Structural Characterization of Polysaccharides Isolated from *Panax notoginseng* Medicinal Residue and Its Protective Effect on Myelosuppression Induced by Cyclophosphamide

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This study aims to establish the isolation and purification method of polysaccharides from medicinal residue of *Panax notoginseng* (PPN). The structure and protective effect of PPN on myelosuppression mice were investigated. One neutral polysaccharide (NPPN) and five acidic polysaccharides (APPN I, APPN II-A, APPN II-B, APPN III-A, and APPN III-B) were obtained. The results confirmed that NPPN, APPN I and APPN II-A are glycan with 1, 4 main chains. APPN III-A is a glycan. APPN II-B and APPN III-B are homogalacturonan pectin with 1, 4 main chains. This study demonstrated that NPPN played a bone marrow protective role in myelosuppression mice induced by cyclophosphamide. NPPN could relieve cell cycle arrest, reduce the apoptosis rate of marrow cells, and improve granulocyte-macrophage colony-stimulating (GM-CSF), thermoplastic polyolefin (TPO) and erythropoietin (EPO) serum level, which contributes to promoting the proliferation of hematopoietic cells.

**Keywords:** polysaccharide, *Panax notoginseng*, purification, structural characterization, myelosuppression.

**Introduction**

*Panax notoginseng* (Burk) F. H. Chen is a valuable Chinese traditional medicinal plant, which belongs to the Panax genus under the Araliaceae family. *Panax notoginseng* has a long history in Chinese traditional medicine. It has good blood tonic function. The pharmacological action is to promote blood circulation to remove blood stasis. Its active ingredients include saponin, polysaccharides, flavonoids, etc. Polysaccharide is one of the major components of *Panax notoginseng*. According to research findings, PPN has many pharmacological activities, for instance, antitumor, antioxidant, hepatoprotective effect, and immunomodulatory activity. At present, *Panax notoginseng* has been used in extracting saponins. The production process would create large numbers of *Panax notoginseng* medicinal residue. According to statistics, 30–50 tons of *Panax notoginseng* polysaccharides are discarded with unemployed medicine residue every year. Based on previous, we obtained the polysaccharide from *Panax notoginseng* medicinal residue through water extraction followed by alcohol precipitation method. However, the purity of the polysaccharide was low, and the structure was not determined.

At present, systematic research of the structure of PPN is few. Liu et al. was only studied a novel polysaccharide from the 0.3 M NaCl eluting compound. The preliminary characterization of 0.2 M
NaCl eluting fractions was determined by Feng et al.\textsuperscript{[2]} Meanwhile, a study from Wang et al. was persistent only to the acidic polysaccharide.\textsuperscript{[1]} The other structure research about NPPN was not a system.\textsuperscript{[7]} As a result, those studies contain very limited information on the detailed structures of PPN and hinder the utilization of Panax notoginseng. Hence, to better utilize Panax notoginseng resources, we extracted and further purified polysaccharides from the medicinal residue, and analyzed the structural features of each polysaccharide component. We hope to provide evidence for further study on the structure-activity relationship and provide ingredients for functional food.

The malignant tumor is one of the most severe diseases threatening human life. Chemotherapy is the primary treatment for cancer, but most chemotherapeutic drugs can lead to blood cells reduction, bone marrow suppression, immunosuppression, etc.\textsuperscript{[8]} Myelosuppression is the most common dose-limiting side effect that results treatment termination.\textsuperscript{[9]} This seriously affected the therapeutic effect, even dying at last. The current treatments for myelosuppression are medicines (recombinant human granulocyte stimulating factor) and transfusion. However, because of the severe side-effects and high cost, their clinical application is very limited. Previous research indicated that many polysaccharides have a good protecting and treating effect on chemotherapeutic drug caused bone marrow suppression. Salidroside could inhibit bone marrow cell apoptosis and accelerate the cell cycle in bone marrow depressed mice.\textsuperscript{[10]} Basilicum polysaccharides could promote hematopoietic cell proliferation.\textsuperscript{[11]} Astragalus polysaccharides have a preventive effect on myelosuppression after chemotherapy.\textsuperscript{[12,13]} At the same time, our laboratory found that neutral polysaccharides from Panax notoginseng could increase the number of red blood cells, white blood cells, and lymphocytes in bone marrow suppressed mice induced by cyclophosphamide (CTX), and enhance cellular and humoral immunity. These results suggest that Panax notoginseng polysaccharide had a protective effect on bone marrow.\textsuperscript{[6]} However, there was no study on the therapeutic effect of Panax notoginseng polysaccharide in myelosuppression mice induced by CTX.

In addition, we found the bone marrow protection effect and explored the working mechanism of Panax notoginseng polysaccharide in this study. Therefore, we hope to provide evidence for the therapeutic effect of Panax notoginseng polysaccharide in myelosuppression and the material basis and pharmacodynamics study of blood replenishing by Panax notoginseng.

**Results and Discussion**

**Purification of Polysaccharides**

We purified crude polysaccharide Panax notoginseng (CPPN) using a DEAE Sepharose Fast Flow anion-exchange chromatography column for stepwise elution with distilled water and 0.1, 0.2, 0.3 M NaCl solutions. Four fractions (NPPN, APPN I, APPN II, APPN III) were detected and collected based on the elution curve (Figure 1a). We purified the APPN II on a Sephadex G-75 column to obtain APPN II-A and APPN II-B (Figure 1b). Similarly, the eluates from the 0.3 M NaCl fractions (APPN III) were further purified on a Sephadex G-100 column to successfully obtain APPN III-A and APPN III-B (Figure 1c). The yields of the NPPN, APPN I, APPN II-A, APPN II-B, APPN III-A, and APPN III-B are shown in Table 1.

**Structural Characterization of Polysaccharides**

**Determination of Total Sugar Content and Molecular Weight**

The results of total sugar content determination and molecular weight are shown in Table 1. NPPN contained a high neutral sugar content (96.40%). The average molecular weight (Mw) of NPPN was 232.50 kDa. APPN I contained a high neutral sugar content (80.87%). The average molecular weight (Mw) of APPN I was 489.90 kDa. APPN II-A contained a high neutral sugar content (70.46%). The average molecular weight (Mw) of APPN II-A was 450.10 kDa. APPN II-B contained a high neutral sugar content (39.86%). The average molecular weight (Mw) of APPN II-B was 336.10 kDa. APPN III-A contained a high neutral sugar content (20.17%). The average molecular weight (Mw) of APPN III-A was 363.10 kDa. APPN III-B contained a high neutral sugar content (30.69%). The average molecular weight (Mw) of APPN III-B was 56.28 kDa. The yield rate, neutral sugar, uronic acid, carbohydrate, molecular weight, and D (Mw/Mn) are shown in Table 1.

| Sample   | Yield rate (%) | Neutral sugar (%) | Uronic acid (%) | Carbohydrate (%) | Mw (kDa)   | Mn (kDa) | D (Mw/Mn) |
|----------|----------------|-------------------|----------------|------------------|------------|----------|-----------|
| NPPN     | 5.72           | 96.40             | –              | 96.40            | 232.50     | 29.12    | 7.98      |
| APPN I   | 3.89           | 80.87             | 15.02          | 95.89            | 489.90     | 19.72    | 24.85     |
| APPN II-A| 27.20          | 70.46             | 15.02          | 90.63            | 450.10     | 91.34    | 4.93      |
| APPN II-B| 58.80          | 6.28              | 85.34          | 91.62            | 363.10     | 12.51    | 2.29      |
| APPN III-A| 15.50        | 39.86             | 30.69          | 70.56            | 336.10     | 89.43    | 3.76      |
| APPN III-B| 73.40         | 5.89              | 77.07          | 82.96            | 56.28      | 25.11    | 2.24      |

Notes: Carbohydrate (%) = neutral sugar (%) + uronic acid (%).
content (80.87\%) and low uronic acid (15.02\%). The high-performance gel permeation chromatography (HPGPC) chromatograph of APPN–I (Figure 2B) presented multiple peaks with a $M_w$ of 489.90 kDa, indicating APPN I was a heteropolysaccharide. The neutral sugar and uronic acid of APPN II-A were 70.46\% and 20.17\%. APPN II-B contained low neutral sugar (6.28\%) and high uronic acid content (85.34\%). The $M_w$ of APPN II-A and APPN II-B were 450.10 and 28.60 kDa, respectively. APPN III-A and APPN III-B were obtained by gel chromatography. The molecular weight and carbohydrate profiles of APPN II-A and APPN III-B were similar to APPN II-A and APPN II-B. The neutral sugar and uronic acid of APPN III-A were 39.86\% and 30.69\%, respectively. APPN III-B contained low neutral sugar content (5.89\%) and high uronic acid content (77.07\%). The molecular weight of APPN III-A and APPN III-B were 336.10 and 56.28 kDa, respectively. The HPGPC chromatograph of NPPN, APPN II-A, APPN II-B, APPN III-A and APPN III-B (Figure 2A, C, D, E, F) showed single peaks, demonstrating that those were homogeneous polysaccharides.

Monosaccharide Composition

The results of monosaccharide compositions are given in Figure 3. The monosaccharide compositions of PPN were identified by retention times of reference stand-
ards. NPPN was composed of Ara, Gal, Glc, and Man, and the molar ratio was 3.76:18.58:76.85:0.80. According to the monosaccharide composition, NPPN was classified as a neutral polysaccharide, and principally formed by Glc. The result is consistent with previous literatures.\textsuperscript{13,14} APPN I was composed of Ara, Gal, Glc, Man, GalA and GlcA, and the molar ratio was 11.47:34.82:43.48:2.28:5.66:2.29. APPN II-A was composed of Ara, Gal, Glc, GalA and GlcA, and the molar ratio was 11.04:39.59:39.80:7.03:2.54. In comparison, APPN II-B was composed of Ara, Gal, Glc, and GalA with a molar ratio of 1.49:1.64:2.50:94.36. APPN III-A

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**Figure 2.** HPGPC chromatography of PPN fractions. The molecular weight and purity of NPPN (A), APPN I (B), APPN II-A (C), APPN II-B (D), APPN III-A (E) and APPN III-B (F) were determined by HPGPC.
was composed of Fuc, Ara, Gal, Glc, Xyl, Man, GalA and GlcA, and the molar ratio was 1.61:9.45:39.25:16.61:1.11:1.74:26.66:3.57. APPN III-B was composed of Ara, Gal, Glc and GalA, and the molar ratio was 1.22:1.52:2.90:94.36. Experimental results showed that APPN I, APPN II-A, APPN II-B, APPN III-A and APPN III-B were acidic polysaccharides. The monosaccharide compositions of APPN III-B is

Figure 3. Structural characterization of PPN. (a) The HPAEC-PAD chromatograms of PPN fractions. (A) The chromatograms of mixed standard monosaccharides (Note: 1. Fuc 2. Ara 3. Gal 4. Glc 5. Xyl 6. Man 7. Fru 8. Rib 9. GalA 10. GlcA 11. ManA). The monosaccharide composition of NPPN (B), APPN I (C), APPN II-A (D), APPN II-B (E), APPN III-A (F) and APPN III-B (G) have been ascertained. (b) ATR-FT-IR spectra of NPPN (A), APPN I (B), APPN II-A (C), APPN II-B (D), APPN III-A (E) and APPN III-B (F).
different from previous study because of different extraction and purification methods.[4] The monosaccharide compositions of APPN II-B are similar to APPN III-B, but more detailed structure of those fractions needs further investigation. Furthermore, these results suggest that APPN II-A and APPN III-B are probably pectins because of particularly rich in GlcA.

Methylation Analysis

We used methylation analysis to obtain bond structure information of each polysaccharides fraction. We found that NPPN contained seven linkages: T-Linked Glcp (14.05%), 1,4-Linked Manp (1.17%), 1,6-Linked Glcp (2.81%), 1,4-Linked Glcp (70.22%), 1,6-Linked Galp (3.54%), 1,3,4-Linked Galp (1.57%), 1,4,6-Linked Galp (6.63%, Table 2). To sum up, the backbone of NPPN was comprised of 1, 4-Glcp.

Methylation analysis showed that APPN I was composed of T-Linked Araf (0.94%), T-Linked Glcp (7.08%), T-Linked Galp (2.59%), 1,3-Linked Galp (3.05%), 1,4-Linked Glcp (64.30%), 1,6-Linked Galp (7.28%), 1,4,6-Linked Glcp (4.87%), 1,3,6-Linked Galp (6.20%), T-Linked GlcAp (0.79%, Table 3). Having said all of the above, the backbone of APPN I was made up of 1,4-Glcp.

APPN II-A was made up of T-Linked Araf (2.23%), T-Linked Glcp (5.68%), T-Linked Galp (5.79%), 1,3-Linked Galp (3.35%), 1,4,Linked Glcp (34.51%), 1,6-Linked Galp (7.05%), 1,3,4-Linked Glcp (3.50%), 1,4,6-Linked Glcp (3.65%), 1,3,6-Linked Galp (8.52%). Moreover, the chain connected at T-Linked GlcAp (3.88%), 1, 4-Linked GlcAp (5.35%, Table 4).

According to the result of methylation analysis, APPN II-B consisted of T-Linked GalAp (8.31%), 1,4-Linked GalAp (83.80%), 1,3,4-Linked GalAp (2.09%), 1,4,6-Linked GalAp (2.46%), 1,4-Linked Glcp (3.35%). This result suggested that APPN II-B was a homogalacturonan (HG) pectin.[15,16]

As shown in Table 6, APPN III-B contained four linkages: T-Linked GalAp (4.95%), 1,4-Linked GalAp (82.32%), 1,3,4-Linked GalAp (3.71%), 1,4-Linked Glcp (3.35%). This result suggested that APPN III-B was an HG pectin, too.[15,16]

Infrared Spectrum Analysis

The attenuated total reflection flourier transformed infrared spectroscopy (ATR-FT-IR) spectrum of NPPN, APPN I, APPN II-A, APPN II-B, APPN III-A and APPN III-B were demonstrated in Figure 3.

Table 2. Methylation analysis of NPPN.

| Retention time (min) | PMAAs | Linkage types | Relative molar ration (%) | m/z |
|----------------------|-------|---------------|---------------------------|-----|
| 9.71                 | 2,3,4,6-Me4-Glcp | Glcp-(1→) | 14.05 | 59, 71, 102, 118, 129, 145, 162, 205 |
| 13.85                | 2,3,6-Me3-Manp   | →4)-Manp-(1→) | 1.17 | 57, 71, 102, 118, 129, 142, 162, 173, 233 |
| 16.65                | 2,3,4-Me3-Glcp   | →6)-Glcp-(1→) | 2.81 | 59, 71, 87, 99, 118, 129, 173 |
| 15.03                | 2,3,6-Me3-Glcp   | →4)-Glcp-(1→) | 70.22 | 59, 71, 87, 99, 118, 129, 142, 159, 173 |
| 16.41                | 2,3,4-Me3-Galp   | →6)-Galp-(1→) | 3.54 | 59, 71, 87, 99, 118, 129, 143, 159, 173 |
| 17.19                | 2,6-Me2-Galp     | →3,4)-Galp-(1→) | 1.57 | 59, 71, 87, 99, 118, 129, 143, 159, 185 |
| 19.29                | 2,3-Me2-Galp     | →4,6)-Galp-(1→) | 6.63 | 59, 85, 102, 118, 127, 142, 159, 187, 201, 261 |

Table 3. Methylation analysis of APPN I.

| Retention time (min) | PMAAs | Linkage types | Relative molar ration(%) | m/z |
|----------------------|-------|---------------|--------------------------|-----|
| 6.31                 | 2,3,5-Me3-Araf | Araf-(1→) | 0.49 | 57, 71, 87, 102, 118, 129, 145 |
| 9.71                 | 2,3,4,6-Me2-Glcp | Glcp-(1→) | 7.08 | 59, 71, 87, 102, 118, 129, 145, 162, 205 |
| 9.71                 | 2,3,4,6-Me2-GlcAp | GlcAp-(1→) | 0.79 | 59, 71, 87, 102, 118, 129, 145, 162, 205 |
| 10.75                | 2,3,4,6-Me2-Galp | Galp-(1→) | 2.59 | 71, 87, 101, 118, 129, 161, 174, 234 |
| 13.77                | 2,4,6-Me2-Galp   | →3)-Galp-(1→) | 3.05 | 71, 87, 102, 118, 129, 162, 189, 233 |
| 14.65                | 2,3,6-Me2-Galp   | →4)-GalAp-(1→) | 3.35 | 71, 87, 102, 118, 129, 162, 233 |
| 15.06                | 2,3,6-Me2-Glcp   | →4)-Glcp-(1→) | 64.30 | 71, 87, 102, 118, 129, 162, 233 |
| 16.43                | 2,3,4-Me2-Galp   | →6)-Galp-(1→) | 7.28 | 59, 71, 87, 102, 118, 129, 162, 189, 233 |
| 19.28                | 2,3-Me2-Glcp     | →4,6)-Glcp-(1→) | 4.87 | 59, 85, 102, 118, 127, 142, 201, 261 |
| 19.92                | 2,4-Me2-Galp     | →3,6)-Galp-(1→) | 6.20 | 59, 87, 101, 118, 129, 139, 189, 234, 305 |
The ATR-FT-IR spectra exhibited absorption bands at 2918, 1416, 1371, and 1234 cm\(^{-1}\), which are typical bands for carbohydrates.\(^{[17]}\) 1148, 1077 and 1015 cm\(^{-1}\) were the absorption of pyranoside. 850 cm\(^{-1}\) was attributed to C/H deformation vibration of \(\alpha\)-pyranose, which indicated NPPN was an \(\alpha\)-pyranoside. Similarly, APPN I was an \(\alpha\)-pyranoside. The weak absorption at 895 cm\(^{-1}\) originated from the C/H deformation vibration of \(\beta\)-pyranoside. The results showed that APPN II-A and APPN III-A were \(\beta\)-pyranosides. Whereas 891 and 832 cm\(^{-1}\) resulted from the absorption of \(\alpha\) and \(\beta\) pyranoside, indicating that APPN II-B and APPN III-B existed in \(\alpha\) and \(\beta\) pyranoside. A strong absorption band with a peak at 1607 cm\(^{-1}\) was attributed to the stretching of –COOH.\(^{[4]}\) According to the results of ATR-FT-IR, NPPN was a neutral polysaccharide, APPN I, APPN II-A, APPN II-B, APPN III-A and APPN III-B were acidic polysaccharides. These results are consistent with the conclusions of monosaccharide composition analysis and methylation analysis.

### Scanning Electron Microscope Analysis

A scanning electron microscope (SEM) was used to analyze the surface morphology of polysaccharides. The morphological properties of polysaccharides are shown in Figure 4. The SEM image of NPPN (A) showed a random coil and a small proportion of rod-like structure. SEM analysis showed that APPN I (B) was presented an irregular structure as a random coil and spheroidal structure. As shown in Figure 4C, APPN II-B exhibited spheroidal structure under SEM observed. APPN II-B (D) is characterized by a banded structure. The morphological properties of APPN III-A showed a

| Table 4. Methylation analysis of APPN II-A. |
|----------------|----------------|----------------|----------------|
| Retention time (min) | PMAAs | Linkage types | Relative molar ratio (%) | m/z |
|----------------|----------------|----------------|----------------|----------------|
| 6.58 | 2,3,5-Me3-Araf | Araf-(1→2) | 2.23 | 57, 71, 87, 102, 118, 129, 145 |
| 9.71 | 2,3,4,6-Me4-Glc | Glc-(1→3) | 5.68 | 59, 71, 87, 102, 118, 129, 145, 162, 205 |
| 9.71 | 2,3,4,6-Me4-Glc | GlcAp-(1→6) | 3.88 | 59, 71, 87, 102, 118, 129, 145, 162, 205 |
| 10.75 | 2,3,4,6-Me4-Galp | Galp-(1→2) | 5.79 | 59, 71, 87, 102, 118, 129, 145, 174, 205 |
| 13.77 | 2,4,6-Me3-Galp | Gal-(1→3) | 5.31 | 59, 74, 87, 101, 118, 129, 143, 161, 174, 202, 217, 234 |
| 14.66 | 2,3,6-Me3-Galp | Gal-(1→4) | 14.53 | 59, 71, 87, 118, 131, 142, 173, 189, 203 |
| 14.66 | 2,3,6-Me3-Galp | GalAp-(1→4) | 5.35 | 59, 71, 87, 118, 131, 142, 173, 189, 203 |
| 15.00 | 2,3,6-Me3-Glcp | Glcp-(1→4) | 34.51 | 59, 71, 87, 118, 129, 143, 159, 173, 189 |
| 16.41 | 2,3,4-Me3-Galp | Glcp-(1→6) | 7.05 | 59, 71, 87, 99, 118, 129, 143, 159, 173, 189 |
| 17.19 | 2,3,6-Me2-Galp | Glp-(1→4) | 5.09 | 59, 71, 102, 118, 129, 159, 201, 231, 261 |
| 19.28 | 2,3-Me2-Galp | Glcp-(1→4,6) | 3.65 | 59, 87,118, 129, 160, 189, 234 |
| 19.91 | 2,4-Me2-Galp | Glcp-(1→3,6) | 8.52 | 59, 87, 118, 129, 160, 185, 203, 231, 305 |

| Table 5. Methylation analysis of APPN II-B. |
|----------------|----------------|----------------|----------------|
| Retention time (min) | PMAAs | Linkage types | Relative molar ratio (%) | m/z |
|----------------|----------------|----------------|----------------|----------------|
| 10.75 | 2,3,4,6-Me3-Galp | GalAp-(1→4) | 8.31 | 57, 73, 88, 102, 118, 131, 147, 163, 207 |
| 14.73 | 2,3,6-Me2-Galp | GalAp-(1→4) | 83.80 | 59, 87, 102, 118, 129, 162, 175, 235 |
| 14.98 | 2,3,4,6-Me3-Glc | Glc-(1→4) | 3.35 | 59, 71, 87, 102, 118, 129, 162, 173, 233 |
| 16.70 | 2,3,4-Me2-Galp | GlcAp-(1→4) | 2.09 | 71, 85, 99, 118, 131, 186, 218, 307 |
| 19.70 | 2,3,4-Me2-Galp | GlcAp-(1→4,6) | 2.46 | 71, 87, 102, 118, 129, 162, 203, 263 |

| Table 6. Methylation analysis of APPN III-B. |
|----------------|----------------|----------------|----------------|
| Retention time (min) | PMAAs | Linkage types | Relative molar ratio (%) | m/z |
|----------------|----------------|----------------|----------------|----------------|
| 10.71 | 2,3,4,6-Me4-Galp | GalAp-(1→4) | 4.95 | 60, 73, 89, 102, 118, 131, 147, 163, 207 |
| 14.65 | 2,3,6-Me3-Galp | GalAp-(1→4) | 82.32 | 59, 87, 102, 118, 129, 162, 175, 235 |
| 14.94 | 2,3,6-Me3-Glcp | Glcp-(1→4) | 8.46 | 71, 87, 102, 118, 129, 162, 233 |
| 16.67 | 2,3,4-Me2-Galp | Glcp-(1→3,4) | 3.71 | 60, 71, 118, 131, 186, 232, 307, 338 |
catenulate and spherical chain with rough and uneven surfaces. APPN III-B (F) presented lamella sheets with a smooth surface.

**Figure 4.** Photomicrographs of NPPN (A, magnified 700 times), APPN I (B, magnified 800 times), APPN II-A (C, magnified 800 times), APPN II-B (D, magnified 800 times), APPN III-A (E, magnified 800 times), APPN III-B (F, magnified 800 times).

**Bone Marrow Protection of NPPN**

*Effect of NPPN on hematological Parameters on Myelosuppression Mice Induced by Cyclophosphamide*

In order to investigate the effect of NPPN on peripheral blood count, red blood cells (RBCs), hemoglobin concentration (Hb), packed cell volume (PCV), platelet (PLT), white blood cells (WBCs), lymphocyte
(LYM), and neutrophils were counted. The results of blood routine tests are shown in Figure 5. The number of RBC, Hb, PCV, PLT, WBC, LYM and neutrophil in the model group decreased significantly. Experimental results indicated that CTX injured hemopoiesis, and the myelosuppression model was established successfully. Compared with the model group, WBC and Hb counts of the high dosage NPPN group were increased, and PCV were elevated in all NPPN dosage group. The number of PLT in medium and high dosage NPPN groups was higher than that of the model group. Furthermore, WBC and neutrophil numbers in NPPN high dosage group were increased. The number of neutrophils in the high-dose NPPN group had increased to the same level as the normal group. In treatments with medium dosage NPPN, the lymphocyte number was increased. There was no significant difference between the high dosage NPPN group and the rh G-CSF group in the WBC number and Hb. The improvement effect of PLT counts in NPPN-M dosage was remarkable compared with Diyushengbai tablet group. The number of WBC in the high dosage NPPN group had no significant difference than that of in the Diyushengbai tablet group. Meanwhile, there was no significant differences in LYM number between the NPPN-M group and the rh G-CSF group. The results suggested that NPPN can accelerate recovery of the hematopoietic function in mice.

Effect of NPPN on Cell Cycle and Apoptosis Rate on Myelosuppression Mice Induced by Cyclophosphamide

Like other cytotoxic drugs, CTX can induce cell cycle arrest and apoptosis. We injected CTX into mice to establish a myelosuppression model and observed the effects of NPPN on bone marrow cell proliferation (Figure 6). Compared with the normal group, the apoptosis rate was significantly higher in the model group. The above results indicated that the myelosuppression mice model was established. After being treated with NPPN, the apoptosis rates in all NPPN treatment group were decreased. The effect in the medium dosage NPPN group is better than that in the diyushengbai tablet group, and no significant difference than that of in rh G-CSF group.

Flow cytometry revealed that cyclophosphamide induced S-phase arrest in bone marrow cells. After the NPPN treatments, the S-phase fraction of the cell was decreased compared with the model group. However, there was no significant difference in regulating cycle progression between all NPPN and the rh G-CSF groups. Furthermore, the effect with low dosage NPPN is better than the diyushengbai tablet group. Those results suggested that NPPN can change the cell cycle distribution, induce cell cycle progression from S into the G2/M phase and promote bone marrow cell proliferation.

Effect of NPPN on Hematopoietic Growth Factor on Myelosuppression Mice Induced by Cyclophosphamide

Hematopoietic growth factors are important cytokines, which play a major role in blood cell building and regulation. To explore the effect of NPPN on hematopoietic growth factor, the serum level of GM-CSF, TPO and EPO were measured by ELISA. As shown in Figure 6, the levels of GM-CSF, TPO and EPO of mice in the normal group can maintain at a certain range. After CTX administration, three hematopoietic growth factors were elevated. The serum levels of GM-CSF in all three dosages of NPPN were increased compared to the model group. There was no significant difference in the GM-CSF concentration among high dosage NPPN group mice and the diyushengbai tablet group mice. The serum TPO level was significantly increased in the medium and high NPPN group compared to the model group. Compared to the model group, all three dosages NPPN could elevate EPO concentration in various degrees. The serum TPO and EPO levels showed no significant difference between the high dosage NPPN and the rh G-CSF groups. Meanwhile, the EPO levels between medium and high dosage NPPN group and diyushengbai tablet groups were not significantly different. Our preliminary results suggest that NPPN promote differentiation and maturation of hematopoietic stem cells.

Discussion

In this study, CPPN was purified by the DEAE Sepharose Fast Flow anion exchange chromatography with gradient elution consisting of 0, 0.1, 0.2 and 0.3 M NaCl solution. One neutral polysaccharide and three acidic polysaccharides were obtained. Then, APPN II and APPN III were further purified by different cross-linking degrees of polydextran gel. The results showed that the elution curves of NPPN and APPN I were a single symmetrical peak, which indicated a homogeneous component of these two fractions.

Polysaccharide is a class of macromolecular compounds with complex structure. Molecular weight and distribution are important to polysaccharide structural research. The purified product of polysaccharides is a
Figure 5. Effect of NPPN on hematological indexes in mice with myelosuppression (\(\pm s, n=5\)) WBC (A), LYM (B), neutrophil (C), RBC (D), Hb (E), PLT (F) as well as PCV (G) were determined by automatic hematology analyzer. *\(P < 0.05\), **\(P < 0.01\), vs. CTX, *\(P < 0.05\), **\(P < 0.01\), vs. Diyushengbai tablet, \#\#\#\(P < 0.001\), vs. rh G-CSF.
Figure 6. Bone marrow protection of NPPN (± s, n = 5). (a) Effect of NPPN on apoptosis rate and cell cycle in mice with myelosuppression. Apoptosis rate (A) and cell cycle (B) of bone marrow cells were determined by flow cytometry according to the kit maintain instruments. (b) Effect of NPPN on serum level of GM-CSF, TPO and EPO in mice with myelosuppression. The serum level of GM-CSF (A), TPO (B) and EPO (C) were determined by enzyme-linked double antibody sandwich method. *P < 0.05, **P < 0.01, vs. CTX, *P < 0.05, **P < 0.01, vs. Diyushengbai tablet, ∆P < 0.05, ∆∆P < 0.01, vs. rh G-CSF.
homogeneous component with certain molecular weight distribution. Therefore, the polydispersity of polysaccharides could measure by HPGPC. The polysaccharide was seen as a homogeneous polysaccharide if chromatogram was presented as a single symmetrical peak.

The purity of the PPN was determined by the gel filtration chromatography. The APPN I elution curve from Sephadex gel represented a single peak, but the result was not consistent with the results of HPGPC. It is caused by three reasons. Firstly, the results of HPGPC are closely related to the standard substance. In the study, there is a different character between the standard substance and samples. It always leads to results inconsistent with the fact. APPN I potentially appeared at different retention times because of the complicated structure. Besides, the mobile phase has a great influence on HPGPC chromatography.\[18\] The results showed that APPN I had different aggregation states in 0.1 M NaCl solution, and ultimately led to had a difference in retention time. At last, APPN I is still a heteropolysaccharide that includes two or more components. The standard gel filtration chromatography has low efficiency, so that it is difficult to separate polysaccharides. Probably an unsuited gel filtration chromatography pack was chosen. The appropriate purification and purity identification methods of APPN I need further development.

Polysaccharides are divided into acidic polysaccharides and neutral polysaccharides according to the characteristic group. Acidic polysaccharides include heparin, chondroitin, pectin, carrageenan, etc. Pectin is particularly rich in galacturonic acid that occurs on a wide range of plant cell walls. The structure of pectin frequently consists of four domains: homogalacturonan (HG), rhamnogalactan-I (RG–I), rhamnogalactan-II (RG-II) and arabinogalactan (AG). Those domains are combined by covalent bond or noncovalent bond.\[15\] HG consists of Gal-AC linked by α-1, 4 glucosidic bonds. The backbone of RG–I was composed of Rha and GalA that linked by -2Î±-L-Rhap(1,4)Î±-D-GalAp-(1-glucosidic bond. The side chain of Rha is composed of galactosan, arban and arabinogalactan.\[19\] The backbone of RG-II was composed of GalA that is linked by α-1,4 glucosidic bond. The molecular weight of RG-II is relatively small (3–12 kDa).\[16\] AG has two domains: AG-I and AG-II. The skeletal structure of AG-I is formed in β-1,4-Galp glucosidic bond. AG-II was linked by β-1,6-Galp glucosidic bond.\[20\]

The results of monosaccharide composition analysis showed that APPN II-B and APPN III-B contain large amounts of GalA. In the meantime, methylation analysis results indicated that GalA was linked by1,4 glucosidic bond. All these showed that APPN II-B and APPN III-B could be HG pectin. The types of monosaccharides and glucosidic bond of APPN II-B were very similar to APPN III-B. However, the molecular weights and morphological properties of the above two polysaccharides were different. Thus, our preliminary conclusion is that APPN II-B and APPN III-B were different degrees of polymerization of pectin. The fine structure differences between APPN II-B and APPN III-B require further investigation.

The findings showed that the structure of APPN I and APPN II-A were complex, and detailed structures need to be further elucidated.

Methylation analysis is widely employed in the study of structural analysis of polysaccharides but also has insufficiency. As a result, the accuracy of pentose methylation analysis is low. Take arabinose as an example. Eight bonding ways are known. However, the database recorded only three bonding ways. In addition, PMAAs from pentose have a low response value in GC/MS. If the percentages of those parts are low that it is difficult to be accurately detected.

The carbodiimide-sodium borohydride method was applied in uronic acid reduction. However, the application of the methylation method in APPN III-A structure analysis was unfeasible. When DMSO was added into the tube after APPN III-A reduction, a white precipitate was generated. The precipitate was difficult to disperse and dissolve. Hence, the methylation analysis of APPN III-A could not get it done. Through analysis, we believe that the existence of sulfate, protein and amidogen in APPN III-A has a great impact on the process of uronic acid reduction. The carbodiimide-sodium borohydride method only applies to uronic acid reduction. Therefore, those factors may lead to reaction fails. Furthermore, we suspect that APPN III-A maybe includes an unknown group. We cannot use current methods to analyze the unknown polysaccharides. Therefore, this research did not analyze the elaboration structure of APPN III-A. Other techniques will be applied to analyze the APPN III-A structure in the future.

Though we got the monosaccharide composition, bonding structure and anomeric carbon types, the information of linked locus, branches location and numbers, constitutional repeating units and spatial configuration of PPN need further exploration.

CTX is a chemotherapeutic drug used in tumor treatment. The side effects include bone marrow suppression, immunosuppression and gastrointestinal reaction, threatening a patient’s life.\[21\] Some studies
found that CTX induces cell apoptosis by Fas/FasL pathway.[22] Meanwhile, CTX can activate ATM/ATR-Chk1/Chk2 pathway and induce cell cycle arrest at S-phase.[23,24] In the present study, we showed that cyclophosphamide could induce cell cycle arrest at the S-phase, which is consistent with previous studies. NPPN significantly decreased bone marrow cell apoptosis rate and resumed cell cycle progression. It is possible that NPPN could protect bone marrow cells from toxic cyclophosphamide metabolites injury and promote cell proliferation.

Hematopoietic stem cells are precursor cells that normally go on to produce blood cells. Hematopoietic growth factors are an important part of cytokines, which play an important role in the mechanism of blood building and regulation. Hematopoietic growth factors could regulate hematopoietic cell survival, proliferation and differentiation. There are many kinds of hematopoietic growth factors that play different roles in hemopoietic systems, such as GM-CSF, EPO, TPO, IL-3, IL-6, etc.[25] In this study, we found that the levels of GM-CSF, TPO, and EPO were improved in different degrees after NPPN treatment. The hematological parameters results showed that NPPN could increase the WBC, LYM, neutrophil, RBC, Hb, PLT and PCV counts in the peripheral blood. These results indicated that NPPN could promote the differentiation and maturation of hematopoietic stem cells.

Conclusion

In summary, CPPN was further isolated and purified to obtain one neutral polysaccharide and five acidic polysaccharides named NPPN, APPN I, APPN II-A, APPN II-B, APPN III-A and APPN III-B. NPPN (Mw: 232.50 kDa) was comprised of Ara, Gal, Glc, and Man. The main chain of NPPN was α-1, 4-Glcp glycosidic linkage. NPPN existed in random chain and rod-like. APPN I (Mw: 489.90 kDa) was comprised of Ara, Gal, Glc, Man, GalA, and GlcA. APPN I’s main chain was formed by α-1, 4-Glcp glycosidic linkage. APPN I existed as a random chain and spherical. APPN II-A (Mw: 450.10 kDa) was comprised of Ara, Gal, Glc, GalA, and GlcA. APPN II-A’s main chain was formed by β-1, 4-Glcp glycosidic linkage. APPN II-A showed spheroidal structure. APPN II-B (Mw: 28.60 kDa) was comprised of Ara, Gal, Glc, and GalA. APPN II-B was an HG pectin. APPN II-B’s main chain was formed by 1, 4-GalAp glycosidic linkage. APPN II-B existed as a banded chain. APPN III-A (Mw: 336.10 kDa) was comprised of Fru, Ara, Gal, Glc, Xyl, Man, GalA, and GlcA. APPN III-A was linked by β pyranoside. Additionally, APPN III-A existed as a catenulate and spherical chain with rough and uneven surfaces. APPN III-B (Mw 56.28 kDa) was comprised of Ara, Gal, Glc, and GalA. APPN III-B was a HG pectin. APPN III-B’s main chain was formed by α and β pyranoside. APPN III-B existed as a lamellate chain.

In the current study, we have demonstrated that NPPN could promote the recovery of hematopoietic function in CTX-induced myelosuppression mice through increasing peripheral blood counts, relieving cell cycle arrest, decreasing the apoptosis rate of marrow bone cells, improving the serum level of GM-CSF, TPO and EPO, promoting bone marrow cells differentiation and maturation.

Experimental Section

Experimental Animals

Female Kunming mice (8 weeks old, 18–22 g) were purchased from the Laboratory Animal Center of Kunming Medical University (Kunming, China; the license number: SYXX (Dian) K2020-0006). Mice were housed in a 12 h light-dark cycle and with 20±4 °C. All animals were given two weeks to adapt to the new environment. And then, mice without abnormal were used as the experimental subjects. The qualification of the major investigator was approved by Yunnan Provincial Science and Technology Department (No. LA2018221). All animal procedures in this study were according to the General Requirements for Animal Experiment and approved by the Ethics Committee of Kunming Medical University (ethical permission number: kmmu20211572).

Purification of Polysaccharides

The extraction of CPPN from Panax notoginseng medicinal residue has been described previously.[3,6,26] CPPN was dissolved in distilled water and isolated by a DEAE Sepharose Fast Flow anion-exchange chromatography column (GE Healthcare, Little Chalfont, UK, 2.4 cm × 50 cm) for gradient elution with distilled water and 0.1, 0.2 and 0.3 M NaCl solutions. The effluent was collected (5 mL/tube). The elution curve was proposed according to the results of the anthrone-sulfuric acid method. One neutral polysaccharide and three acidic polysaccharides were obtained and named NPPN, APPN I, APPN II, APPN III, respectively. APPN II was further separated by Sephadex G-75 column (Solarbio, Beijing, China, 1.6 cm × 100 cm) with distilled water to obtain two polysacchar-
ide fractions (APPN II-A and APPN II-B). APPN III was further purified by Sephadex G-100 column (Soralbio, Beijing, China, 1.6 cm×100 cm) with distilled water. Two purified polysaccharide factions (APPN III-A and APPN III-B) was obtained.

**Structural Characterization of Polysaccharides**

**Determination of Total Sugar Content**

The anthrone-sulfuric acid method was used to measure the neutral sugar contents.[7,27] The m-hydroxy-diphenyl method was applied to analyze the uronic acid contents of PPN.[28,29]

**Determination of the Molecular Weight**

The molecular weight was measured by high-performance gel permeation chromatography (HPGPC) with an Agilent 1260 HPLC system (Agilent Technologies, Santa Clara, CA, USA), including Shodex Ohpak SB-805-HQ column (Shodex, Japan), and a refractive index detector. The mobile phase was 0.1 mol L−1 NaCl solution at a flow rate of 0.5 mL min−1. The Column temperature and detector temperature were 35 °C.

Dextran standards were used to build a standard curve. We injected 20 μL every polysaccharide fraction into the HPGPC at 10 mg mL−1, and then analyzed the weight average molecular weight, number molecular weight and coefficient of dispersion (D, D = Mw/Mn).

**Monosaccharide Composition Analysis**

The monosaccharide composition of PPN was determined by high-performance anion exchange chromatography-pulsed amperometric detector (HPAEC-PAD).[30] Each polysaccharide sample (5 mg) was hydrolyzed in 1 mL of 2 M TFA at 121 °C for 2 h. The hydrolyzed products were dried under nitrogen, and then we washed the product with methanol. The above process was repeated three times. Dried samples were dissolved in distilled water, and the monosaccharide composition was determined by ICS 5000 ion chromatography (Thermo Fisher Scientific, Waltham, MA, USA) using a Dionex™ CarboPac™ PA20 column (3.0×150 mm). We used Glc, Fru, Ara, Fuc, Gal, Man, Rib, Xyl, GlcA, GaIA and ManA (Sigma-Aldrich, St. Louis, MO, USA) to establish a standard curve. Five μL polysaccharide fraction was injected into the ion chromatography, and then analyzed the monosaccharide composition of each sample. The chromatographic condition was as follows: mobile phase A was distilled water; mobile phase B was 100 mM NaOH; mobile phase C was 100 mM NaOH/200 mM NaAC; column temperature: 30 °C; flow rate: 1 mL min−1; analysis time: 60 min.

**Uronic Acid Reduction and Methylation Analysis**

We analyzed the bond structure of NPPN according to the Needs method.[31] We dissolved 1 mg NPPN in 500 μL DMSO, and added 50 μL 120 mg mL−1 DMSO/NaOH solution. After 30 min, 10 μL methyl iodide was added, and the mixture was reacted for 10 min. After that, we added 10 μL methyl iodide and reacted another 10 min. We finally added 10 μL methyl iodide and reacted for 60 min. We stopped this reaction by adding 500 μL dichloromethane and 1 mL distilled water and extracted the methylate with dichloromethane and distilled water. The methylate was hydrolyzed in 100 μL of 2 M TFA at 121 °C for 1.5 h. The hydrolyzed products were dried by rotary evaporators. We sequentially added 50 μL 2 M ammonium hydroxide and 50 μL 1 M sodium borodeuteride (NaBD₄) at room temperature for 2.5 h. Whereafter, we stopped this reaction by adding 20 μL acetic acid. The hydrolyzed products were dried under nitrogen, and washed the product with methanol. Added 250 μL acetic anhydride into the dried sample at 100 °C for 2.5 h. We stopped this reaction by adding 500 μL dichloromethane and 1 mL distilled water and extracted the methylate with dichloromethane and distilled water. We dialyzed the partially methylated alditol acetates (PMAAs) by 6890A-5975C gas chromatography-mass spectrometry (Agilent Technologies, Santa Clara, CA, USA) equipped with a BPX70 column. Helium gas was used as the carrier gas at a flow rate of 1 mL min−1, the split ratio was 10:1. GC of PMAAs was carried out at temperature programmed from 140 °C (2 min) to 230 °C (3 min) with a rate of 3 °C/min. Mass spectrometer conditions were: ionization mode: E1, electron energy: 70 eV, interface temperature: 230 °C, quadrupole temperature: 100 °C, mass scan range: 30–600.

The method of uronic acid reduction was conducted as described previously.[32] We dissolved 3 mg acidic polysaccharide in 1 mL distilled water and then added 200 μL 0.2 M 2-Morpholinoethanesulfonic acid and 200 μL 500 mg mL−1 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-p-toluene-sulfonate at room temperature for 2 h. The sample was divided into two parts after adding 1 mL of...
imidazole. Then, we severally added 1 mL 30 mg mL\(^{-1}\) sodium borohydride (NaBH\(_4\)), 1 mL 30 mg mL\(^{-1}\) NaBD\(_4\) for 3 h. We stopped this reaction by adding 200 \(\mu\)L of glacial acetic acid. The reaction products were dialyzed (MWCO 3.5 kD) through distilled water for 48 h and then lyophilized the solution. Gradually, we obtained methylated polysaccharides were got by hydrolysis, reduction, and acetylation as described previously. Finally, we analyzed the reaction products by GC/MS, which the condition was carried out as previously described.

**Infrared Spectrum Analysis**

We analyzed polysaccharides with an attenuated total reflection flourier transformed infrared spectroscopy (ATR-FT-IR, Thermo Fisher Scientific, Waltham, MA, USA) in the 4000 – 400 cm\(^{-1}\) vibration region, and the number of the scan was 16 times.

**Scanning Electron Microscope Analysis**

One mg sample was placed in the working stage, and then conductive gold was be covered the sample. We observed the morphological properties of polysaccharides by Scanning Electron Microscope (SEM, S300N, Hitachi production institute, Japan).

**Bone Marrow Protection of NPPN**

**Establishment and Grouping of Cyclophosphamide-Induced Myelosuppression Mice Model**

SPF Kunming mice were divided into seven groups randomly, five in each group. The seven groups were: normal group, model group, low, medium, and high doses of NPPN groups (100 mg kg\(^{-1}\), 200 mg kg\(^{-1}\), 400 mg kg\(^{-1}\)), Dijushengbai tablet group (100 mg kg\(^{-1}\), Tianfu, Chengdu, China) and recombinant human granulocyte colony-stimulating factor (rh G-CSF) injection (0.125 mg kg\(^{-1}\), Shuanglu, Beijing, China) group. The low, medium, and high doses of NPPN groups, Dijushengbai tablet group were given corresponding medicines by intragastric administration for 12 days. Saline was given to the normal group with the same method as the experimental groups. On the 6\(^{\text{th}}\) day, the other groups except the normal group were intraperitoneally injected with CTX (Baxter Oncology GmbH, Germany, 80 mg kg\(^{-1}\).d) for 5 days. On the 11\(^{\text{th}}\) day, rh G-CSF group was subcutaneously injected with rh G-CSF injection for 2 days. The mice were given free access to food for 12 h and then were killed after the last dosage.

**Determination of Hematological Indexes**

Blood was sampled from each group of mice eyes, and the count of blood cells was detected by XT-2000i automated hematology analyzer (Sysmex, Japan).

**Determination of Bone Marrow Nucleated Cells Apoptosis Rate**

Bone marrow nucleated cells were obtained from the thighbone of mice, the cells were purified by a 300 mesh nylon screen. The cells were centrifuged at 1000 r min\(^{-1}\) for 5 min. Cells were resuspended in the 1 mL PBS, counted live cells, and the number of marrow nucleated cells was adjusted to 1 \(\times\) 10\(^6\) cells/mL. The cell suspension was centrifuged at 1000 r min\(^{-1}\) for 5 min. And then, the supernatant was discarded. Cells were resuspended in the 500 \(\mu\)L 1 \(\times\) Binding Buffer, 5 \(\mu\)L Annexin V-FITC and 10 \(\mu\)L PI was added subsequently. The test tubes were incubated for 5 min at room temperature, protected from light. The apoptosis rate of bone marrow nucleated was measured by Partec flow cytometry (Partec, Germany) according to the Annexin V-FITC/PI apoptosis detection kit (MultiSciences Biotech Co., Ltd., Hangzhou, China).

**Analyses of Cell Cycle**

We obtained bone marrow nucleated cells as described earlier. The cell suspension was centrifuged at 1000 r min\(^{-1}\) for 5 min. After that, the supernatant was discarded. Then, cells were resuspended in the 1 mL PBS. The cell suspension was added into 3 mL –20°C anhydrous ethanol, and then fixed cell at –20°C for 24 h. The cell suspension was centrifuged at 1000 r min\(^{-1}\) for 5 min. The supernatant was discarded. Then, we added 500 mL PBS into the test tube. After 15 min, the supernatant was removed by centrifugation, and the precipitate was added 10 \(\mu\)L intraprep permeabilization reagent and 1 mL DNA staining solution. The test tubes were placed under the conditions of protection from light at room temperature for 30 min. The cell cycle of bone marrow nucleated was determined by flow cytometry according to the cell cycle detection kit (MultiSciences Biotech Co., Ltd., Hangzhou, China).
Determination of the Serum Level of GM-CSF, TPO, EPO

We detected the serum levels of GM-CSF, TPO, and EPO by enzyme-linked double antibody sandwich technique. We took blood samples at the inner canthus of the eye and put in the refrigerator for 2 h. Serum was collected after centrifugation at 12000 r min$^{-1}$ for 10 min. According to the manufacturer's instructions, the serum levels of GM-CSF, TPO, and EPO were examined using diagnostic kits (Shanghai Enzyme-linked Biotechnology Co., Ltd, Shanghai, China).

Statistical Analysis

All experimental data were expressed as the mean ± standard deviation. Data were analyzed by one-way analysis of variance (ANOVA). A less than 0.05 probability (P < 0.05) is considered a statistically significant difference between the two groups.

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Author Contribution Statement

YHL completed the design of the study, performed the experiments, and wrote the manuscript; TC has completed the experimental design and manuscript revision; YQZ coordinated and edited the manuscript; HW manipulated the experiment operation instruction, SL, HYQ and MDP participated in the experiment. All the authors have read and approved the final manuscript.

References

[1] S. L. Feng, H. R. Cheng, Z. Xu, S. C. Feng, M. Yuan, Y. Huang, J. Q. Liao, C. B. Ding, 'Antioxidant and anti-aging activities and structural elucidation of polysaccharides from Panax notoginseng root', Process Biochem. 2019, 78, 189 – 199.
[2] C. X. Wang, L. Y. Zheng, S. N. Liu, X. X. Guo, Y. Qu, M. J. Gao, X. M. Cui, Y. Yang, 'A novel acidic polysaccharide from the residue of Panax notoginseng and its hepatoprotective effect on alcoholic liver damage in mice', Int. J. Biol. Macromol. 2020, 149, 1084 – 1097.
[3] H. Y. Li, L. L. Gu, Y. Y. Zhong, Y. J. Chen, L. Zhang, A. R. Zhang, R. W. Sobol, T. Chen, J. F. Li, ‘Administration of polysaccharide from Panax notoginseng prolonged the survival of H22 tumor-bearing mice’, Oncotarget 2016, 9, 3433 – 3441.
[4] S. N. Liu, Y. Yang, Y. Qu, X. X. Guo, X. Y. Yang, X. M. Cui, C. X. Wang, ‘Structural characterization of a novel polysaccharide from Panax notoginseng residue and its immunomodulatory activity on bone marrow dendritic cells’, Int. J. Biol. Macromol. 2020, 161, 797 – 809.
[5] Y. Zhu, F. Pettolino, S. L. Mau, Y. C. Shen, C. F. Chen, Y. C. Kuo, A. Bacic, ‘Immunostimulatory polysaccharide-rich fractions from Panax notoginseng’, Planta Med. 2006, 72, 1193 – 1199.
[6] Y. H. Liu, H. Y. Qin, Y. Y. Zhong, S. Li, H. J. Wang, H. Wang, L. L. Chen, X. Tang, Y. L. Li, Z. Y. Qian, H. Y. Li, L. Zhang, T. Chen, ‘Neutral polysaccharide from Panax notoginseng enhanced cyclophosphamide antitumor efficacy in hepatoma H22-bearing mice’, BMC Cancer 2021, 21.
[7] W. T. Su, J. Q. Xie, X. H. Pan, J. J. Liu, J. P. Si, ‘Temporal and spatial variation of polysaccharides and alcohol-soluble extractives of Polygonatum cyrtonema’, China J. Chin. Mater. Med. 2019, 44, 270 – 273.
[8] L. Friberg, A. Henningsson, H. Maas, L. Nguyen, M. Karlsson, ‘Model of chemotherapy-induced myelosuppression with parameter consistency across drugs’, J. Clin. Oncol. 2003, 20, 4713 – 4721.
[9] R. S. Epstein, M. S. Aapro, U. K. Basu Roy, T. Salimi, J. Krenitsky, M. L. Leone-Perkins, C. Girman, C. Schlusser, Crawford, ‘Patient burden and real-world management of chemotherapy-induced myelosuppression: results from an online survey of patients with solid tumors’, Adv. Ther. 2020, 37, 3606 – 3618.
[10] X. S. Zhang, B. D. Zhu, X. Q. Huang, Y. F. Chen, ‘Effect of salidroside on bone marrow cell cycle and expression of apoptosis-related proteins in bone marrow cells of bone marrow depressed anemia mice’, J. Sichuan Univ. (Medical Sciences Edition) 2005, 06, 65 – 68, 91.
[11] B. B. Feng, ‘A study of the protection of basil polysaccharide myelosuppression caused by cyclophosphamide chemotherapy. Master’, Shandong Traditional Chinese Medicine University 2017.
[12] Z. P. Zheng, W. B. Yang, N. Li, Y. J. Bo, S. Y. Xing, Q. Shao, L. B. Huang, ‘Clinical observation on injection of astragalus polysaccharides protecting marrow suppression of non-small-cell lung cancer after chemotherapy’, Chin. Tradit. Herb Drugs 2013, 44, 208 – 209.
[13] W. R. Bao, Q. W. Zhang, H. M. Zheng, L. F. Li, M. Liu, H. Y. Cheng, T. L. Kong, G. Zhang, A. P. Lu, Z. X. Bian, D. Ma, C. H. Leung, Q. B. Han, ‘Radix Astragali polysaccharide RAP directly protects hematopoietic stem cells from chemotherapy-induced myelosuppression by increasing FOS expression’, Int. J. Biol. Macromol. 2021, 183, 1715 – 1722.
Comparisons of isolation methods, structural features, and bioactivities of the polysaccharides from three common panax species: a review of recent progress, *Molecules* 2021, 26, 4997.

D. Mohnen, ‘Pectin structure and biosynthesis’, *Curr. Opin. Plant Biol.* 2008, 11, 266 – 277.

B. Yapo, ‘ChemInform abstract: pectin rhamnogalacturonan II: the ‘small stem with four branches’ in the primary cell walls of plants’, *Int. J. Carbohydr. Chem.* 2011, 43.

Y. F. Wang, G. H. Hou, J. L. Li, M. M. Surhio, M. Ye, ‘Structure characterization, carboxymethylated and sulfated modifications, and antioxidant and hypoglycemic activities in vitro of a polysaccharide from *Lachnum sp*’, *Process Biochem.* 2018, 72, 177 – 187.

X. Zhao, H. Yang, T. L. Song, G. L. Yu, ‘Study on influence factors on relative molecular weight determination of grifola frondosa beta-glucan for injection by HPGPC’, *Chin. Pharm. J.* 2006, 23, 1777 – 1780.

B. Yapo, ‘Rhamnogalacturonan-I: A structurally puzzling and functionally versatile polysaccharide from plant cell walls and Mucilages’, *Polym. Rev.* 2011, 51, 391 – 413.

T. Tryfona, H. C. Liang, T. Kotake, Y. Tsumuraya, E. Stephens, P. Dupree, ‘Structural characterization of *Arabidopsis* leaf arabinogalactan polysaccharides’, *Plant Physiol.* 2012, 160, 653 – 666.

H. Wang, M. Y. Wang, J. Chen, Y. Tang, J. Dou, J. Yu, T. Xi, C. L. Zhou, ‘A polysaccharide from *Strongylocentrotus nudus* eggs protects against myelosuppression and immunosuppression in cyclophosphamide-treated mice’, *Int. Immunopharmacol.* 2011, 11, 1946 – 1953.

N. Saxena, P. Yadav, O. Kumar, ‘The Fas/Fas ligand apoptotic pathway is involved in abrin-induced apoptosis’, *Toxicol. Sci.* 2013, 135, 103 – 118.

M. Goldstein, W. P. Roos, B. Kaina, ‘Apoptotic death induced by the cyclophosphamide analog mafosfamide in human lymphoblastoid cells: contribution of DNA replication, transcription inhibition and Chk/p53 signaling’, *Toxicol. Appl. Pharmacol.* 2008, 229, 20 – 32.

J. Vera, Y. Raatz, O. Wolkenhauer, T. Kottek, A. Bhattacharya, J. Simon, M. Kunz, ‘Chk1 and Wee1 control genotoxic stress induced G2-M arrest in melanoma cells’, *Cell. Signaling* 2015, 27, 951 – 960.

M. Ogawa, ‘Differentiation and proliferation of hematopoietic stem cells’, *Blood* 1993, 81, 2844 – 2853.

S. Y. Zhao, T. Chen, Y. W. Zhang, X. W. Gan, T. L. Wu, N. Xiao, R. P. Yang, Y. Guo, ‘Extraction and determination of polysaccharide in *Panax notoginseng*, *West Chin. J. Pharm. Sci.* 2011, 26, 481 – 483.

R. Q. Song, T. G. Nan, Y. Yuan, Y. Jin, Q. Yang, M. Zhang, K. Y. Hu, ‘Study on polysaccharide content and monosaccharide composition of Polyporus umbellatus from different production areas’, *China J. Chin. Mater. Med.* 2019, 44, 3608 – 3614.

N. Blumenkranz, G. Asboe-Hansen, ‘New method for quantitative determination of uronic acids’, *Anal. Biochem.* 1973, 54, 484 – 489.

M. K. Chan, Y. Yu, S. Wulamu, Y. Wang, Q. Wang, Y. Zhou, L. Sun, ‘Structural analysis of water-soluble polysaccharides isolated from *Panax notoginseng*, *Int. J. Biol. Macromol.* 2020, 155, 376 – 385.

J. H. Xie, M. Y. Shen, S. P. Nie, X. Liu, H. Zhang, M. Y. Xie, ‘Analysis of monosaccharide composition of *Cyclocarya paliurus* polysaccharide with anion exchange chromatography’, *Carbohydr. Polym.* 2013, 98, 976 – 981.

P. W. Needs, R. R. Selvendran, ‘Avoiding oxidative degradation during sodium hydroxide/methyl iodide-mediated carbohydrate methylation in dimethyl sulfoxide’, *Carbohydr. Res.* 1993, 245, 1 – 10.

R. L. Taylor, H. E. Conrad, ‘Stoichiometric depolymerization of polyuronides and glycosaminoglycanurans to monosaccharides following reduction of their carbohydrate-activated carboxyl groups’, *Biochemistry* 1972, 11, 1383 – 1388.

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