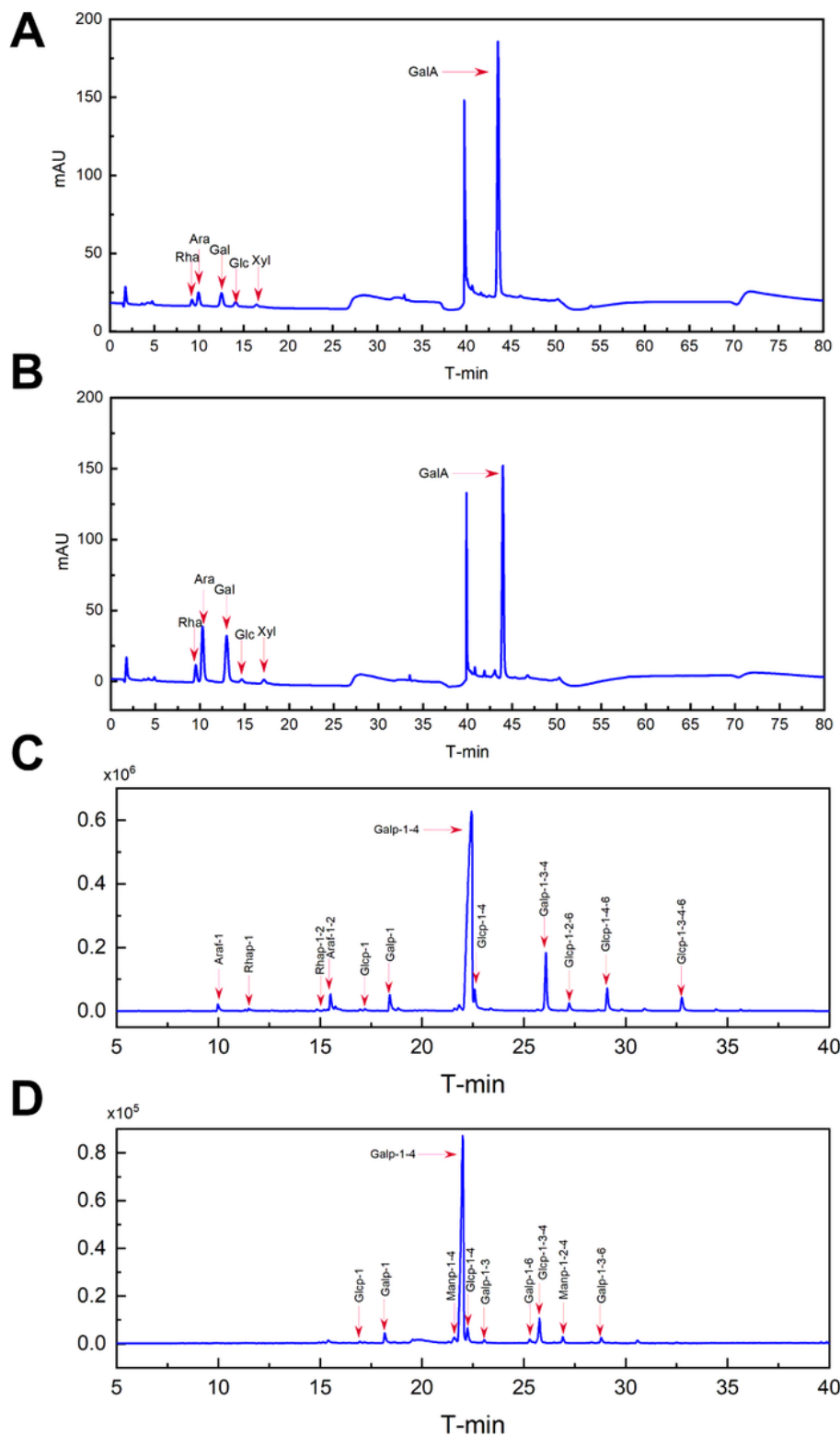


Figure 2

Structural identification of the two polysaccharides from *Zanthoxylum bungeanum* Maxim. (A) Total ion flow chromatograms of WZBP-1-2 for monosaccharide composition. (B) Total ion flow chromatograms of WZBP-2 for monosaccharide composition. (C) Results of methylation analysis of WZBP-1-2. (D) Results of methylation analysis of WZBP-2.

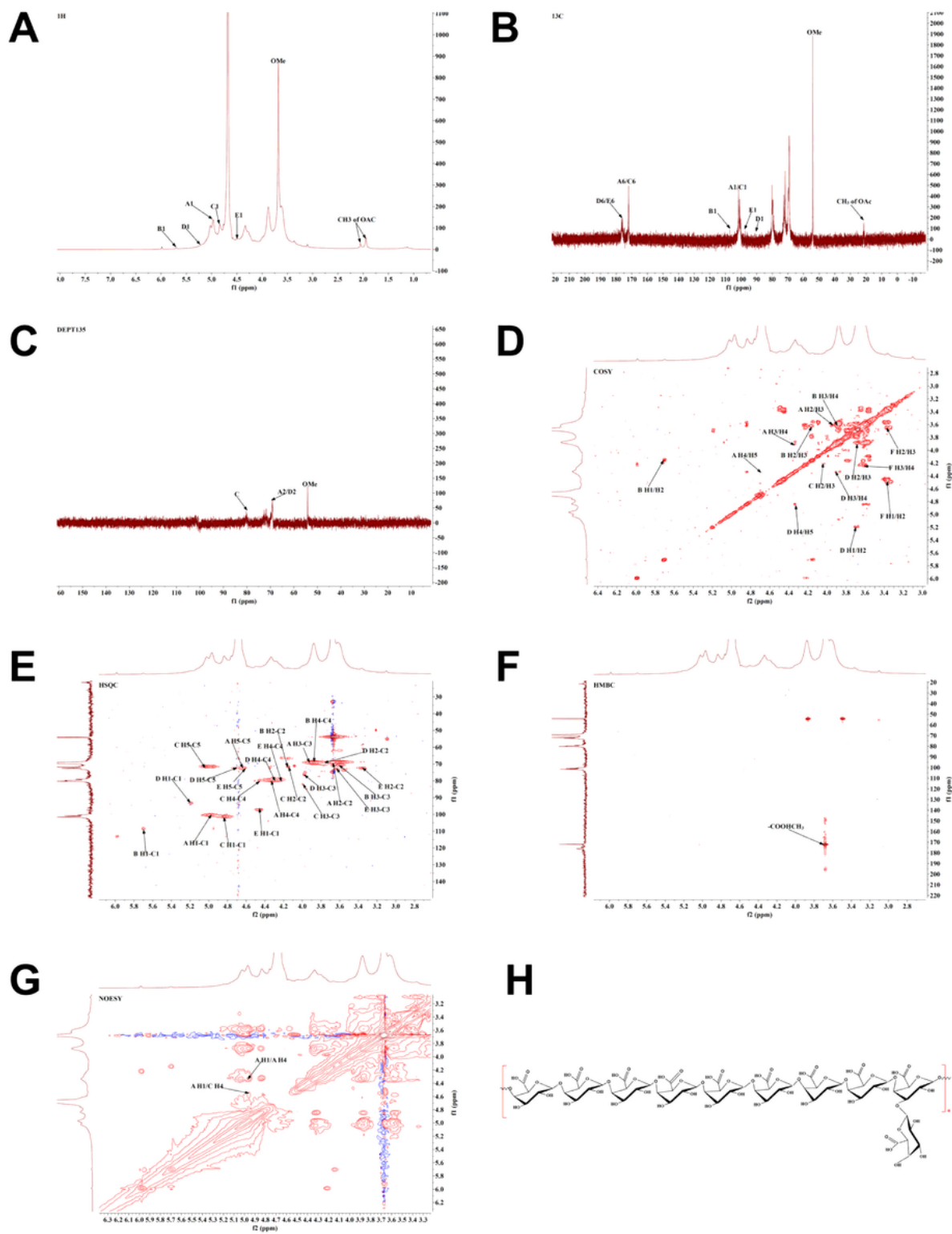


Figure 3

NMR data of WZBP-1-2. (A) ^1H spectra. (B) ^{13}C spectra. (C) Dept-135 spectra. (D) ^1H - ^1H COSY spectra. (E) HSQC spectra. (F) HMBC spectra. (G) NOESY spectra. (H) Structure of WZBP-1-2.

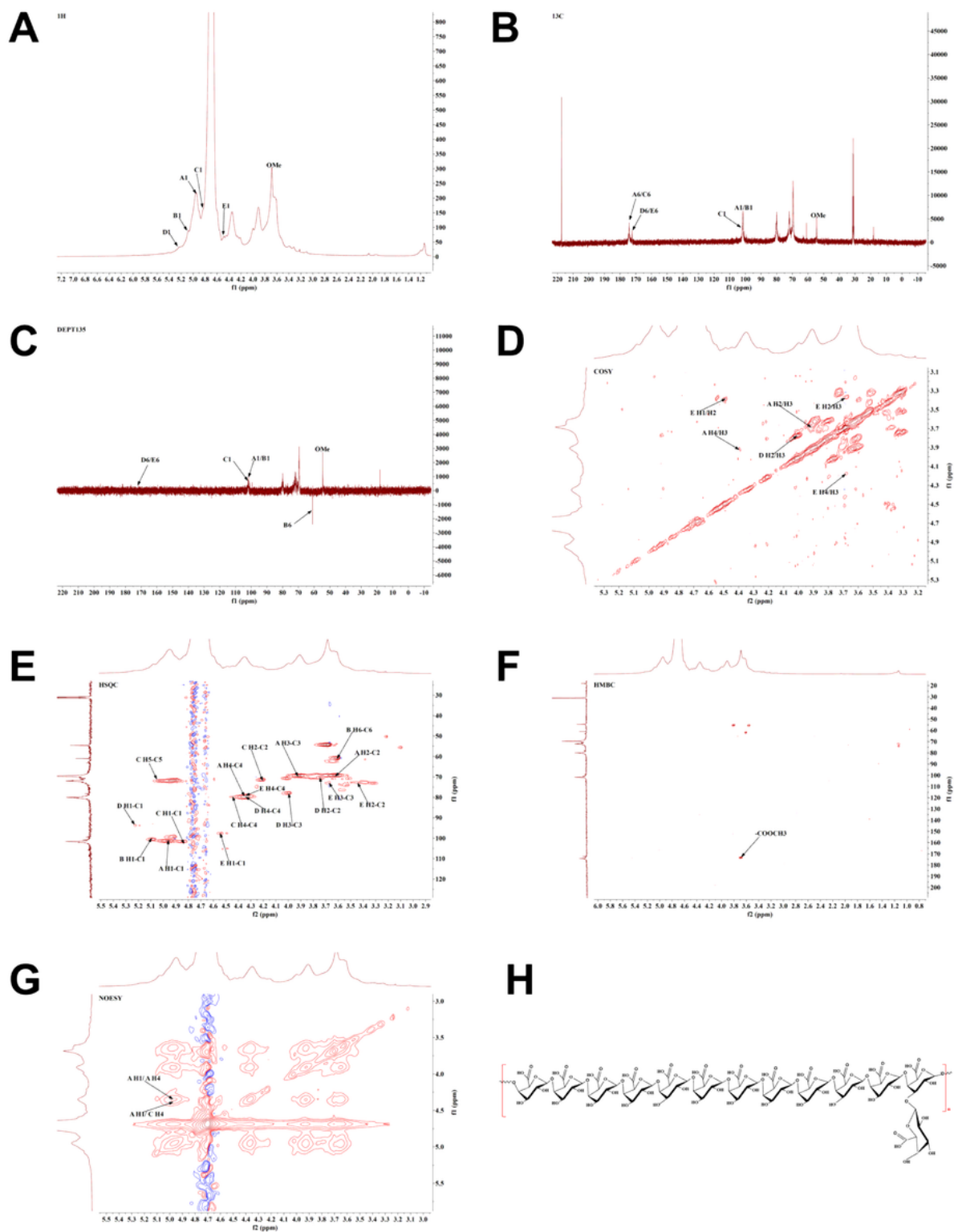


Figure 4

NMR analysis of WZBP-2. (A) ^1H spectra. (B) ^{13}C spectra. (C) Dept-135 spectra. (D) ^1H - ^1H COSY spectra. (E) HSQC spectra. (F) HMBC spectra. (G) NOESY spectra. (H) Structure of WZBP-2.

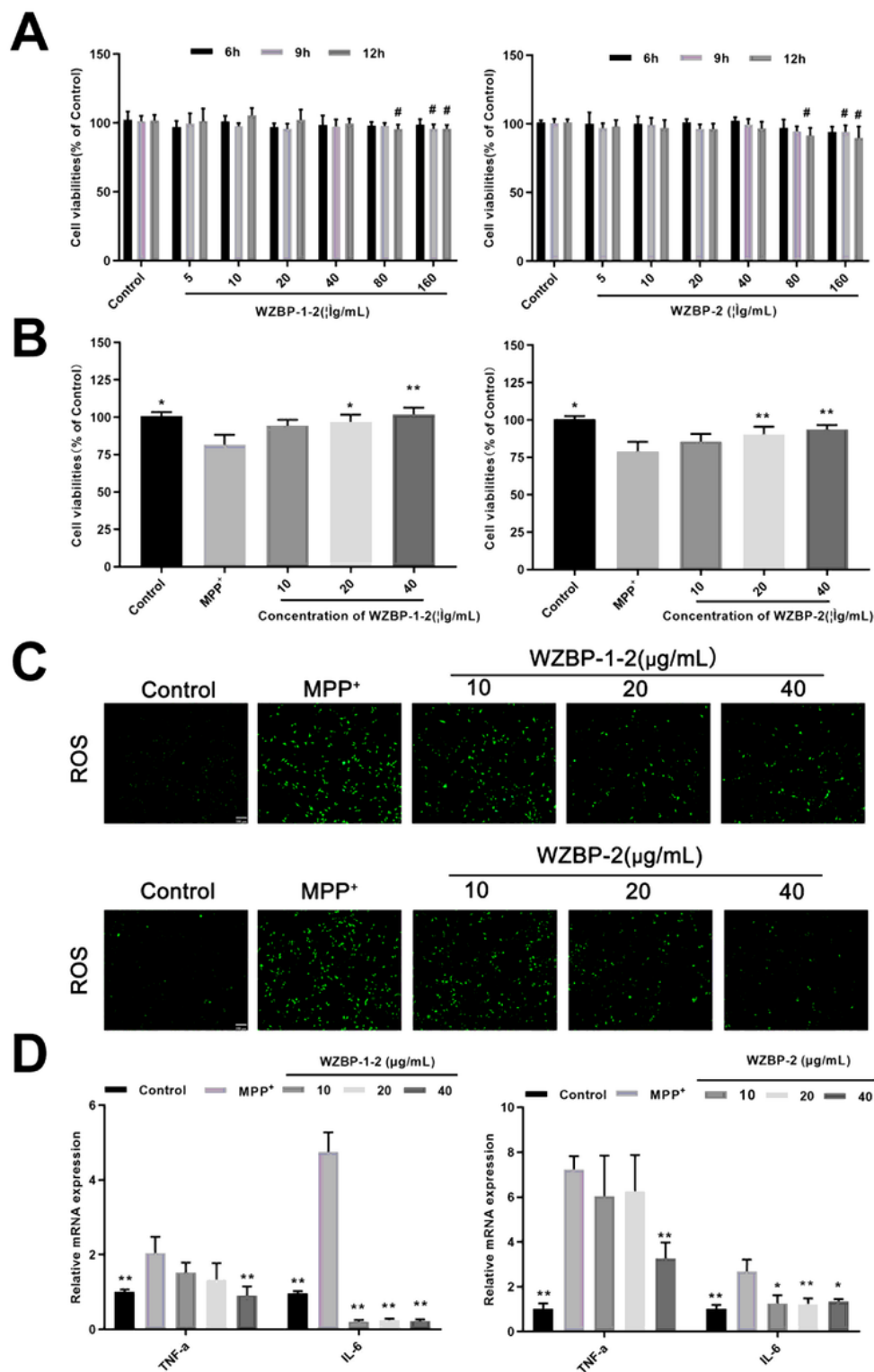


Figure 5

WZBP-1-2 and WZBP-2 prevented MPP⁺-induced MN9D cell damage. (A) Effects of WZBP-1-2 and WZBP-2 on cell viability. (B) Effects of WZBP-1-2 and WZBP-2 on MN9D cell viability induced by MPP⁺. (C) Effects of WZBP-1-2 and WZBP-2 on ROS levels in MN9D cells induced by MPP⁺ (200 ×). (D) Effects of

WZBP-1-2 and WZBP-2 on the mRNA levels of TNF-α and IL-6 in MPP⁺-induced MN9D cells. Data are expressed as mean±SD (*n*=3). **P*<.05, ***P*<.01 compared with MPP⁺ model groups.

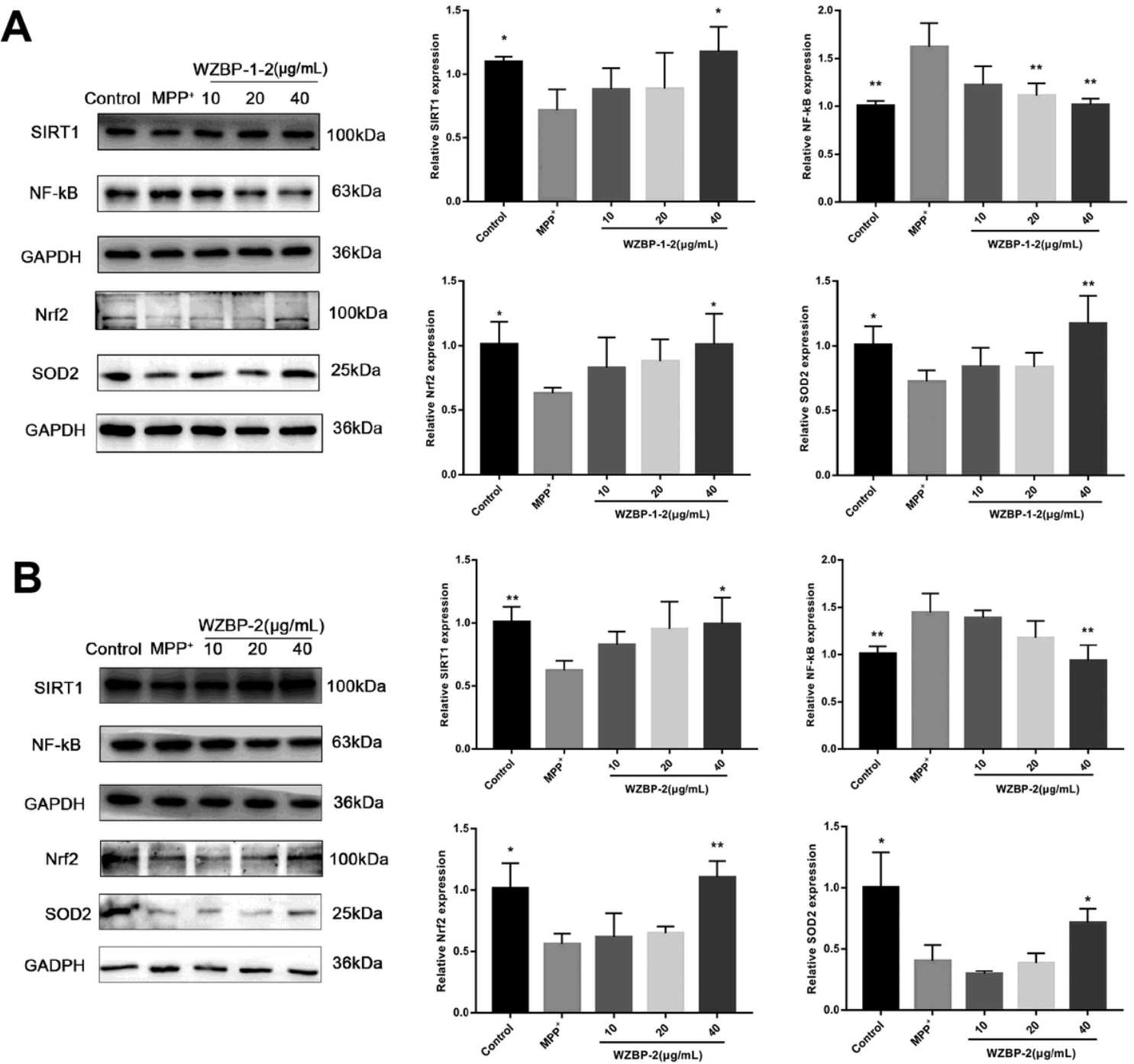


Figure 6

WZBP-1-2 and WZBP-2 activated SIRT1-mediated Nrf2/NF-κB pathway in MN9D cells induced by MPP⁺. (A) Effects of WZBP-1-2 on the protein levels of SIRT1, NF-κB, Nrf2 and SOD2 in MN9D cells by western blot assay. (B) Effects of WZBP-2 on the protein levels of SIRT1, NF-κB, Nrf2 and SOD2 in MN9D cells by Western blot assay. Data were expressed as mean±SD (*n*=3). **P*<.05, ***P*<.01 compared with MPP⁺ model groups.

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