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Original Article

Structural characterization and a novel water-soluble polysaccharide from *Fomes Fomentarius*

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Abstract: At present, there is no report on the anti-hypoxia activity of fomes fomentarius polysaccharide. Therefore, our aim was to evaluate the anti-hypoxia activity of the purified exopolysaccharide of Paragonimus fordii. The extracellular polysaccharide of Trichophyton was screened and purified by DEAE-52 chromatographic column, and the fraction was FFEP-1 (molecular weight = 3.08×10^5 DA). The analysis of monosaccharide components showed that FFEP-1 was mainly constructed by galactose, mannose and glucose. The main chain of FFEP-1 is composed of β-1,2-connected GALP β-1,2,3-connected GALP α-1,3-connected GLCP β-1,3,4-linked Manp composition, and α-1,3-linked Manps are identified in O-2 and O-3 α-1-galp at the end and α-1-terminal GLCP substitution. The possible structure of FFEP-1 was determined by one-dimensional and two-dimensional NMR and methylation analyses. The anti-hypoxia effect of FFEP-1 was studied through various experiments, which showed that FFEP-1 had a similar anti-hypoxia effect on propranolol hydrochloride. The anti-hypoxia effect of FFEP-1 might be explained by increasing the number of red blood cells and hemoglobin content.

Keywords: *Fomes Fomentarius*, polysaccharide, structure, anti-anoxia

Introduction

The F. Fomentarius as wood-rotting fungus belongs to *Basidiomycota*, *Basidiomycetes*, *Polyporales*, *Polyporaceae* and *Fomes*. The *F. Fomentarius* has been used for treating stasis, silt, diuresis and pain for hundreds of years. A previous study has documented that the fruiting bodies contain a variety of active substances [1]. Numerous studies relating to the polysaccharide isolated from *F. Fomentarius* have also been performed. Chen et al. investigated culture conditions EPS (Expandable Polystyrene) from *F. Fomentarius* by orthogonal matrix [2]. Same research team also investigated the intervention function of the intracellular polysaccharide (IPS) from *F. Fomentarius* on SGC-7901 and MKN-45 [3]. Kim et al. isolated MFKF-AP1β from *F. Fomentarius* and their study showed that MFKF-AP1β inhibited lung cancer A549 cell growth [4]. In addition to antitumor effect, Gao et al. also found its extra effects in mice [5]. Although various activities of polysaccharides from *F. Fomentarius* have been demonstrated [6-8], no existing data focuses on the chemical structure analysis of polysaccharides from *F. Fomentarius*. To better understand the structural basis of the various activities of polysaccharides from *F. Fomentarius*, we aimed to figure out polysaccharide from fermentation broth after submerged culture of *F. Fomentarius* through monosaccharide composition analysis, FT-IR spectrometer analysis and NMR analysis.

Hypoxia is defined as the reduction of oxygen available to the body tissues. In mammals, the body tissue function is impaired under hypoxia, which may cause various physiological abnormalities and pathological symptoms. It is important to find substances that can improve the body's ability to resist hypoxia. The polysaccharides have been shown to have the ability to improve the body's anti-anoxic ability through multiple mechanisms. Dong et al. found a main fraction obtained from the aqueous polysaccharide of *Cordyceps militaris* [9]. Li et al. reported the anti-hypoxic potential of crude polysaccharide [10]. However, as far as we know, there is no report about the anti-hypoxia

activity of polysaccharides from *F. Fomentarius*. Thus, the anti-hypoxia activity of purified fraction of the extracellular polysaccharide of *F. Fomentarius* was evaluated in this study.

Materials and methods

Strains and fermentation elements

The *F. Fomentarius* was selected and preserved by North Minzu University. Fermentations were conducted in a 20 L auto-control bioreactor under the conditions of temperature 31°C, speed 150 rpm, pH 7.5 for 10 days. The fermentation medium was sucrose 15 g/L, casein peptone 7.5 g/L, KH_2PO_4 1.0 g/L, $MgSO_4$ 0.5 g/L and VB_2 0.625 mg/L for monitoring elements.

F. Fomentarius extracellular polysaccharide

The broth was collected through filtration at the end of the fermentation. 95% ethanol was added to the fermentation broth (3:1) to precipitate the crude polysaccharide. After redissolved by deionized water, deproteinized by trichloromethane and decolorized by 30% H_2O_2, the polysaccharide solution was then dialyzed for 48 h.

For purification, 1 mL of polysaccharide solution was loaded in the column of DEAE-52. The sample was eluted with 0.0, 0.2, 0.5 M NaCl in 0.5 mL/min. The phenol-sulfuric acid was performed to select carbohydrate content, and 3 fractions were obtained, named FFEP-1, FFEP-2 and FFEP-3. The content of FFEP-1 was the highest. Due to the heavy workload of the experiment, we selected FFEP-1 as the research object. The main fraction (FFEP-1) was pooled together for elution curves plotting.

Homogeneity and molecular weight

Homogeneity and weight were investigated *via* HPGPC and ELSD. Columns were Ultrahydrogel™ 2000 and Ultrahydrogel™ 500. The temperature was 35°C. The phase was 0.02 mol/L of KH_2PO_4. The concentration was 1.0 mg/mL. The injection was 20 μL. MW was found *via* dextran standards.

Monosaccharide composition analysis

The FFEP-1 (2 g) was solved in 30 mL of water and added to Zinc acetate solution and potassium ferricyanide, and then added in 80 mL water. Pretreated sample was obtained by oscillation for 1 h at room temperature, centrifuge, filtration and fixed capacity to 100 ml, successively. To get hydrolytic sample, one milliliter of pretreated sample was added to 1.0 mL of TFA 4 mol/L, dried and put in water bath at 70°C under the atmosphere of nitrogen. Then PMP derivatization was carried out. The 0.5 mL of 0.5 mol/L PMP and 0.3 mol/L NaOH was added to the monosaccharide samples obtained after the hydrolytic drying, cooled in normal temperature, added to 0.5 mL of 0.3 mol/L HCl, and mixed well after extraction by chloroform for three times. Then, the water layer was collected to obtain the derivatized monosaccharide for analysis.

Thermo u3000 liquid chromatograph equipped with Ultraviolet detector was used to identify the Monosaccharide composition of FFEP-1, columns: Thermo C18, T: 25°C, mobile: 0.1 mol/L pH 6.7 KH_2PO_4, concentration: 1.0 $mg{\cdot}mL^{-1}$, and injection: 10 μL. An ultraviolet detector with 245 nm was used.

Methylation analysis

Methylation was performed *via* Needs and Selvendran [11], and 50 mg of FFEP-1 was solved in 4 mL of dimethylsulfoxide. NaOH 50 mg was added, oscillated for 30 min to get the liquid coagulated. Methyl iodide was oscillated and added with water. PH was 25% acetic acid. The acetylating agent was solved in chloroform *via* GC-MS by HP-5 column, with a T of 50°C and then 250°C. The monosaccharides were obtained *via* the same method.

FT-IR analysis

The FT-IR analysis of FFEP-1 was conducted using a FT-IR spectrometer, and FFEP-1 was grounded by KBr and pressed to pellets by scanning 4000 to 400 cm^{-1} [12].

1D and 2D NMR analysis

The sample was dissolved in D_2O and 1D NMR, and 2D NMR spectra was obtained at 25°C. The ^1H, ^{13}C, spectra were analyzed using DEPT-135, COSY, HSQC and HMBC.

The anti-hypoxia activity of FFEP-1

Sixty-five male Kunming mice (20 ± 2 g) were purchased from Laboratory Animal Center of Ningxia Medical University (Certificate number:

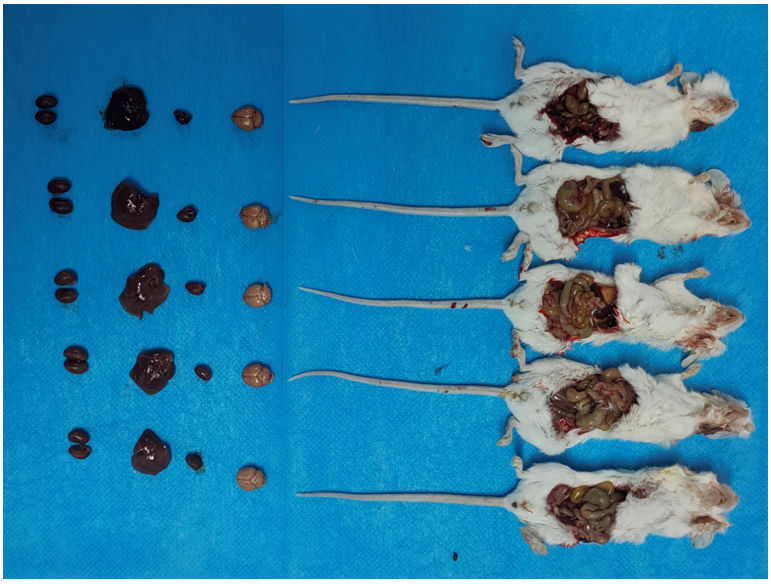

Figure 1. Some solid images of experimental mice.

No. 2020003). Our experiments were conducted in accordance with the guidelines for care of laboratory animals and approved by the Ethics Committee on Animal Experiments of the Ningxia Medical University. The mice were adaptively fed for one week under standard condition, with free drinking and eating before and during the experiment. All mice were fasted for 12 h prior to any experimental operation. Control mice were given saline gavage. Mice in FFEP-1 treatment groups with low, middle and high dosages were given 50, 100 and 200 mg/kg BW FFEP-1, respectively, whereas mice in positive group were treated with 30 mg/kg propranolol hydrochloride. Gavage was performed once a day for 3 weeks. Anoxia experiment under normal pressure, sodium nitrite poisoning or acute brain ischemia was carried out 60 min later after the last administration.

Normobarie hypoxia assay: For anoxia experiment under normal pressure, each mouse was put into a 250 mL container containing 15 g calcium hydroxide. The survival time of mice under oxygen deprivation was recorded from the time sealing the container till the mice stop breathing.

Sodium nitrite toxicosis assay: Each mouse was intraperitoneally injected with Sodium nitrite (200 mg/kg BW) once. The survival time of the mice was from the injection of sodium nitrite to the mice stop breathing.

Acute cerebral hypoxia experiment: Three mice were randomly selected from each group, anesthetized with Ether, and decapitated from the neck quickly. The survival time of the mice was recorded from the neck fracture to the mice stop breathing. In addition, the mice were dissected immediately After the neck was broken, the whole brain, heart, liver and kidneys were separated for the determination of MDA content in the tissues by ELISA kit.

Hematological parameters determination: Before eyeball-removal, anesthetic was used in the mice. Blood samples were collected by exsanguination via eyeball-removal and then measured by an XE-2100 automated hematology analyzer for red blood cell (RBC) and hemoglobin (HGB).

Statistical analysis

Measurement values were presented as mean± standard deviation, and differences between groups were investigated by one-way ANOVA followed by post hoc Turkey using SPSS (Statistical Product Service Solutions).

Results

Separation and purification

FFEP was isolated from *Fomes Fomentarius,* and it was selected in DEAE-52. The 3 fractions were obtained water, 0.2 M and 0.5 M NaCl eluted fractions, named FFEP-1, FFEP-2 and FFEP-3, respectively (**Figure 2A**).

Homogeneity and MW

The regression was obtained for log [Mw] and number via Y = 19.971-0.5457X; R^2 = 0.9967. The HPLC of FFEP-1 was presented in **Figure 2B**, which reveals an Mw of about 3.08 × 10^5 Da. The chromatogram of the FFEP-1 was reported peak that FFEP-1 was polysaccharide.

FT-IR spectrum

FT-IR of FFEP-1 is presented in **Figure 2C**. The absorption 3396 cm^{-1} is hydroxyl stretching for

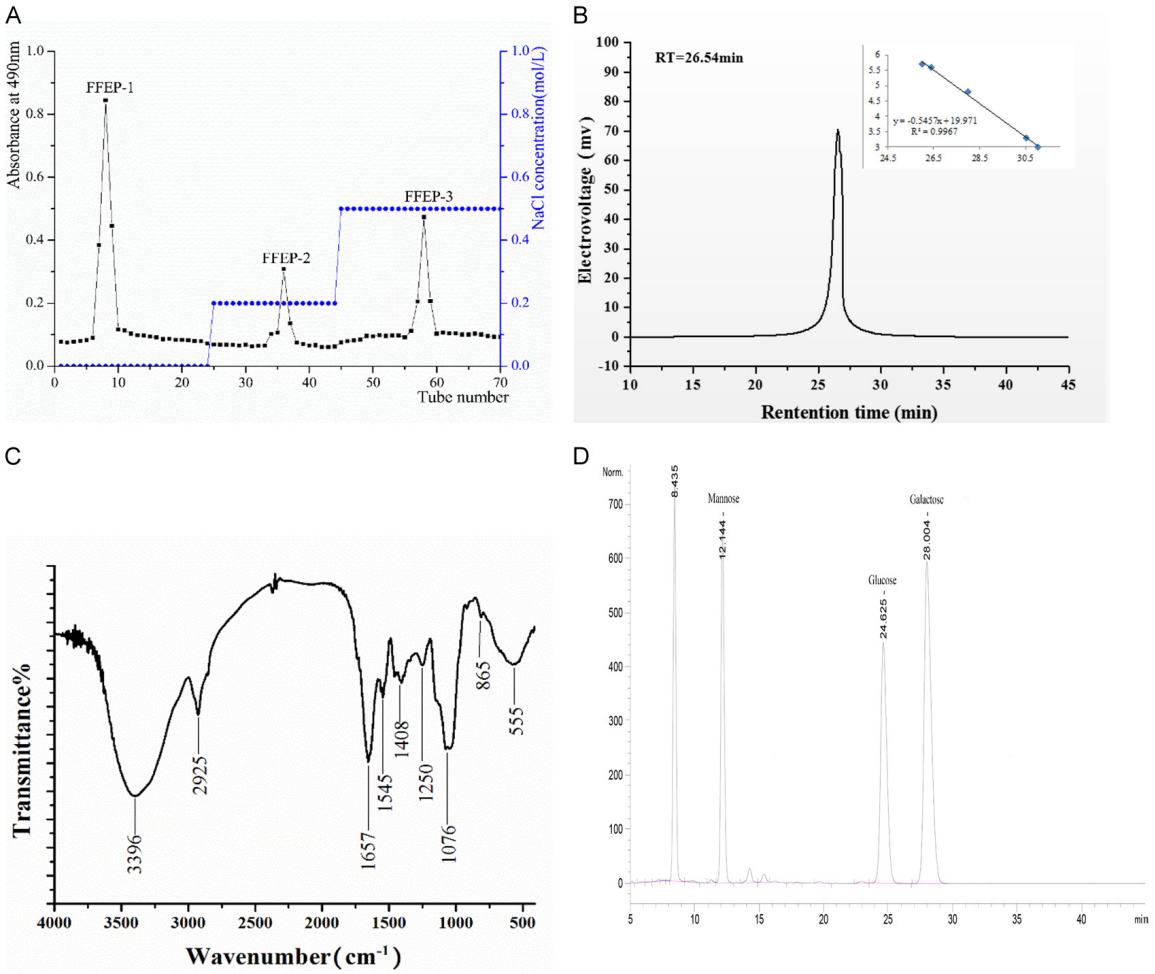

Figure 2. Composition and glycosidic bonds of monosaccharides. A: Chromatogram of FFEP in DEAE-52 eluted with 0.0, 0.2, 0.5 M NaCl. B: HPLC Chromatogram of FFEP-1. C: FT-IR of FFEP-1 and methylated FFEP-1. D: Monosaccharide composition of FFEP-1.

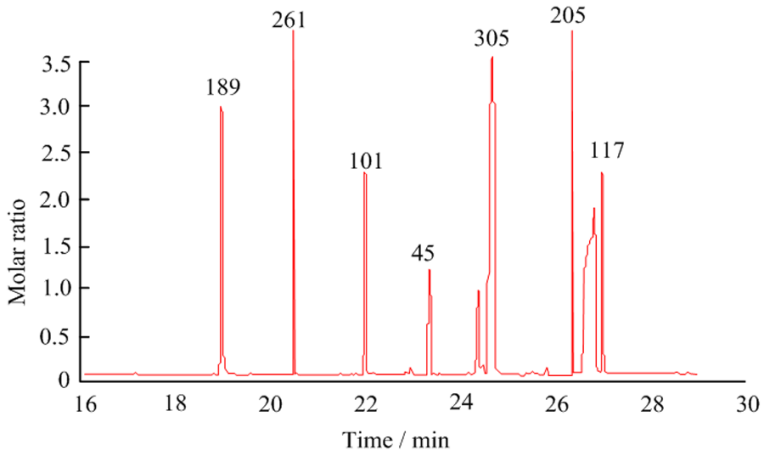

Figure 3. Total ion chromatography of gas chromatography.

inter- and intra-hydroxyl [13]. Bands 2925 cm^{-1} is belong to peak C-H vibration of CH and CH_2 [14]. The 1657 cm^{-1} is C = O asymmetric vibration. The peak 1408 cm^{-1} attributed to deformation vibration of CH showed that FFEP-1 included pyranose rings [15].

The 1250 cm^{-1} is anomeric hydrogen, and 825-860 cm^{-1} is anomeric H [16]. 1076 cm^{-1} is hydroxyl vibration, and 555 cm^{-1} is CCO vibration. In 970-920 cm^{-1} the bands are C-O-C glycosidic of FFEP-1 [17].

Monosaccharide composition and glycosidic linkages

In acetylated monosaccharide in GC-MS the FFEP-1 was selected accurately. Mannose, glucose and galactose are the main monosaccharide in FFEP-1 in the molar ratio of 0.18:0.24:0.58 (shown in **Figure 3**). FFEP-1 has

Table 1. GC-MS of methylated FFEP-1 for alcohol-precipitation extracts of fermentation broth of *Fomes Fomentarius*

Methylated units	linkages	Molar ratio	Major mass fragments (m/z)
3,4,6-Me_3-Gal*p*	1,2-linked Gal*p*	2.75	71, 87, 99, 101, 129, 161, 189
4,6-Me_2-Gal*p*	1,2,3-linked Gal*p*	1.55	71, 87, 101, 127, 129, 161, 217, 261
2,4,6-Me_3-Glc*p*	1,3-linked Glc*p*	1.03	43, 45, 71, 87, 101, 118, 129, 161, 174, 217, 234, 277
2,3,4,6-Me_4-Glc*p*	1-terminal Glc*p*	1.41	43, 45, 71, 87, 102, 118, 129, 145, 161, 162, 205
2,6-Me_4-Man*p*	1,3,4-linked Man*p*	1.37	87, 143, 159, 185, 245, 261, 305
2,3,4,6-Me_4-Gal*p*	1-terminal Gal*p*	1.55	101, 117, 129, 145, 161, 205
2,4,6-Me_3-Man*p*	1,3-linked Man*p*	0.34	45, 117, 161, 233

8 linkage patterns: 1,2-linked Gal*p*, 1,2,3-linked Gal*p*, 1,3-linked Glc*p*, 1-terminal Glc*p*, 1,3,4-linked Man*p*, 1-terminal Gal*p*, and 1,3-linked Man*p* with relative ratio of 2.75:1.55:1.03:1.41 :1.37:1.55:0.34, in accordance with that of monosaccharide compositions (**Table 1**, **Figure 2D**).

1D and 2D NMR

NMR of FFEP-1 is presented in **Figure 4**. The spectrum of polysaccharide is reported in **Table 2** and it is derived from ^{1}H, ^{13}C, DEPT-135, COSY, HSQC and HMBC. Residues were designated as A-G. Remarkably, ^{13}C and DEPT-135 NMR spectra showed unsubstituted C-6 at 62.55 ppm and around 61.00 ppm, which verified that there was no substituted C-6. β-1,2-linked Gal*p*, β-1,2,3-linked Gal*p*, α-1,3-linked Glc*p*, α-1-terminal Glc*p*, β-1,3,4-linked Man*p*, α-1-terminal Gal*p* and α-1,3-linked Man*p* were selected from NMR and are presented in **Table 2**.

The proton shift of A was obtained in δ 4.74 and showed the β-linked residue. 1H resonance for H-2 was in δ 3.19 (**Figure 2**). The H-3, H-4, H-5 shifts are reported in **Table 2**. The chemical shifts of carbons of A were obtained in HSQC [18]. Shifts of C-2 (δ 74.16) indicates that A is 1,2-linked β-D-galactose-pyranose [19].

The residue B was in δ 4.55 and showed β-linked residue, and cross-peak H1/H2 was obtained in COSY. H-2 was in δ 3.46, and H-6 was in HSQC. In B, shift C-2 was in δ 75.85, and C-3 was in δ 74.16. It is indicated that B is 1,2,3-linked β-D-galactopyranose [20].

Coupling of $J_{H\text{-}1,\ H\text{-}2}$ was lower than 2 Hz showed that C was α-configuration [21]. In COSY the intense peak proposed H-2 shift of A in δ 3.5 ppm. The H-3, H-4, H-5, H-6 and H-6' shifts are reported in **Table 2**.

Moreover, carbon signal at δ 92.11 ppm obtained in HSQC is consistent with the literatures [18]. The ^{13}C signals were reported in non-anomeric in HSQC. The shifts of C are close to methyl glycoside of α-D-glucose [18], indicating that C is 1-linked α-D-glucopyranose.

The anomeric in δ 5.11 showed that the D was α-linked, and 1H for H2, H3 and H4 of D were in 1H-1H COSY. The H-5, H-6a and H-6b were in HSQC and COSY. Shifts of D were close to methyl glycoside of α-D-glucose. So, D is 1-linked α-D-glucopyranose.

The signal of E was in δ 4.94 showed that β-linked and proton shift of H-2, H-3 and H-4 was in COSY. H-5, H-6a and H-6b were in HSQC and COSY. The shifts of C-3 were in δ 75.20 and C-4 was in δ 71.43. So, E is a 1,3,4-linked β-D-Man*p*.

The anomeric of F was in δ 5.22, and it was α-linked. The proton shift of H-2 was δ 3.75 in COSY and H-3 and H-4 were in COSY. The H-5, H-6a and H-6b were in HSQC and COSY. The carbon shifts in C-1 to C-6 were in HSQC (**Table 2**), which is close to methyl glycoside of α-D-galactose, so F is 1-linked α-D-galactopyranose.

The anomeric of E was in δ 5.03, and it was α-linked. Negative peak in δ 61.17 ppm of DEPT-135 was in C-6 of galactose. The shifts of C-3 were in δ 75.20 carbon signals, so G is 1,3-linked α-D-Man*p*.

In A-G, the glycosidic of FFEP-1 was deduced for $^{1}H/^{13}C$ correlations in HMBC. The cross peaks of residues were investigated and obtained *via* HMBC (**Figure 4**; **Table 3**).

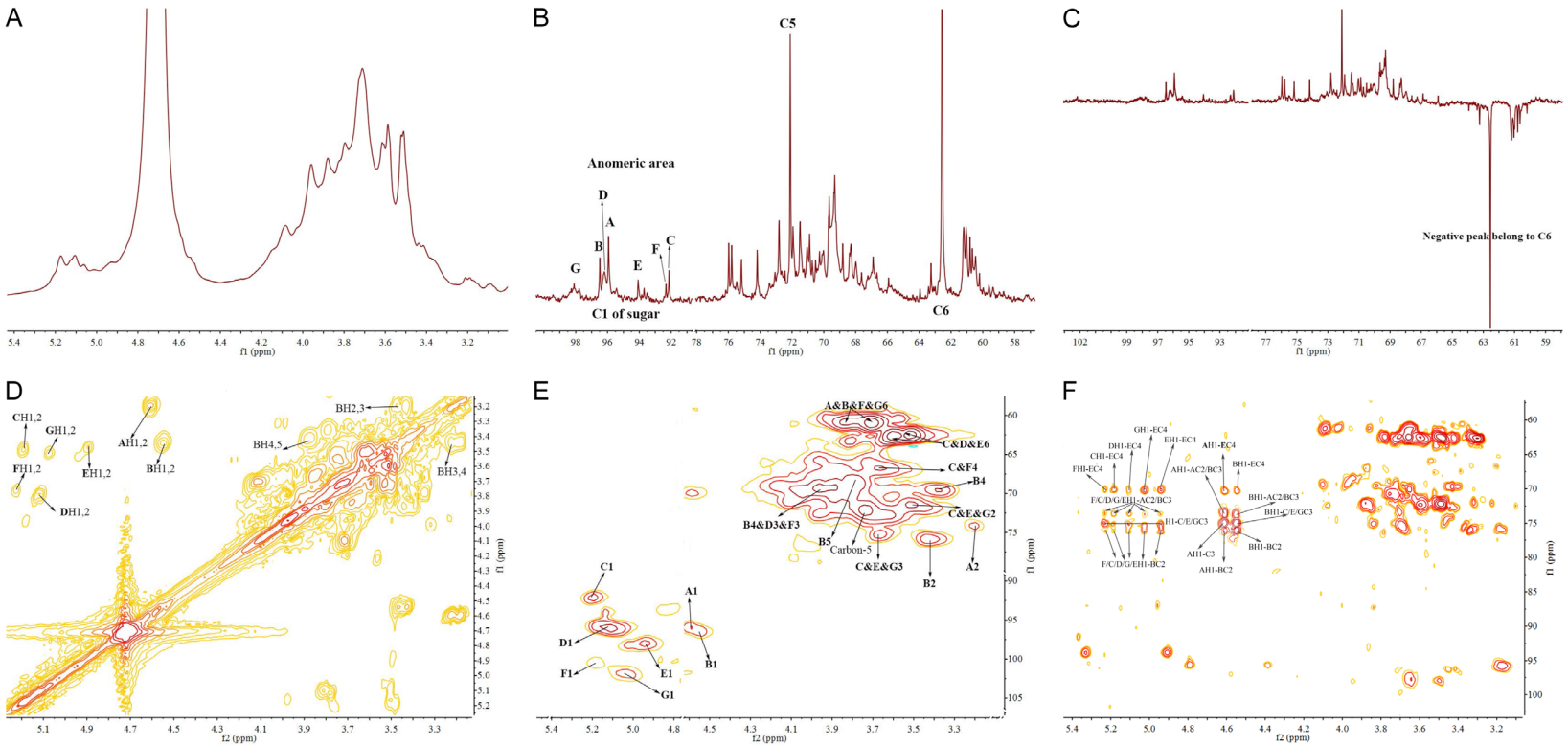

Figure 4. 1H, 13C, DEPT-135, HSQC, HMBC, COSY NMR spectra of FFEP-1. Note: The seven residues in FFEP-1 are labeled as A-G; in complex COSY spectrum protons correlations were labeled partially; in HMBC spectrum C-H multiple bond correlations were labeled with more than one possibility.

Table 2. ^{1}H and ^{13}C data for FFEP-1

Residue	$^{1}H/^{13}C$					
	1	2	3	4	5	6
A	4.61	3.19	3.43	3.88	3.74	3.82; 3.71
β-1,2-linked Gal*p*	95.98	74.16	71.43	/	72.06	61.17
B	4.55	3.46	3.21	4.03	3.79	3.82; 3.71
β-1,2,3-linked Gal*p*	96.46	75.85	74.16	69.32	68.88	61.24
C	5.19	3.5	3.68	3.59	3.74	3.60; 3.52
α-1,3-linked Glc*p*	92.11	71.43	75.2	/	72.33	62.55
D	5.11	3.76	4.04	3.90	3.75	3.59
α-1-terminal Glc*p*	96.06	72.14	69.32	69.2	72.14	62.52
E	4.94	3.50	3.62	3.48	3.74	/
β-1,3,4-linked Man*p*	98.01	71.48	75.20	71.43	72.16	62.66
F	5.22	3.75	4.10	3.60	3.74	3.82
α-1-terminal Gal*p*	100.52	72.14	69.32	/	72.33	62.46
G	5.03	3.51	3.62	3.88	3.74	/
α-1,3-linked Man*p*	101.89	71.43	75.20	65.0	72.06	61.17

Note: / stands for not detected.

Table 3. Data of HMBC for FFEP-1 of *Fomes Fomentarius*

Residues	Sugar linkage	H-1	Observed connectivities		
		δ_H	δ_C	Residues	Atom
A	β-1,2-linked Gal*p*	4.61	74.16	A/B	C-2/C-3
			75.85	B	C-2
			75.20	C/E/G	C-3
			71.43	E	C-4
B	β-1,2,3-linked Gal*p*	4.55	74.16	A/B	C-2/C-3
			75.85	B	C-2
			75.20	C/E/G	C-3
			71.43	E	C-4
C	α-1,3-linked Glc*p*	5.19	74.16	A/B	C-2/C-3
			75.85	B	C-2
			75.20	C/E/G	C-3
			71.43	E	C-4
D	α-1-terminal Glc*p*	5.11	74.16	A/B	C-2/C-3
			75.85	B	C-2
			75.20	C/E/G	C-3
			71.43	E	C-4
E	β-1,3,4-linked Man*p*	4.94	74.16	A/B	C-2/C-3
			75.85	B	C-2
			75.20	C/E/G	C-3
			71.43	E	C-4
F	α-1-terminal Gal*p*	5.22	74.16	A/B	C-2/C-3
			75.85	B	C-2
			75.20	C/E/G	C-3
			71.43	E	C-4
G	α-1,3-linked Man*p*	5.03	74.16	A/B	C-2/C-3
			75.85	B	C-2
			75.20	C/E/G	C-3
			71.43	E	C-4

Table 4. Effect of FFEP-1 on mice weight (n = 13)

Group	Before drug/g	After drug/g	Weight growth ratio/%
Control	21.62 ± 4.15	25.23 ± 5.30	16.70 ± 4.68
FFEP-1 50 mg·kg^{-1}	21.78 ± 4.37	26.54 ± 5.43	21.85 ± 4.38[a]
FFEP-1 100 mg·kg^{-1}	21.50 ± 3.06	27.80 ± 4.79[a]	29.30 ± 5.05[a]
FFEP-1 200 mg·kg^{-1}	21.54 ± 2.54	29.67 ± 2.67[a]	37.74 ± 3.81[b]
PHT 30 mg·kg^{-1}	21.57 ± 3.62	25.67 ± 2.73	19.01 ± 4.47

Notes: [a]P < 0.01 in comparison to control, [b]P < 0.05 in comparison to control.

The intense cross peaks (δ 4.61/74.16) for HMBC (**Figure 2**) were in $^3J_{AH1, BC3}$, which showed correlations for H-1 (δ 4.61) of β-1,2-linked Gal*p* and C-3 of β-1,2,3-linked Gal*p* through 1,3-O-glycosidic. Peaks (BH1/BC3; BH1/AC2; BH1/CC3) showed consecutive linkages of B via 1,3-O-glycosidic, correlations for H-1 of B and C2 of A via 1,2-O-glycosidic, for H-1 of B and C3 of C by 1,3-O-glycosidic. The peak (δ 5.19/71.43; δ 5.11/75.20; δ 5.22/75.85) showed correlations H-1 of C and C-4 of E, for H-1 of D and C-3 of E, for H-1 of F and C-2 of B. Cross peaks (4.94/75.20; 4.94/74.16) were obtained as correlations for H-1 of E and C-3 of C (EH1/CC3) or C-3 of G (EH1/GC3) and A (EH1/AC2). There was also consecutive linkages of residue E by 1,4-O-glycosidic. Peaks (5.03/75.20; 5.03/71.43) were obtained as correlations for H-1 of G and C-3 of G (GH1/GC3), C-4 of E (GH1/EC4), which showed that G was linked for G and E via 1,3-O-glycosidic, 1,4-O-glycosidic.

The inference showed that FFEP-1 was substituted in O-2 and O-3 by α-1-terminal Gal*p* and α-1-terminal Glc*p*. The structure is presented in **Figure 3**.

Anti-anoxia potential of FFEP-1

The effect of FFEP-1 on the weight gain of mice: The mouse weight in different group was monitored during experimental period and reported in **Figure 1**; **Table 4**. There was no difference in mouse weight between each group prior to the treatment of drugs. At the end of experiment, mice treated with 100 or 200 mg·kg^{-1} were heavier than control mice (P < 0.01).

The anti-hypoxia activity of FFEP-1 under normal pressures in mice: The survival analysis of mice in a closed container was determined and showed in **Figure 5A**. Mice in FFEP-1 middle and high dose group survived about 20 min, which was longer than that in the controls. In addition, the survival time in FFEP-1 middle and high dose groups was similar to that in the PHT group, indicating the anti-hypoxia activity of FFEP-1 under normal pressures was of great value.

Anti-hypoxia potential of FFEP-1 in sodium nitrite poisoning mice: Sodium nitrite induces the transform of Fe^{2+} in hemoglobin molecules into Fe^{3+}, resulting in hypoxia of tissue cells. After intraperitoneal injection of sodium nitrite at a dosage of 200 mg/kg BW, the mice survived about 20 min (**Figure 5B**). When the mice were pretreated with 50, 100 and 200 mg/kg FFEP-1, the survival time increased to 25, 30 and 40 min, respectively, showing a dose-dependent anti-hypoxia effect of FFEP-1 in sodium nitrite poisoning mice. The mice pretreated with PHT survived about 30 min, which was similar to the treated with 100 mg/kg FFEP-1.

The anti-hypoxia activity of FFEP-1 under acute cerebral ischemia in mice: The antioxidant activity was tested using five white mice, and the anti-hypoxic activity of FFEP-1 was also evaluated in mice under acute cerebral ischemia conditions. The results are shown in **Figure 5C**. After acute cerebral ischemia, the mice died in less than 30 s. Treatment with 200 mg/kg FFEP-1 or 30 mg/kg PHT significantly prolonged the survival for more than 1 minute. Overall, the results suggest that FFEP-1 improves hypoxia survival in mice induced by sodium nitrite poisoning or acute cerebral ischemia.

As one of the products of membrane lipid peroxidation, MDA can indirectly estimate the degree of biological oxidation. The MDA level in different tissues was determined after the mice died (**Table 5**). In comparison to controls, the levels of MDA in various tissues of FFEP-1 and PHT treated groups decreased. However, only the FFEP-1 high dose group had a signifi-

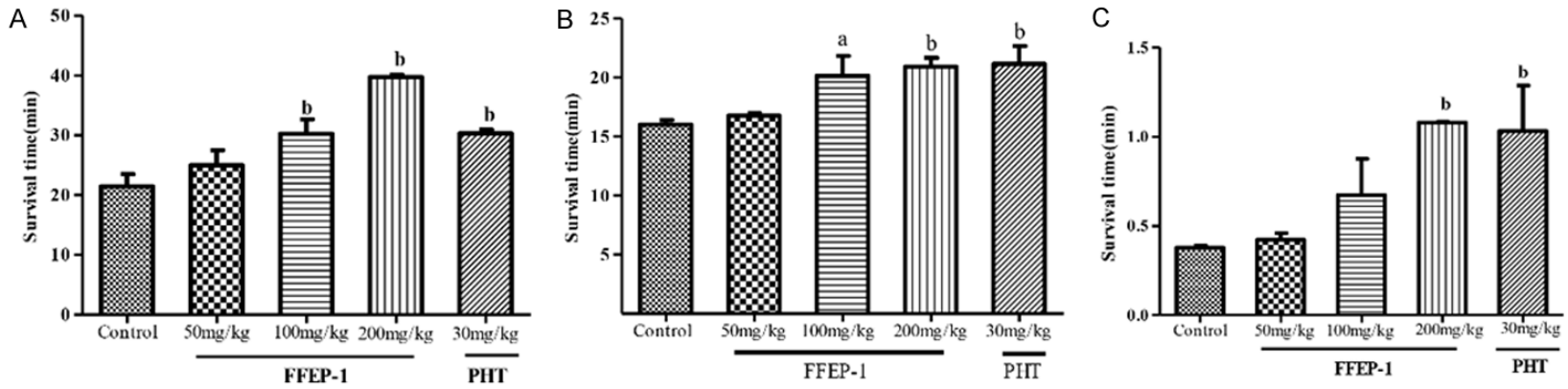

Figure 5. Effect of FFEP-1a for mice survival in hypoxia test (A), sodium nitrite toxicosis (B), and under acute cerebral ischemia (C).

Table 5. FFEP-1 effect on MDA content (mg/mgpro, X ± SD) in different tissues of mice

Group	Brain	Heart	Liver	Kidney
Control	0.173 ± 0.127	0.323 ± 0.219	0.438 ± 0.925	1.572 ± 0.963
FFEP-1 50 mg·kg^{-1}	0.123 ± 0.046^{b}	0.275 ± 0.434	0.348 ± 0.578	1.381 ± 0.116
FFEP-1 100 mg·kg^{-1}	0.119 ± 0.154^{b}	0.265 ± 0.182	0.358 ± 0.320	1.455 ± 0.749
FFEP-1 200 mg·kg^{-1}	0.118 ± 0.120^{b}	0.248 ± 0.423^{a}	0.289 ± 0.525^{b}	1.147 ± 0.619^{b}
PHT 30 mg·kg^{-1}	0.137 ± 0.205^{b}	0.321 ± 0.633	0.322 ± 0.718^{a}	1.259 ± 0.192^{a}

Notes: $^{a}P < 0.01$ in comparison to control, $^{b}P < 0.05$ in comparison to control.

Table 6. FFEP-1 effect on mice parameters

	Control	Dose of FFEP-1a (mg·kg^{-1})			PHT (mg·kg^{-1})
	Dosage	50	100	200	30
RBC/1 × 10^{12} L^{-1}	6.80 ± 1.03	7.71 ± 0.54	8.04 ± 0.12^{a}	8.63 ± 0.44^{b}	8.61 ± 0.34^{b}
HGB/g·L^{-1}	129.7 ± 24.50	156.3 ± 1.15^{a}	154.7 ± 4.51^{a}	177.0 ± 6.25^{b}	162.0 ± 7.94^{b}

Notes: $^{a}P < 0.01$ in comparison to control, $^{b}P < 0.05$ in comparison to control.

cantly lower MDA content than the controls in all detected tissues.

FFEP-1 effect on blood red number and hemoglobin concentration: To explore the molecular mechanisms underlying the anti-hypoxic activity of FFEP-1, we measured erythrocyte counts and hemoglobin in different mice. Hemoglobin (Hb) is a protein responsible for carrying oxygen in higher organisms. The main component of red blood cells is hemoglobin, which accounts for about 32% of its wet weight and 97% of its dry weight. The content of hemoglobin in the human body is an important indicator for evaluating the prevalence of anemia and iron nutritional status. Therefore, a convenient and effective method for detecting hemoglobin concentration, especially the method for detecting hemoglobin concentration at the single cell level, is very useful for evaluating human health status. Red blood cells carry oxygen through hemoglobin, and 90% of red blood cells are made up of hemoglobin. Hemoglobin concentration and red blood cell count are important indicators of the human oxygen transport system. Our results showed that FFEP-1 at doses of 100 and 200 mg/kg improved erythrocyte number and HGB concentration in mouse blood (**Table 6**), which may contribute to the anti-hypoxic activity of FFEP-1.

Discussion

Since 1950s, it has been found that fungal polysaccharides have anti-tumor and antioxidant activities [22, 23]. In recent years, the research about fungal polysaccharides has attracted more and more attention worldwide. Artificial cultivation of fungi takes a long time and produces very little active ingredients. Compared with the artificial cultivation, the active metabolite obtained through deep fermentation is more stable, the process is more easily controlled, the time is shorter, and there is no heavy metal pollution. Thus, deep fermentation is considered to be an effective means to develop and utilize fungi resources [24-26]. In this study, we isolated extracellular polysaccharide of submerged fermentation broth of *F. Fomentarius*, and studied the molecular structure and anti-hypoxia activity of the main fraction *in vivo*.

At present, the research on fungal polysaccharides is mainly concentrated on the following aspects: optimization of fermentation, conditions of extraction and purification, structural analysis, molecular modification and bioactivity study. Among them, structural analysis and activity are the most extensively studied. The preliminary structure of extracellular polysaccharide isolated from *F. Fomentarius* was generalized through determination of molecular weight, monosaccharide composition analysis, methylation, FT-IR, and 1D and 2D NMR. The data proposed FFEP-1 with MW = 425 kDa, is composed of mannose, glucose and galactose with a molar ratio of 0.18:0.24:0.58. FFEP-1 is consisted of eight linkage patterns, including β-1,2-linked Gal*p*, β-1,2,3-linked Gal*p*, α-1,3-linked Glc*p*, α-1-terminal Glc*p*, β-1,3,4-linked Man*p*, α-1-terminal Gal*p* and α-1,3-linked Man*p*

with relative ratio of 2.75:1.55:1.03:1.41:1.37:1.55:0.34 respectively. Park et al. separated MFKF-AP1β via fruiting of *F. Fomentarius* by DEAE-sepharose FF and A-sepharose 4B [27]. In contrast with our research, MW of MFKF-AP1β is 12 kDa by HPLC, and MFKF-AP1β is xylose. We believed that the main reason for this huge difference is due to the different sources of polysaccharide. The polysaccharide in this study was secretion of fungus into the culture medium, and the polysaccharide in the study performed by Park et al. was isolated. In addition, the chromatographic columns used in the purification of polysaccharides may also contribute to this difference.

A variety of bioactivity of polysaccharide from *F. Fomentarius* has been confirmed, including antitumor effect [3, 4, 7, 28], antiviral activity [29], immunomodulatory activity [5, 8], antibacterial and cytotoxic activities [6]. However, no study has been carried out about the anti-anoxia activity of polysaccharide produced by *F. Fomentarius*. In the pre-experiment, *F. Fomentarius* fruiting body was directly used as mouse feed, which was compared with FFEP-1. However, due to the high lignin content of fruiting body, the mice could not eat. In our study, the hypoxia tolerance of *F. Fomentarius* had not been determined, which is a limitation which needs further investigation.

The anti-hypoxia activity of fungal polysaccharides has been widely described. The sources of these polysaccharides include *Cordyceps militaris* [9], *Agaricus bisporus (Lange) Sing*. Chaidam [30], *Lachnum* sp. [31], *Sipunculus nudus L* [32] and *Cordyceps Sinensis* [33]. To determine the relationship between anti hypoxic function and structure of these polysaccharides, we obtained the presumptive structure of these polysaccharides exhibited anti hypoxic effect from the published literatures. The purified polysaccharides extracted from *Cordyceps militaris* (MW: 37.842 kDa), and it has L-rhamnose, L-arabinose, D-mannose, D-galactose [9]. The chain conformation analysis indicated that LEP-1 had no triple helical compound [31]. Comparing the monosaccharide composition of these three polysaccharides with anti-hypoxic activity, it was found that mannose was contained in all these polysaccharides. Glucose and galactose are present in 2 kinds of Polysaccharides, whereas L-rhamnose and L-arabinose are only found in polysaccharides extracted from *Cordyceps militaris*. This indicated that polysaccharides rich in mannose, glucose and galactose may have an anti-hypoxia function. The connection patterns of these three polysaccharides were significantly different, indicating that the way of monosaccharide attachment may have no significant effect on the anti-hypoxia effect of polysaccharides. However, due to the limited number of studies on the anoxic activity of fungal polysaccharides, these conclusions are based on a small sample size. More research needs to be included in order to reach a convincing conclusion.

In this study, a new type of polysaccharide (FFEP-1) was obtained through deep fermentation of *F. Fomentarius* and column separation using Sephadex G-100. Chemical structure of FFEP-1 is drawn through monosaccharide composition and methylation method, FT-IR and 1D, 2D NMR. Studies on anti-anoxic activity of FFEP-1 indicated that FFEP-1 could effectively prolong the survival time of hypoxic mice induced by oxygen exhaustion, sodium nitrite poisoning or acute cerebral ischemia. This work proposed a theoretical basis for the development of the *F. Fomentarius* resources.

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Disclosure of conflict of interest

None.

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