



Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: <https://www.sciopen.com/journal/2097-0765>

What are the structural and bioactive differences between fresh/dried *Rehmanniae Radix* or *Rehmanniae Radix preparata* oligosaccharides and polysaccharides? A review of bioavailable possessions

Li Wang^a, Yupeng Yang^a, Yahui Jia^a, Chunnan Wen^a, Shucheng Huang^b, Zhiqiang Zhang^c, Qiuling Ma^c, Zhongyi Zhang^d, Xiao Li^{e*}, Hui-Min David Wang^{f,*}, Bingji Ma^{a,*}

^aDepartment of Traditional Chinese Medicine, College of Agronomy, Henan Agricultural University, Zhengzhou 450046, China

^bCollege of Veterinary Medicine, Henan Agricultural University, Zhengzhou 450046, China

^cThe Second School of Clinical Medicine, Henan University of Chinese Medicine, Zhengzhou, 450046, China

^dFujian Agriculture and Forestry University, Fuzhou 350002, China

^eHenan High Tech Industry Co., Ltd., Henan Academy of Sciences, Zhengzhou 450000, China

^fGraduate Institute of Biomedical Engineering, National Chung Hsing University, Taiwan, China

ABSTRACT: *Rehmannia glutinosa* (RG), officially recognized as a medicinal and edible substance in China since 2024, exhibits significant potential for pharmaceutical and functional food applications. Its processed forms—Fresh *Rehmanniae Radix* (FRR, Xiandihuang), Dried *Rehmanniae Radix* (DRR, Shengdihuang), and *Rehmanniae Radix* Preparata (RRP, Shudihuang)—display distinct oligosaccharide (RGO) and polysaccharide (RGP) profiles that directly influence their bioactivities. While FRR/DRR are rich in stachyose-type RGOs with hypoglycemic and hepatoprotective effects, RRP is rich in polysaccharides with immunomodulatory, anti-aging, and anti-inflammatory properties. Despite their therapeutic promise, systematic comparisons of these saccharides across processing methods remain scarce. This review comprehensively analyzes extraction techniques, structural features, bioactivities, and structure-activity relationships of RGOs/RGPs, highlighting their mechanisms in metabolic regulation, gut microbiota modulation, and multi-organ protection, while bridging traditional applications with modern scientific validation. Notably, RGPs demonstrate enhanced bioavailability and therapeutic efficacy when formulated into liposomes or nano-adjuvants. This study systematically examines processing-induced structural transformations, bioactivities and safety profiles to establish theoretical foundations for advancing RGOs/RGPs development and proposes novel research directions. The findings provide critical guidance for developing RG-based functional ingredients targeting diabetes management, immune modulation, and preventive healthcare, with expected applications in pharmaceuticals, health foods, and nutritional supplements.

Keywords: *Rehmannia glutinosa*, oligo/polysaccharide, separation, structure, bioactivity, future prospects

1. Introduction

Rehmannia glutinosa Libosch. is the fresh or dry root of the therapeutic herb in the Scrophulariaceae family. It was first recorded in “*Shennong's Classic of Materia Medica*”, which was listed as “top-grade”

*Corresponding author

Xiaol_sunny@163.com; davidw@dragon.nchu.edu.tw;
mbj123@sina.com

Received 2 December 2024

Received in revised form 17 January 2025

Accepted 4 April 2025

medicine material and widely used in China, South Korea, Japan, and other Asian countries ^[1-2]. RG is spread throughout Henan, Hebei, Jiangsu, Shandong, and others. DRR is obtained by gradually baking the FRR at a temperature typically below 60°C until it reaches approximately 80% dryness ^[3]. The primary steaming methods to obtain RRP include traditional processing (e.g., nine cycles of steaming and sun-drying, often with rice wine as an adjunct), pharmacopoeia-specified steaming (with optional wine or water as adjuncts), and modern high-pressure steaming (with optional adjuncts) ^[2]. FRR can clear blood heat, cool blood, and hemostasis; DRR has the function of clearing heat and cooling blood, nourishing Yin, and accelerating the production of body fluids; RRP has the clinical activity of replenishing blood, nourishing “yin”, and reinforcing vital essence and marrow ^[4], which indicate that RG has many pharmacological actions. RG is a typical representative Chinese medicine of raw and processed treatment in clinical practice. The view that *Rehmannia glutinosa* has the properties of “Sheng Qing, Shu Bu” is widely accepted within traditional Chinese medicine.

The various active ingredients, such as iridoid glycosides (catalpol), saccharides, and phenylethanoid (acteoside), are rich in RG ^[5]. The contents of small molecular substances (such as catalpol, rehmannioside D, acetone, oligosaccharide, and jugal) were decreased. The content of 5-Hydroxymethylfurfural (5-HMF) and polysaccharides was significantly increased ^[6]. While some controversy exists regarding saccharide composition changes during processing, current research demonstrates distinct profiles across RG preparations. FRR contains stachyose as its predominant saccharide (20.19%-68.27% oligosaccharide content), while DRR primarily consists of sucrose, raffinose, and residual stachyose. The substantial oligosaccharide content, particularly stachyose, in both FRR and DRR establishes these forms as valuable sources of dietary fiber and potential prebiotic agents ^[7-8]. RRP demonstrates a dual transformation: (1) breakdown of oligosaccharides or polysaccharides into simpler sugars (fructose, glucose, melibiose and manninotriose), accompanied by (2) significant accumulation of polysaccharides (3.45%-21.13%) ^[2,9-10]. These accumulated polysaccharide component-likely derive from the hydrolysis of RG cell wall constituents during steaming-serve as the key bioactive substances responsible for the synergistic pharmacological effects of RRP ^[2]. Furthermore, more and more pieces of evidence have proved that the RGOs and RGPs had a variety of biological activities, such as antioxidant, neuroprotective, anti-inflammatory, antiviral, anti-tumor, hypoglycemic, immunomodulatory, renoprotective, hepatoprotective, anti-fatigue, gastroprotective and other bioactivities with low toxicity and side effects ^[4,10-14].

Currently, no review systematically categorizes and summarizes the composition and efficacy of RGOs and RGPs based on the processing methods and usage forms of *Rehmannia Radix*. However, conducting a classification study of carbohydrate components according to the various forms of *Rehmannia Radix* is significant for optimizing processing techniques, exploring the different mechanisms of action among fresh and processing *Rehmannia Radix*, and better serving clinical and industrial applications. This review reviewed the properties and bioactivities of RGPs and RGOs. It specifically included the extraction, isolation, purification, structural characterization, and biological activities of oligo/polysaccharides in FRR, DRR, and

RRP. This could provide a reference for the product development and clinical application of RG saccharides, and it is beneficial for improving the quality standard of RG.

2. Overview for extraction and purification of RG saccharides

Given the distinct compositional profiles of saccharides across FRR, DRR, and RRP, standardized extraction and purification protocols are essential to ensure the reproducibility of their bioactivities. The following section systematically evaluates the current methodologies for isolating these functionally important carbohydrates.

Due to RGOs/RGPs significant role in functional foods, medicine, and healthcare, there has been growing interest in extraction methods for oligosaccharides/polysaccharides from RG, as this process can determine the products' yield and bioactivities. Before the extraction, isolation, and purification of RGPs/RGOs, pretreatment with acetone, petroleum ether, or diethyl ether is necessary to remove non-polysaccharide and non-oligosaccharide molecules, including certain pigments and lipid-soluble components. Conventional hot water extraction (HWE) and boiling water extraction (BWE) represent the primary methods for isolating RGOs/RGPs. Comparative analyses reveal that both HWE and ultrasonic-assisted extraction yield RGOs with comparable oligosaccharide profiles (sucrose, raffinose, and stachyose) and significant antioxidant capacity [8]. BWE-derived RGPs are predominantly composed of galactose and arabinose, demonstrating notable immunomodulatory and hematinic properties [2]. In contrast, microwave-assisted extraction shows distinct selectivity, preferentially extracting raffinose, stachyose, and verbascose [15]. Further purification is required to clarify the composition, molecular weight (MW), and basic chemical structures of RGOs/RGPs. Notably, during the hot water extraction process, the colors of FRR and DRR solutions gradually change from light to black. In contrast, preparing RRP leads to Maillard and caramelization reactions, resulting in a deep black color of the extraction solution [16]. This results in very dark crude polysaccharides, posing significant challenges for subsequent studies on their functional mechanisms and structure-activity relationships. Therefore, decolorization of RGOs/RGPs is both critical and technically challenging. The effect of different macroporous resins (HP20, DM130, X-5, S-8, D-101, NKA-II, KA-9, LS-700B, XDA-1, HPD-400, AB-8, LS-300B, and LS-305) on *Rehmannia Radix* polysaccharides was identified, and the result indicated that the decolorization abilities of DM130, HPD-400 and LS-700B were better [17-18]. AB-8 decolorized RGP has a more potent antioxidant activity than induced RGP decolorized by H₂O₂, which could destroy some glycosidic bonds in polysaccharides [19]. After decolorizing the crude polysaccharides, the neutral and acidic RGOs/RGPs were isolated by anion exchange chromatography (DEAE-52 cellulose, DEAE-32, DEAE-Sephadex A-25, DEAE-Sephacel, and DEAE Sepharose FF) using gradient salt elution, and the RGOs/RGPs with different MWs were isolated by gel filtration column chromatography (Sephadex G-25, G-50, G-75, G-100; Sephadex LH-20; Superdex-200; Sephacryl S-100; Sephacryl S-200HR). The specific methods for obtaining oligo/polysaccharides of RG reported by literature are shown in Figure 1 and Table 1-3.

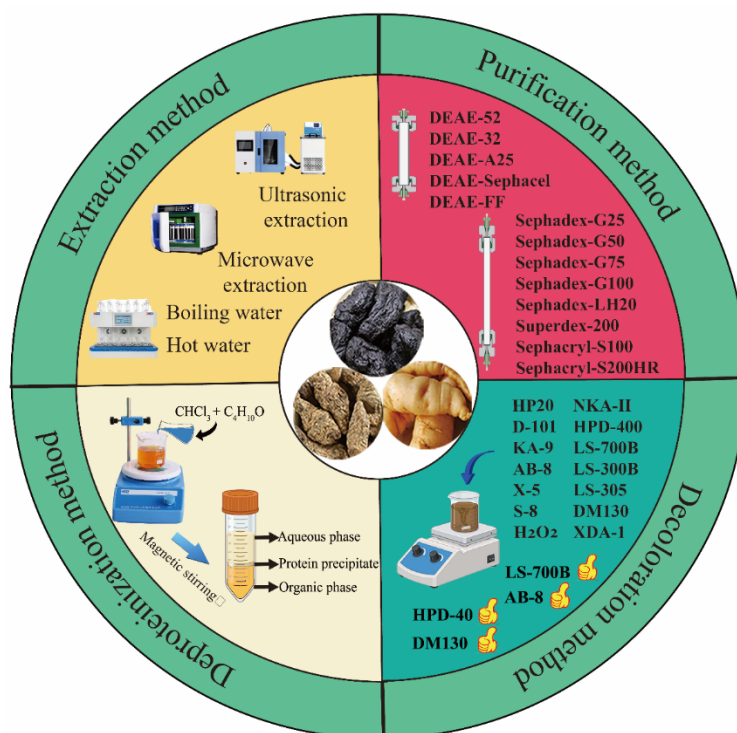


Fig. 1. Systematic flowchart for the extraction, separation, and purification of RGOs/RGPs.

3. Structural transformation for RG saccharides

The structural characteristics of oligo/polysaccharides, including monosaccharide composition, MW, branching degree, type of glycosidic linkage, and spatial configuration, critically determine their bioactivities. To comprehensively elucidate the structural characteristics of oligo/polysaccharides, an integrated analytical strategy was implemented, combining advanced chromatographic, and spectroscopic techniques with classical chemical degradation methods. For molecular weight distribution and purity, high-performance gel permeation chromatography (HPGPC) coupled with multi-angle laser light scattering (MALLS) and dynamic/static light scattering (DLS/SLS) were employed. Monosaccharide composition was determined through complete acid hydrolysis followed by high-performance anion-exchange chromatography (HPAEC), matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS), gas chromatography-mass spectrometry (GC-MS), or high-performance liquid chromatography (HPLC) analysis. Glycosidic linkage configuration (pyranose/furanose rings) was characterized by Fourier transform infrared spectroscopy (FT-IR) and nuclear magnetic resonance (NMR) spectroscopy, while linkage position and branching patterns were resolved via methylation analysis (utilizing GC-MS), periodate oxidation, and Smith degradation. All quantified structural parameters—including monosaccharide ratios, molecular weight distributions, and glycosidic linkage patterns—are systematically presented in Tables 1–3.

Table 1 The Fresh *Rehmanniae Radix* source, extraction, purification, molecular weight, yield, monosaccharide composition, backbone chain, and bioactivities of RGOs/RGPs.

Sources	Extraction and purification	Molecular weight (kDa)	Yield (%)	monosaccharide composition and backbone chain	Bioactivities	References
Oligosaccharide, (Anguo)	Hot water extraction: 1:3 (w/v), cation exchange resin and charcoal with water, 5%-30% ethanol, 3.5% ammonia water	ND	20.19	monosaccharides bisaccharides, trisacchrides (manninotriose), tetrose (stachyose)	Significant hypoglycemic effect	[7]
Oligosaccharide, (Wen county)	Hot water and ultrasonic extraction methods: 60 °C, 1:20 (w/v), 10 min, 200 W for ultrasonic extraction; 4 h, 80 °C for water extraction, Savage deproteinized, HP-20 decolorization, DEAE-cellulose, Sephadex G-50	ND	50.79	Sucrose:raffinose:stachyose=1.53:3.80:79.73 (GRCP);	Antioxidant activities	[8]
		ND	68.27	Sucrose:raffinose:stachyose=2.38:3.18:80.99 (GRSP)		
Oligosaccharide, (Anguo)	Hot water extraction: 1:3 (w/v); cation exchange resin and charcoal with water, 5%-30% ethanol, 3.5% ammonia water	ND	1.16	Stachyose:manninotriose=2:1	Anti-diabetic activity	[12]
Oligosaccharide, (Wuzhi county)	Hot water extraction: 90 °C, 1 h, activated carbon and clay (80 °C, 30 min), 001 × 7, D201 cation/anion exchange resin, D201, D101 macroporous adsorption resin	ND	ND	Sucrose:raffinose:stachyose:verbascose=7.52:5.19:81.02:4.85	Ameliorate intestinal inflammation, protect mice intestinal function	[14]
Polysaccharide, (Wen county)	Boiling water extraction: 100 °C, 1:20 (w/v), 3 h, 3 volumes of 95% ethanol precipitation	125 (13.1%), 169 (69.6%) and 121 (17.3%)	0.61	Glucose:galactose:mannose:rhamnose:arabinose:galacturonic acid:glucuronic acid=5.8:24.8:1.0:9.1:24.7:30.0:1.3	Blood-enriching activity, immunomodulatory activity	[2]
Polysaccharide, (Taipei, Taiwan)	Hot water extraction: 80 °C, 1:100 (w/w), 6 h, 4 volumes of 95% precipitation	2.03 (95.6%) and 0.6	0.55	Glucose:galactose:mannose=4:2:1	Anti-inflammatory, anti-cancer	[9]

Table 2 The dried *Rehmanniae Radix* source, extraction, purification, molecular weight, yield, monosaccharide composition, backbone chain, and bioactivities of RGOs/RGPs.

Sources	Extraction and purification	Molecular weight (kDa)	Yield (%)	Monosaccharide composition and backbone chain	Bioactivities	References
Oligosaccharide, Qinyang	Ethanol extraction: 50% ethanol (1:10, w/v), 80 °C, 4 h, 7 volumes of ethanol, diatomaceous earth and charcoal (1:1 w/w), crude RGOs were eluted by 20%, 40%, 60%, and 80% aqueous alcoholic solution, respectively	ND	31.3	Sucrose:raffinose:stachyose=7.1:23.7:61.7	Prevent oxidative stress-induced liver injury	[13]
Oligosaccharide, (Taipei, Taiwan)	Boiling water extraction: 100 °C, 1:9 (w/v), 2 h, 3 volumes of 95% ethanol	ND	ND	Carbohydrate:protein:fat: moisture:ash=70.04:4.72:0.29:9.24:0.04	Inhibit proliferation and stimulate p53-mediated apoptosis in HCC cells	[39]
Oligosaccharide, Henan	Microwave assisted extraction: RDR/60% ethanol=1:1 (g/L), and the microwave assisted extraction at 500 W and 80 °C for 10 min; a MCI gel CHP20P column for decolorization, Sephadex G-25, Sephadex LH 20 and Bio-Gel P2 columns	ND	1.72, 7.52, 0.51	Raffinose, stachyose and verbascose	ND	[15]
Oligosaccharide, (Beijing)	Boiling water extraction: 100 °C, 1:10 (w/v), 3 times, precipitation with a final concentration of 50% ethanol	ND	28.83, 24.30	Sucrose:raffinose: mannotriose:stachyose: melibiose:glucose =5.31:12.10:24.66:21.99:7.14:10.01 (OPRR); Sucrose:raffinose: mannotriose: stachyose:melibiose:glucose =8.01:6.00:15.40:29.11:5.69:6.78 (ODRR)	Neuroprotective activity	[79]
Polysaccharide, (Taipei, Taiwan)	Hot water extraction: 80 °C, 1:100 (w/w), 6 h, 4 volumes of 95% ethanol precipitation	1937.0,1057.0, 540.0, 20.0 and 1.9 1937 and 1057 (73.6%)	4.45	Glucose:galactose:mannose=7:3:2	Anti-inflammatory, anti-cancer	[9]
Polysaccharide, (China)	Boiling water extraction: 100 °C, 2 h, 20.0 mL/g and three extractions; DEAE-32, Sephacryl S-200HR	ND	ND	Crude polysaccharide: 12.6%; purified polysaccharide: 3.1%	Enhance the non-specific immunity, antioxidant activity and anti- <i>A. hydrophila</i> activity of the common carp	[11]
Polysaccharide, (Wen county)	Ultrasonic water extraction: 65 °C, 1:12.5, 1 h, ethanol precipitation, AB-8/H ₂ O ₂ , deproteinized by Seavage, DEAE-52, Sephadex	18.964, 3.305	ND	Glucose:galactose:mannose: rhamnose:arabinose: galacturonic acid:glucuronic	Antioxidant activity	[19]

G-100				acid:ribose:xylose:fucose: <i>N</i> -acetyl-glu cosamine=45.34:25.87:0.10:2.90:10.2 9:13.52:0.75:0.04:0.57:0.52:0.11 (RGP-1-A); Glucose:galactose:mannose: rhamnose:arabinose: galacturonic acid:glucuronic acid:ribose:xylose:fucose: <i>N</i> -acetyl-glu cosamine=38.00:56.46:0.23:0.15:2.47: 1.20:0.68 (RGP-2-A)		
Polysaccharide	Boiling water extraction: 1:10 (w/v), 1 h, 3 times, precipitation with a final concentration of 80% ethanol, deproteinized by Sevage, DEAE-52 cellulose, Sephadex G-100	ND	ND	Carbohydrate:protein=83.35:11.20; Glucose:galactose:mannose: rhamnose:arabinose =39.51:56.85:2.38:0.05:1.22	Anti-aging effect	[22]
Polysaccharide (Guangzhou)	Hot water extraction: 80 °C, 1:10 (w/v), 3 h, precipitation with a final concentration of 50%, 70% ethanol, successively, DEAE-Sepharose Fast Flow, Sephadex G-75	3.6	0.57	Glucose:galactose: =23.9:76.1 1,4- α -D-Galp, 1,6- α -D-Galp, and 1,3,4,6- β -D-Galp	Anti-aging acitvity	[23]
Polysaccharide, (China)	Boiling water extraction: 30 min, precipitation with 1% Na ₂ SO ₄ , 5% CTAB, 2 volumes of ethanol; 80% ethanol washing the precipitate; DEAE-Sephadex A-25 column, DEAE-Sephacel, Sephadex G-25	ND	0.825	Galactose:rhamnose: arabinose:galacturonic acid =46.6:5.4:38.9:4.6 1,5-Araf, 1,2,5-Araf, 1,3-Araf, 1,3-Galp, 1,4-Galp, 1,6-Galp, 1,3,6-Galp, 1,4,6-Galp, 1,4-GalpA, 1,2-Rhap, 1,2,4-Rhap (SA); Galactose:rhamnose: arabinose:galacturonic acid =21.7:26.3:37.6:5.4; 1,5-Araf, 1,2,5-Araf, 1,3-Galp, 1,4-Galp, 1,6-Galp, 1,3,6-Galp, 1,4,6-Galp, 1,4-GalpA, 1,2-Rhap, 1,2,4- Rhap (SB)	Anti-complementary activities; reticuloendothelial system	[24]
Polysaccharide, (Guangzhou)	Hot water extraction: 80 °C, 1:10 (w/v), 3 h, precipitation with a final concentration of 50%, 70% ethanol, successively, DEAE-Sepharose Fast Flow, Sephadex G-75	6.7	1.07	Arabinose only; →5)- α -L-Araf-(1→, →3)- α -L-Araf-(1→, →2,5)- α -L-Araf-(1→, and →2,3,5)- α -L-Araf-(1→	Anti-inflammatory activity	[25]
Polysaccharide, (Jiaozuo)	Hot water extraction: 85 °C, 1:10 (w/v), 3 h, 3 times, precipitation with a final concentration of 50% ethanol, deproteinized by Sevage,	4.9, 2.8	ND	→3)-L-Araf-(1→, →5)-L-Araf-(1→, →3,5)-L-Araf-(1→, →2,5)-L-Araf-(1→, D-Manp-(1→,	Hypoglycemic activity	[27]

AB-8 macroporous, DEAE-52, Sephacryl S-100				$\rightarrow 2$)-D-Manp-(1 $\rightarrow$, $\rightarrow 4$)-D-Manp-(1 $\rightarrow$, D-Galp-(1 $\rightarrow$, $\rightarrow 4$)-D-Galp-(1 $\rightarrow$, $\rightarrow 4,6$)-D-Galp-(1 $\rightarrow$, $\rightarrow 6$)-D-Glcp-(1 $\rightarrow$, $\rightarrow 4,6$)-D-Glcp-(1 $\rightarrow$, $\rightarrow 3,6$)-D-Glcp-(1 $\rightarrow$ (RGP70-1-1); $\rightarrow 5$)-L-Araf-(1 $\rightarrow$, $\rightarrow 3,5$)-L-Araf-(1 $\rightarrow$, $\rightarrow 4$)-D-Manp-(1 $\rightarrow$, $\rightarrow 3,6$)-D-Manp-(1 $\rightarrow$, D-Galp-(1 $\rightarrow$, $\rightarrow 6$)-D-Galp-(1 $\rightarrow$, D-Glcp-(1 $\rightarrow$, $\rightarrow 6$)-D-Glcp-(1 $\rightarrow$, $\rightarrow 4,6$)-D-Glcp-(1 $\rightarrow$ (RGP70-1-2) L-Araf-(1 $\rightarrow$, $\rightarrow 3$)-L-Araf-(1 $\rightarrow$, $\rightarrow 5$)-L-Araf-(1 $\rightarrow$, D-Glcp-(1 $\rightarrow$, $\rightarrow 3$, 5)-L-Araf-(1 $\rightarrow$, $\rightarrow 2$, 5)-L-Araf-(1 $\rightarrow$, $\rightarrow 6$)-D-Glcp- (1 $\rightarrow$ and $\rightarrow 2$, 3, 4)-D-Xylp-(1 $\rightarrow$ (RGP 90-1-1); L-Araf-(1 $\rightarrow$, $\rightarrow 3$)-L-Araf-(1 $\rightarrow$, $\rightarrow 5$)-L-Araf-(1 $\rightarrow$, $\rightarrow 3,5$)-L-Araf-(1 $\rightarrow$ and $\rightarrow 6$)-D-Glcp-(1 $\rightarrow$ (RGP90-1-2) Glucose:galactose:mannose: rhamnose:arabinose acid=19.91:61.13:0.90:1.53:16.53 Carbohydrate:protein=65.5:22.0		
Polysaccharide, (Henan)	Hot water extraction: 85 °C, 1:10 (w/v), 3 h, 3 times, precipitation with a final concentration of 50%, 70%, 90% ethanol, deproteinized by Sevage, DEAE-52, Sephadex G-75	13.2, 6.2	ND		Hypoglycemic activity and anti-inflammatory effects	[28]
Polysaccharide (Guangzhou)	Hot water extraction: 80 °C, 1:10 (w/v), 3 h, precipitation with a final concentration of 70% ethanol	3.2-37	23.82		Anti-aging acitivity	[45]
Polysaccharide, (Taipei, Taiwan)	Boiling water extraction: 1:10 (w/v), 2 h, 100 °C	ND	4.30		Anti-inflammatory and anti-fibrotic activities	[79]

Table 3 The *Rehmanniae Radix Preparata* source, extraction, purification, molecular weight, yield, monosaccharide composition, backbone chain and bioactivities of RGOs/RGP.

Sources	Extraction and purification	Molecular weight (kDa)	Yield (%)	Monosaccharide composition and backbone chain	Bioactivities	References
Oligosaccharide, (Taipei, Taiwan)	Hot water extraction: 1:10 (w/v), 80 °C, 4 h, twice, 80% ethanol dissolution, Sephadex LH-20	ND	37.1	Stachyose, mannotriose, fructose, glucose, galactose, melibiose, raffinose	Anti-diabetic activity	[36]
Polysaccharide, (Wen county)	Boiling water extraction: 100 °C, 1:20 (w/v), 3 h, 3 volumes of 95% ethanol precipitation	129, 171	3.45, 7.29	Glucose:galactose:mannose: rhamnose:arabinose: galacturonic acid:glucuronic acid=29.4:35.0:1.4:3.4:9.8:18.3:0.5 (HRP); Glucose:galactose:mannose: rhamnose:arabinose: galacturonic acid:glucuronic acid=10.8:57.8:1.9:5.1:8.8:10.9:1.1 (NRP)	Blood-enriching activity, immunomodulatory activity	[2]
Polysaccharide, (Taipei, Taiwan)	Hot water extraction: 80 °C, 1:100 (w/w), 6 h, 4 volumes of 95% ethanol precipitation	1593.0, 640.6, 280.8, 145.6, 2.3 and 1.2 1593.0, and 640.6 (78.4%)	7.14	Glucose:galactose:mannose =33:4:1	Anti-inflammation and anti-cancer	[8]
Polysaccharide, (Wuzhi County)	Hot water extraction: 98-100 °C, 1:8 (w/v), 2 h, deproteinization using the Sevage method	ND	21.13	Carbohydrate content: 25.82%	Anti-fatigue activity	[10]
Polysaccharide, (Xi'an)	Hot water extraction: Ethanol precipitation, deproteinized by Sevage method, decolorized by 30% H ₂ O ₂ , DEAE-52, Sephadex G-100	1750	ND	Glucose:galactose:mannose: rhamnose:xylose=5.58:1.39:1.0:3.52:10.64 1→, 1→2, 1→3, 1→4, 1→2,6, 1→4,6 or 1→6, 1→2,3, 1→2,3,4, and other glycosidic bonds	Antioxidant activity	[18]
Polysaccharide	Boiling water extraction: 1:10 (w/v), 1 h, 3 times, precipitation with a final concentration of 80% ethanol, deproteinized by Sevage, DEAE-52 cellulose, Sephadex G-100	ND	ND	Carbohydrate:protein=81.22:19.03 Glucose:galactose:mannose: rhamnose:arabinose=44.08:55.23:0.43:0.02:0.23	Anti-aging effect	[22]
Polysaccharide, (Wuzhi County)	Boiling water extraction: 1:30 (w/v), 100°C, deproteinization using the Sevage method, precipitation with a final concentration of 80% ethanol, DEAE Sepharose FF and Superdex-200	11.9, 35.5	8, 2.25	Glucose:galactose:arabinose =5.3:56.6:38.1 →6)-α-D-Galp-(1→6)-α-D-Galp-(1→5)-α-L-Araf -(1→3,5)-α-L-Araf-(1→ (SDH-WA); Glucose:galactose:arabinose:rhamnose:galacturonic acid=9.5:45.0:31.8:3.9:9.9 →2,4)-α-L-Rhap-(1→4)-α-D-GalpA-(1→ (SDH-0.2A)	Immunomodulatory activities	[29]
Polysaccharide, (Shanxi Ci Yuan Biotechnology Co. Ltd.)	ND	63.5	ND	Glucose:galactose:mannose: rhamnose:arabinose =16.45:30.40:0.73:1.00:1.26	Hypoglycemic effect	[40]

3.1 Saccharide composition variation

During the baking process of FRR, stachyose degradation occurs through both enzymatic and thermal mechanisms. Enzymatically, stachyose is primarily broken down by α -galactosidase into sucrose and galactose, while β -glucosidase facilitates the hydrolysis of the glucose-fructose bond. However, β -glucosidase exhibits poor thermal stability, rapidly losing activity at 50°C, whereas α -galactosidase reaches its peak activity at 50°C but sharply declines above 70°C. In processed RG products, the content of stachyose significantly decreases in RRP as a result of these combined effects. Conversely, manninotriose, melibiose, glucose, and fructose increase notably, particularly during the transition from DRR to PRR [20]. Further supporting this, under simulated RRP conditions (100°C, pH 4.8, 18h), sucrose and raffinose hydrolyze into glucose, fructose, and melibiose, while stachyose releases fructofuranosyl, further elevating fructose and mannose levels. Critically, this transformation of tasteless saccharides (stachyose and raffinose) into sweeter monosaccharides (fructose) enhances the “sweet as candy” flavor characteristic of RRP (Figure 1) [16]. Consistent with these mechanisms, Wenzhi RG exhibits lower levels of oligosaccharides (sucrose, raffinose, and stachyose) compared to steamed RG and wine-stewed RG, likely due to differences in processing conditions that influence thermal degradation pathways [21].

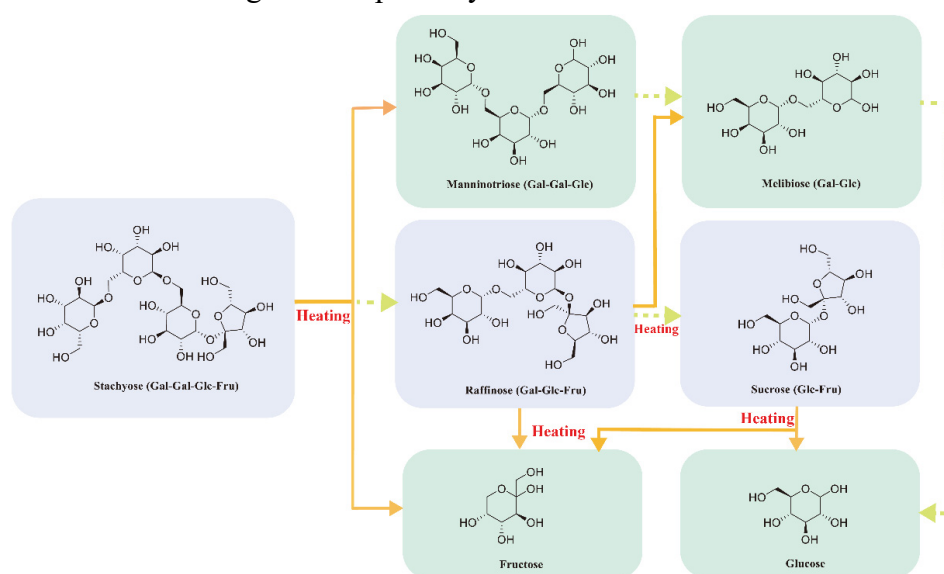


Fig. 2. Chemical transformation pathways of oligosaccharides in *Rehmanniae Radix* during processing. (Solid arrows: Validated degradation pathways; Dotted arrows: Hypothesized or potential degradation pathways)

RGPs mostly contained glucose, galactose, mannose, rhamnose, arabinose, galacturonic acid, and glucuronic acid in ratios of 4:2:1, 5.8:24.8:1.0:9.1:24.7:30.0:1.3, respectively in fresh *Rehmanniae Radix* [2,9]. The monosaccharide composition of RGPs in dried *Rehmanniae Radix* can be categorized into three primary groups: The first group is characterized by the presence of glucose and galactose as the main components, with specific ratios of glucose, galactose, mannose, rhamnose, arabinose, galacturonic acid, and glucuronic acid being 15.6:37.8:2.4:4.0:10.8:24.9:0.9, 39.5:56.6:2.4:0.05:1.2, 7:3:2, and 23.9:76.1 [2,9,22-23]. The second group contains no glucose, featuring galactose, rhamnose, arabinose, and galacturonic acid in the ratios of 46.6:5.4:38.9:4.6 and 21.7:26.3:37.6:5.4, respectively [24]. The third type of RGP is a homogeneous polysaccharide composed solely of arabinose [25].

The RGPs in RRP contained the following monosaccharides in specific ratios: Glucose, galactose, mannose, rhamnose, arabinose, galacturonic acid, and glucuronic acid in the ratios of 29.4:35.0:1.4:3.4:9.8:18.3:0.5, 10.8:57.8:1.9:5.1:8.8:10.9:1.1, 44.1:55.2:0.4:0.02:0.2, and 33:4:1. Additionally, glucose, galactose, arabinose, rhamnose, and galacturonic acid were present in ratios of 5.3:56.6:38.1 and 9.5:45.0:31.8:3.9:9.9, respectively [2,9,22]. Based on the above, it can be concluded that boiling water or boiling water combined with salt and CTAB extraction can yield uronic acid-rich polysaccharides, which are likely pectin-like in nature. This is supported by the presence of arabinose, rhamnose, galactose, and uronic acid, as well as characteristic FT-IR absorption peaks at 1741 cm^{-1} and 1620 cm^{-1} observed in dried *Rehmanniae Radix* [2,24]. This might be attributed to the partial decomposition of the cell wall during prolonged extraction with boiling water. In available documented references, the polysaccharides extracted from *Rehmanniae Radix* consistently contain high levels of galactose, noted for its superior biological activity compared to other common monosaccharides found in plants and microorganisms [26]. Additionally, the processing method also affect the monosaccharide compositions of RGPs. Galactose constituted the predominant monosaccharide in NRP-derived RGP (Nine-cycle-processed RRP), accounting for 57.8% of total sugar content. In contrast, HRP-derived RGP (High-pressure-steamed RRP) exhibited a distinct monosaccharide profile, with galactose (35.0%), glucose (29.4%), and galacturonic acid (18.3%) as the major components [2]. This result directly contributed to differences in their immunomodulatory activities.

3.2 Glycosidic linkage variation

Comprehensive analysis reveals three distinct types of RG saccharides: neutral RGOs, RGPs, and acidic RGPs. Neutral RGOs primarily consist of stachyose-type oligosaccharides (e.g., stachyose, raffinose). The RGPs exhibit structural diversity, classified as either pectin-like polysaccharides or non-pectin heteropolysaccharides. Tables 2 and 3 show that neutral and acidic polysaccharides were obtained from DRR. The glycosidic linkages of RGPs were different according to the different sources, extracted, and purified methods. The pectin-like polysaccharides contained 1,5-Araf, 1,2,5-Araf, 1,3-Araf, 1,3-Galp, 1,4-Galp, 1,6-Galp, 1,3,6-Galp, 1,4,6-Galp, 1,4-GalpA, 1,2-Rhap, 1,2,4-Rhap or $\rightarrow 2,4)\text{-}\alpha\text{-L-Rhap-(1}\rightarrow 4)\text{-}\alpha\text{-D-GalpA-(1}\rightarrow$ [24]. The glycosidic linkages of neutral polysaccharides contained RGP70-1-1, RGP70-1-2, RGP 90-1-1, RGP90-1-2, SDH-WA, respectively [25,27-28]. Detailed structural information can be found in Figure 3, Table 2, 3.

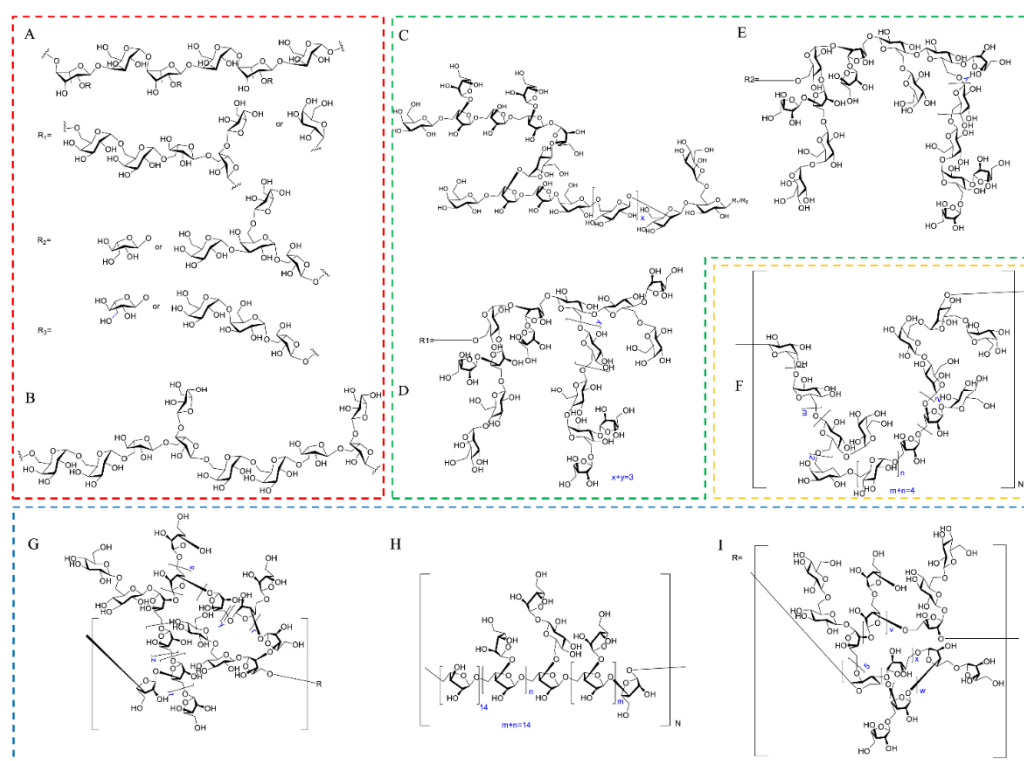


Fig. 3. Potential structures of RGPs. (A: SDH-0.2A; B: SDH-WA; C,D,E: RGP70-1-1; F: RGP70-1-2; G,H: RGP90-1-1; I: RGP90-1-2)

3.3 Molecular weight and yield variation

This structural diversity correlates with pronounced variations in MW and yield. The relatively high MW RGPs in FRR yielded 0.61% (Table 1) [2,8]. Yields of polysaccharides with known MWs from DRR were mostly around 1% (Table 2) [27–28]. The yields of RRP polysaccharides with relatively uniform MWs were mostly concentrated range from 7.14% to 8% [29]. It can be seen from the above results that the MWs of polysaccharides were inclined to uniform heteropolysaccharides, which could be the cleavage of side chains or the rearrangement of chemical bonds within polysaccharide molecules during the steaming process, as well as the degradation of low MW polysaccharides, resulting in a more uniform distribution of MWs [2,30].

4. Bioactivities

According to the pharmacopoeia of the People's Republic of China, FRR and DRR clear heat, while RRP tonifies, demonstrating the differing functions of this herb. Although both FRR and DRR exhibit heat-clearing properties, FRR shows superior clinical efficacy in managing acute hemorrhage, whereas DRR is more effective for treating chronic yin deficiency syndromes. In contrast, RRP demonstrates comprehensive tonic effects, including yin nourishment, blood supplementation, essence fortification, and marrow replenishment. From a traditional Chinese medicine composition perspective, these functional gradients arise from multiple active constituents, particularly RGOs/RGPs, which are among the major bioactive fractions in RG. These compounds exhibit various biological activities, such as anti-diabetic related, anti-aging, anti-inflammatory, and immunomodulatory effects and so on. Polysaccharides, in particular, are widely recognized for their

significant role as tonic ingredients in RRP, contributing to anti-aging, immune modulation, antioxidant effects, liver protection, and improved gut health.

4.1 Anti-diabetic related activities

Traditional Chinese medicine attributes diabetes mellitus to “Yin Deficiency with Dryness-Heat”, where Yin deficiency induces endogenous heat [31]. Medically, this manifests as hyperglycemia due to insulin deficiency/resistance and impaired glucose metabolism. Modern epidemiological studies identify chronic stress, caloric excess, and physical inactivity as key drivers of type 2 diabetes and metabolic syndrome [12,32–33]. These findings underscore diabetes’ systemic impacts and the imperative for early intervention. Plant oligo/polysaccharides exert hypoglycemic effects through multi-target synergistic mechanisms, including: protecting pancreatic β -cell function, enhancing insulin sensitivity, regulating key enzymes, mitigating oxidative stress damage, suppressing inflammatory responses, improving lipid metabolism disorders, and remodeling gut microbiota ecology, thereby synergistically regulating glucose and lipid metabolism homeostasis [33–34].

Both RGOs and RGPs exert anti-diabetic effects through multiple synergistic pathways (Figure 4), including: (1) Enhancement of glucose metabolism by enhancing hepatic glycogen synthesis and inhibiting gluconeogenesis through downregulation of glucose-6-phosphatase activity; (2) Regulation of lipid metabolism through hypothalamic-pituitary-adrenal (HPA) axis and neuroendocrine immunomodulation (NIM) network rebalancing, thereby alleviating chronic stress-induced metabolic dysregulation; (3) Protection of pancreatic β -cell function through promoting insulin synthesis and secretion as evidenced by elevated serum insulin levels and pancreatic insulin content; (4) Exhibiting intestinal α -glucosidase and α -amylase inhibitory activities to reduce postprandial hyperglycemia; (6) Mitigating diabetic complications including hepatic damage (via enhancing antioxidant defenses with increased glutathione (GSH), superoxide dismutase (SOD) and total antioxidant capacity (T-AOC)) and peripheral neuropathy (through HDAC6-mediated Treg regulation). These pleiotropic effects are achieved via molecular mechanisms involving PI3K/AKT signaling activation and p53-mediated apoptosis modulation. The specific mechanisms include the following aspects:

Zhang et al. reported that RGOs, composed of stachyose:manninotriose (2:1), demonstrated a regulatory effect on glucose metabolism in alloxan-induced diabetic rats by increasing hepatic glycogen content and decreasing glucose-6-phosphatase activity. Additionally, it was found that the impact of RGOs on glucose metabolism in hyperglycemic rats depends on the adrenal glands [7]. RGOs also could ameliorate glucose and lipid metabolism in chronic stress rats on a high-fat diet by regulating the hyperactivity of the HPA axis and the dysfunction of the NIM network. This modulation influences glycogen and gluconeogenesis through the HPA axis. Furthermore, RGOs exert comprehensive regulatory effects on the NIM network, impacting immune organs, plasma leptin levels, and plasma free fatty acids levels. By targeting multiple links and targets within the NIM network, RGOs improve impaired glucose metabolism in chronic stress rats on a high-fat diet [12]. Lau et al. found that DRR aqueous extract promoted the healing of diabetic ulcers, as evidenced by a

specific image analysis computer program (IACP) that enabled systematic and automatic determination of wound area. However, this extract did not exhibit significant wound-healing effects in normal rats [35]. The potential mechanism by which RGOs from RRP reduce postprandial glucose levels may involve regulating glucose uptake by inhibiting intestinal α -glucosidase activity and hepatic gluconeogenesis [36].

Patients with diabetes face an increased risk of liver disorders, such as glycogen hepatopathy, non-alcoholic fatty liver disease, cirrhosis, and hepatic fibrosis. The underlying pathophysiological mechanisms of diabetic hepatopathy are multifaceted and include factors such as the accumulation of fatty acids, enhanced production of reactive oxygen species, the formation of advanced glycation end-products, overactivity of polyol pathways, as well as processes like apoptosis, necrosis, and the promotion of fibrosis [37]. Hepatic abnormalities, such as elevated transaminases and alkaline phosphatase levels, are common in diabetes. The increase in these enzyme activities, along with reduced levels of protein and albumin, indicates that liver dysfunction and hepatocyte damage may exacerbate the development of diabetes [38]. Chao et al. reported that RGOs inhibit cell proliferation in a dose-dependent manner and induce p53-mediated apoptosis in both rat and human hepatocellular carcinoma cells [39]. In addition, a wide range of liver damages, including subclinical icteric hepatitis, necroinflammatory hepatitis, cirrhosis, and carcinoma, have been linked to redox imbalance and oxidative stress. RGOs, derived from DRR and composed of sucrose, raffinose, and stachyose in a ratio of 7.1:23.7:61.7, attenuated liver damage in CCl₄-induced mice. This effect was achieved by increasing hepatic levels of antioxidants such as GSH, glutathione peroxidase (GSH-Px), SOD, and T-AOC, while simultaneously reducing elevated hepatic malondialdehyde (MDA) levels, thus preventing reactive oxygen species (ROS)-related liver damage [13].

RGP from DRR can inhibit α -amylase and α -glucosidase activity while also stimulating GLP-1 secretion in STC-1 cells. This effect may be related to the upregulation of PI3K and p-AKT expression [27]. RGP significantly elevated serum insulin levels and pancreatic insulin content in diabetic mice, enhancing basal and glucose-stimulated insulin secretion. Additionally, RGP decreased triglycerides, total cholesterol, and low-density lipoprotein cholesterol levels while raising high-density lipoprotein cholesterol levels. These results indicated that RGP effectively improved hyperglycemia and hyperlipidemia by promoting insulin synthesis and secretion, enhancing liver glycogen synthesis, and reducing liver gluconeogenesis [40]. RGP could ameliorate hyperglycemia by decreasing blood glucose levels, improving glucose tolerance, and increasing serum insulin and the number of islet areas in STZ-induced diabetic mice. RGP could also promote bone formation and protect peripheral nerves, relieve nervous injury by promoting HDAC6 mRNA expression of Treg, and increase Treg number [41].

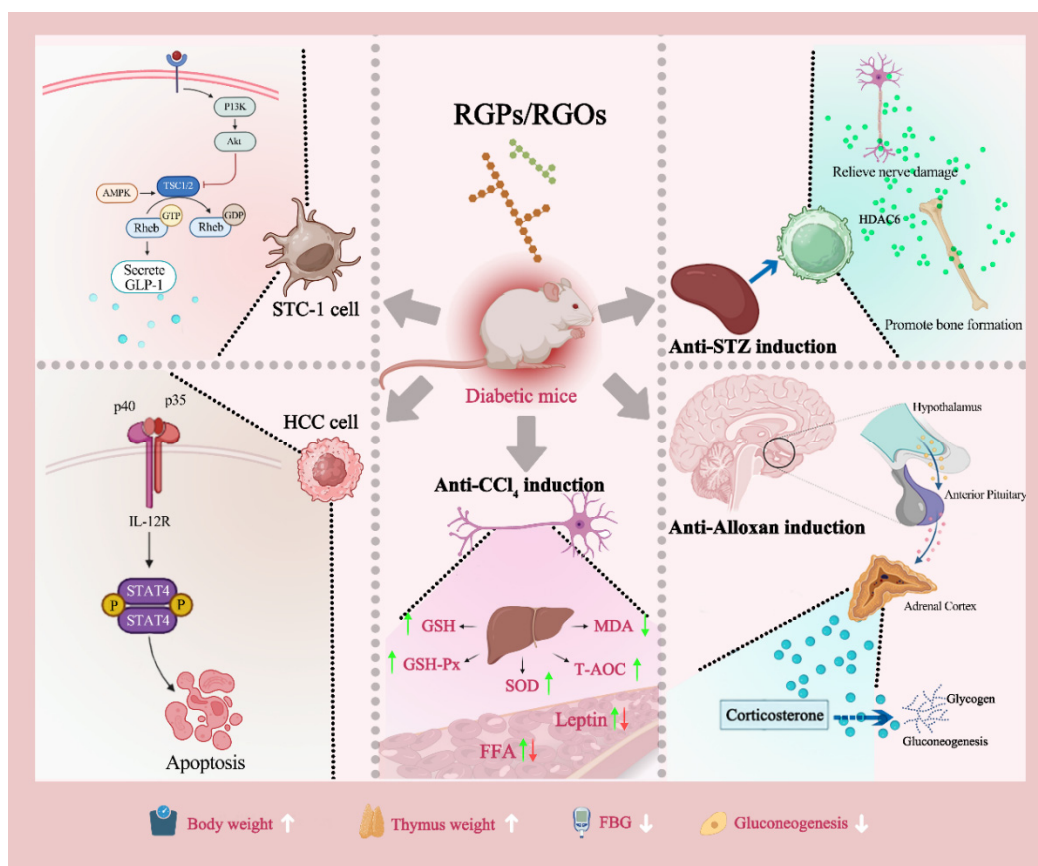


Fig. 4. Anti-diabetic related activities of RGOs/RGPs: RGP exhibits hypoglycemic activity through stimulating GLP-1 secretion in STC-1 cells; RGOs inhibit cell proliferation and induce p53-mediated apoptosis in both rat and human hepatocellular carcinoma cells (HCC); RGOs attenuate liver damage in CCl_4 -induced mice by increasing hepatic levels of antioxidants (GSH, GSH-Px, SOD, T-AOC) and reducing MDA levels; RGP alleviates hyperglycemia and promotes bone formation while enhancing Treg function in STZ-induced diabetic mice; RGOs improve glucose and lipid metabolism in chronic stress rats on a high-fat diet by modulating the HPA Axis and NIM network.

4.2 Anti-aging activity

Aging is an inevitable degenerative process that occurs throughout life. Over time, the functionality of cells, tissues, and organs diminishes, resulting in metabolic imbalances and reductions in immune response, memory, and cognitive abilities. The anti-aging therapies and cosmetics market is valued at approximately USD 60 billion and is projected to grow by 50% over the next five years [42]. The common anti-aging drugs used clinically, such as metformin and rapamycin, may have serious adverse effects with long-term use. As a result, seeking safer and more effective medications to slow the aging process has become a primary objective in anti-aging research [43]. According to the principle of tonifying the kidneys and nourishing essence, RRP is commonly utilized in traditional Chinese medicine for its anti-aging properties. The anti-aging and immunomodulatory activities of the RGP may help elucidate the tonifying properties of RRP, offering scientific support for traditional medicine and the advancement of medicinal products.

Research on the anti-aging effects of RG saccharide has primarily focused on polysaccharides and their effects on the *Caenorhabditis elegans* model. This model is favored for its small size, transparent body, short lifespan, high reproductive output, easy cultivation, and conserved genes related to human aging, making it ideal for in vivo studies of natural compounds [44]. The anti-aging activities of RGP and their underlying mechanisms are illustrated in Figure 5. RG70, a polysaccharide from DRR, could combat aging in

Caenorhabditis elegans by boosting the antioxidant enzyme system, decreasing lipofuscin and reactive oxygen species, upregulating *daf-16*, *skn-1*, and their downstream targets, increasing the abundance of *Eubacterium_siraeum_group* and *Lachnospiraceae_NK4B4_group*, down-regulating *age-1* gene expression, and modulating choline, citrate, and lactate levels in the TCA cycle ^[45]. *Eubacterium* generates acetyl-CoA, which is involved in the TCA cycle, and butyric acid during fermentation ^[46]. *Lachnospiraceae* also produced short-chain fatty acids and modulated intestinal immune functions. The *Eubacterium_siraeum_group* and *Lachnospiraceae_NK4B4_group* show a significant positive correlation with antioxidant biochemical markers (SOD, CAT, GSH-Px, and MDA) in *C. elegans*. This indicates that these bacteria may help slow down the aging process of nematodes by influencing their antioxidant profiles ^[45]. RGP70–1 (purified RG70) significantly extended the lifespan of *Caenorhabditis elegans* by modulating the insulin/IGF-1 signaling (IIS) pathway. It enhanced the nematodes' heat-stress tolerance and increased the levels of SOD, CAT, and GSH-Px. Additionally, RGP70-1 upregulated the expression of critical genes, including *skn-1*, *daf-16*, *gst-4*, *gcs-1*, *ctl-1*, and *sod-3*, while reducing ROS and lipofuscin accumulation ^[23]. Under oxidative stress, *daf-16* and *skn-1* became active in the cytoplasm and translocated to the nucleus, thereby regulating genes (eg. *gcs-1* and *gst-4*) related to oxidative stress ^[47]. Similar studies have reported that RGPs have been reported to extend the lifespan of nematodes by modulating the IIS pathway, particularly through their effects on the key genes *daf-2* and *daf-16*. Specifically, RGPs inhibit the expression of the insulin signaling receptor *daf-2*, which negatively regulates the IIS pathway. Research has shown that *daf-2* acts as an insulin receptor on the cell membrane. It triggers the phosphatidylinositol-3 kinase homolog *age-1*, activating AKT via second messengers produced by *age-1*. This activation results in the phosphorylation of the transcription factor *daf-16*, which hinders its translocation into the nucleus and impedes its transcriptional regulatory functions. Therefore, the knockout of *daf-2* removes its inhibitory effect on *daf-16*, allowing *daf-16* to enter the nucleus and activate its target genes, ultimately contributing to the extended lifespan of nematodes ^[22]. Additionally, RGPs derived from RRP improved the swimming capacity of BALB/c mice by reducing serum urea nitrogen (SUN) accumulation, delaying lactic acid buildup, and enhancing energy storage. Fatigue, one of the most common physiological responses, often occurs in aging and related diseases ^[10].

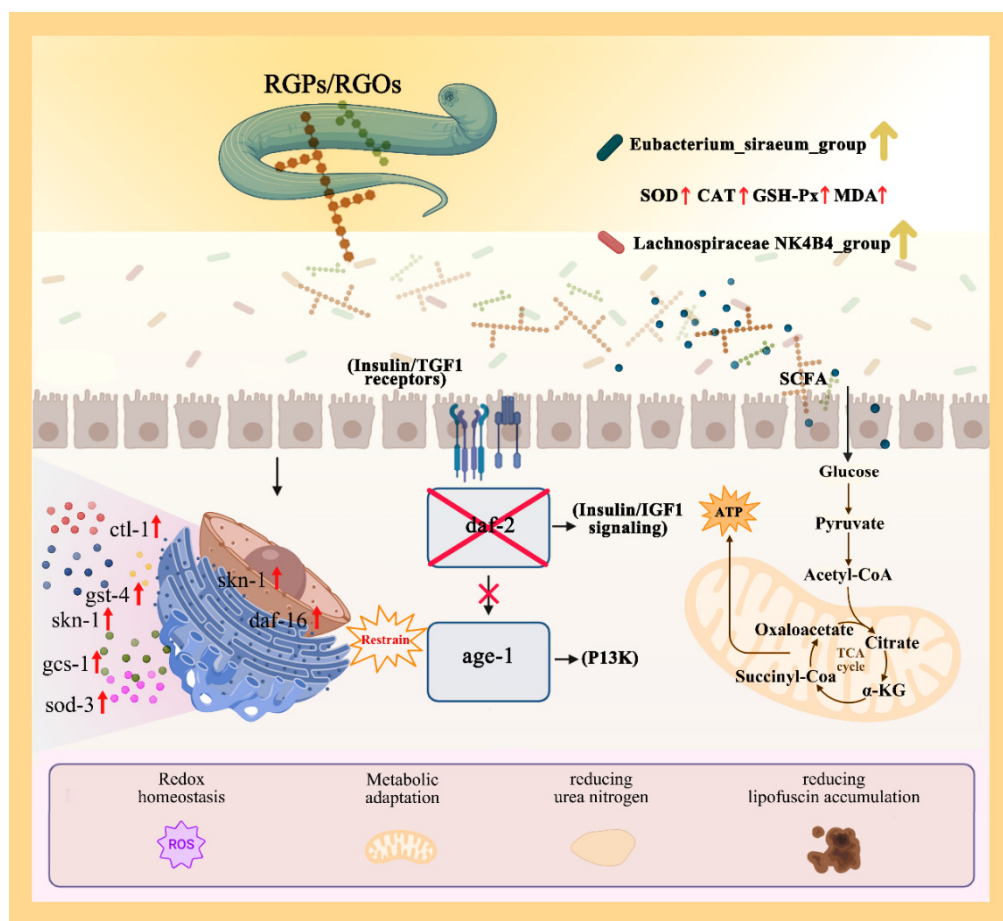


Fig. 5. Anti-aging activities of RGPs: RGPs could combat aging in *Caenorhabditis elegans* by boosting the antioxidant enzyme system, increasing the abundance of *Eubacterium_siraeum_group* and *Lachnospiraceae NK4B4_group*, modulating the insulin/IGF-1 signaling (IIS) pathway, upregulating *skn-1*, *daf-16*, *gst-4*, *gcs-1*, *ctl-1*, and *sod-3*, down-regulating *age-1* gene expression, and modulating choline, citrate, and lactate levels in the TCA cycle.

4.3 Immunomodulatory activities

Natural products demonstrate significantly low toxicity profiles in both humans and animals, while stimulating broad-spectrum immunomodulatory capabilities. Several studies have demonstrated that RGPs can enhance immunomodulatory activities in fish, mice, and human dendritic cells, and the detailed review is as follows (Figure 6):

Interleukin 2 (IL-2) activates Th1 and Th2 cells, While interleukin-4 (IL-4), predominantly produced by Th2 lymphocytes, mast cells, basophils, and eosinophils, mediates allergic responses. Moreover, IL-10, produced by Th0/1/2 cells and monocytes/macrophages, is a key anti-inflammatory cytokine that inhibits Th1 cell production of proinflammatory mediators [48]. SOD is an enzyme that plays a key role in cellular protection against oxidative stress, facilitating the conversion of superoxide anions into hydrogen peroxide. Conversely, CAT is an enzyme that safeguards the body from oxidative damage caused by reactive oxygen species by transforming endogenous hydrogen peroxide into water [49]. RGPs from RRP demonstrated immune and antioxidant activities by increasing serum levels of IL-2, IL-4, and interleukin-10 (IL-10), while also enhancing the activities of GSH, SOD, CAT, and GSH-Px. Additionally, these polysaccharides can reduce the levels of MDA in the skin of mice exposed to ultraviolet B radiation [50].

Lysozyme (LZM) is a low MW alkaline protein commonly found in the mucus, lymphoid tissues, serum, and other bodily fluids of fish. It is essential for nonspecific immune responses and can cleave the β -1,4 glycosidic bonds present within the peptidoglycan structure of bacterial cell walls [51]. Alkaline phosphatase (AKP) and acid phosphatase (ACP) are key hydrolytic enzymes in signal transduction and energy transformation. They also regulate nonspecific immunity and nutrient metabolism [52]. Additionally, total protein (TP) is significant in immune responses, with elevated total serum protein levels believed to indicate a potent innate immune response. RGP (Shangxi Ciyuan Biotech) enhanced nonspecific immune parameters (LZM, SOD, AKP, ACP, TP) and led to an increase in pro-inflammatory cytokines (IL-1 β , IL-8, TNF- α , IFN- γ), as well as growth hormone and insulin-like growth factors (IGF-I and IGF-II). Additionally, it reduced the expression of anti-inflammatory cytokines (IL-10, and TGF- β) in the fish's kidneys, spleen, and gut [17]. A similar research was reported in *Cyprinus carpio* L. that RGP derived from DRR could enhance nonspecific immunity, antioxidant activity, and resistance to *A. hydrophila*. This enhancement was associated with a significant increase in proliferation and phagocytic activities, stimulation of nitric oxide production, and the release of both pro-inflammatory cytokines (such as TNF- α , IL-1 β , IL-6, IL-12) and anti-inflammatory cytokines (including IL-10 and TGF- β). Additionally, there was an increase in enzymatic activities (LZM and AKP) and antioxidant levels (including T-AOC, T-SOD, CAT, GSH, and GSH-Px) [11]. Zhou et al. [29] purified two polysaccharides that did not exhibit cytotoxicity and enhanced innate immunity by dose-dependently increasing the LZM, TNF- α , and IL-6 secretion from RAW264.7 cells.

B and T lymphocytes play crucial roles in an organism's immune response. T lymphocytes mediate cellular immunity and regulate immune responses, while B lymphocytes are mainly involved in humoral immunity. The proliferation of lymphocytes is the most immediate response to immune challenges. RGP (Shangxi Ciyuan Biotech) significantly enhances the proliferation of T and B lymphocytes, both individually and in synergy with phytohemagglutinin and lipopolysaccharide in vitro. Additionally, it increases the levels of IFN- γ and IL-2 secreted by T lymphocytes [1]. Further research showed that RGP can activate human monocyte-derived dendritic cells (MDDCs) and peripheral blood dendritic cells (PBDCs), highlighting its potential as an immune stimulant. RGP active MDDCs play a primary role by triggering the phosphorylation of ERK, p38, and JNK with the p38 signaling pathway. Additionally, RGP enhances the activation of BDCA1⁺ and BDCA3⁺ PBDCs, which in turn boosts the proliferation and cytokine production (specifically IFN- γ and TNF- α) of both CD4⁺ and CD8⁺ T cells, thereby promoting Th1 and Tc1 cell responses. Furthermore, RGP can promote dendritic cell activation, allowing for the presentation of exogenous antigens via MHC class II and endogenous antigens through MHC class I [53].

RGP could activate immune responses in the lungs of C57BL/6 mice when administered intranasally by promoting the activation of dendritic cells (DCs) in the mediastinal lymph node (mLN). This activation is linked to the upregulation of C-C motif chemokine receptor 7 and the increased production of pro-inflammatory cytokines in the lung microenvironment, including IFN- γ and TNF- α . Furthermore, the combination treatment of RGP with OVA led to a significant proliferation of OVA-specific TCR transgenic

CD8⁺ T cells and CD4⁺ T cells in the mLN. Additionally, administering RGP with the tyrosinase-related protein 2 peptide, a self-antigen associated with melanoma, effectively protected the mice from melanoma challenges. These findings suggest that RGP enhances DC activations and facilitates antigen-specific T-cell responses, making it a promising mucosal adjuvant for immunotherapeutic strategies against lung cancer and potentially other malignancies^[54]. Additionally, Qian et al.^[2] reported that polysaccharides from FRR, DRR, and RRP can improve blood-enriching and immunomodulatory activities by elevating the hematocrit, hemoglobin (HGB) levels and stimulating mRNA levels of TNF- α , IL-6, IL-4, and IL-10. Jia et al.^[55] reported that HRP-derived RGP demonstrated immunomodulatory effects by altering gut microbiota and activating the TLR4/NF- κ B signaling pathway in mice with cyclophosphamide-induced immunosuppression. Specifically, RGP increased the abundance of *Lachnospiraceae* and *Erysipelotrichaceae* while reducing *Lactobacillaceae* levels. Additionally, it activated the colonic TLR4/MyD88/NF- κ B pathway and the LC3/Beclin-1/p62 pathway, which promotes autophagy.

RGPs enhance the immune system directly and can be formulated into liposomes or nano-adjuvant to improve their bioavailability and therapeutic efficacy. Huang et al.^[56] created a biomimetic antibacterial vaccine by applying outer membrane vesicles (OMVs) onto the RGP (Ciyuan Biotechnology Co. Ltd) nanocarriers (pRL) through a repeated extrusion process. pRL-OMV demonstrated targeted delivery to lymph nodes and effectively activate dendritic cells, as evidenced by increased production of DC-associated cytokines and enhanced phagocytic activity. Additionally, pRL-OMV significantly promoted the maturation of dendritic cells, leading to mixed solid Th1/Th2/Th17 cellular responses and specific antibody responses against *Bordetella bronchiseptica* infection, with its immune protective effects linked to the genetic regulation of T cell receptor signaling pathways and T cell differentiation. pRL enhances drug-targeting efficiency, significantly stimulates macrophage proliferation, and boosts pro-inflammatory cytokine production, establishing pRL as a potent inducer of M1 phenotype polarization. Macrophages uptake pRL via endocytosis mechanisms such as macropinocytosis and caveolae, which may enhance immunological effectiveness^[57].

Liposomes, spherical vesicles of one or more concentric phospholipid bilayers, are effective adjuvants for vaccine antigens aimed at various bacteria, viruses, and tumors. They have been extensively studied as carriers for drugs and vaccines due to their capability to encapsulate a diverse range of bioactive substances within their aqueous core or embedded within the lipid bilayer. RGP liposome (RGPL) demonstrated effective control over drug release. In vitro, RGPL not only stimulated the maturation of DCs but also enhanced the functionality of mature DCs by increasing the expression of MHC II and CD86 on CD11c⁺ cells. In vivo, RGPL elicited robust antigen-specific immune responses characterized by elevated levels of OVA/PCV-2-specific IgG, maintaining these responses over an extended period. Additionally, RGPL effectively induces immune memory and promotes DC activation in the lymph nodes^[57-58].

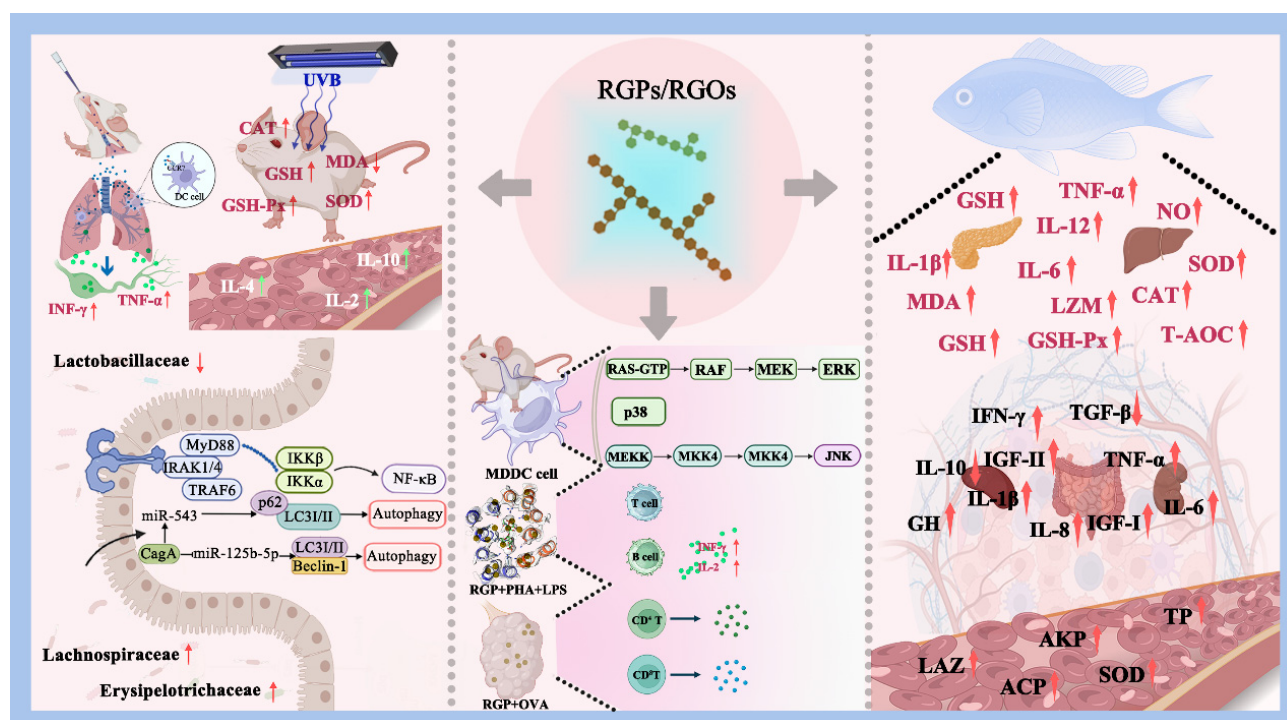


Fig. 6. Immunomodulatory activities of RGOs/RGPs: RGP administered intranasally can activate dendritic cells (DCs) and promote pro-inflammatory cytokine production; RGP enhances immune function and reduce MDA levels in UVB-exposed mouse skin; RGP activates the TLR4/NF-κB signaling pathway, regulates autophagy pathways, and interacts with the gut microbiota to jointly modulate immune regulation; RGP or activate immune cells, thereby exerting immunoregulatory effects; RGP enhances nonspecific immunity, antioxidant activity, and pro-inflammatory cytokine production while modulating anti-inflammatory responses in fish.

4.4 Anti-inflammatory activity

A large number of studies focus on the hypoglycemic activity of RGOs, as well as the immunomodulatory and anti-aging activities of RGPs. In addition, RGPs could anti-inflammatory, suppress busulfan-induced aplastic anemia, improve osteoporosis, attenuate colitis, and antioxidant, antiviral, and anti-cancer activities, which could, directly and indirectly, realize the “nourishing Yin, supplementing blood, boosting essence, and filling the marrow.”

Supplementation with RGP (Shangxi Ciyuan Biotech) significantly alleviated DSS-induced colitis, likely by restoring the disrupted intestinal microbiome (*Lactobacillus*, *Alistipes*, and *Lachnospiraceae_NK4A136*) and its metabolites. This restoration enhances the intestinal epithelial barrier, increases the expression of tight junction proteins (ZO-1, claudin-1, and occludin), and reduces the overproduction of pro-inflammatory factors (TNF-α, IL-1β, and IL-6,) via the NF-κB signaling pathway [59]. RGP extracted from DRR demonstrated significant anti-inflammatory effects in the LPS-stimulated RAW264.7 model by reducing nitric oxide secretion and iNOS expression in a dose-dependent manner, likely via the NF-κB/IκBα signaling pathway [28]. RGP from DRR suppressed ROS production, reduced the levels of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6), and diminished the translocation of NF-κB-p65 from the cytoplasm to the nucleus [25].

4.5 Hematopoietic, Osteogenic and Myotrophic Effects

RGP could inhibit induced aplastic anemia by suppressing the HIF-1 α /NF- κ B signaling pathway, effectively reversing busulfan-induced inflammation and hematopoietic dysfunction. Additionally, RGP reduced the M1/M2 macrophage ratio and corrected cytokine imbalances, upregulating the expression of IL-4, IL-10, and VEGF while downregulating IL-6, IL-12, TNF- α , MCP-1, IL-1 β , and EPO. Furthermore, RGP increased the quantities of HGB, white blood cells, red blood cells, and platelets [60]. The development and aging of the skeletal and muscular systems are interconnected, with osteoporosis formation linked to the decline in coordination between bones and skeletal muscles. RGP (Shanghai Ronghe Pharmaceutical Technology Development Co., Ltd.) could significantly improve the bone structure of osteoporotic rats by promoting bone formation, inhibiting bone resorption, and enhancing skeletal muscle antioxidant status. Additionally, RGP reduced the expression of muscle-specific E3 ubiquitin-protein ligases (MAFbx and MuRF1) and the transcription factor FOXO1, suggesting its potential mechanism for mitigating muscle atrophy through inhibition of the ubiquitin-proteasome pathway [61].

4.6 Other activities

Pseudorabies is an acute and reportable infection primarily caused by the pseudorabies virus (PRV), which leads to reproductive issues, encephalomyelitis, and reproductive failure in pigs. PRV poses a significant threat to the global swine industry. Existing vaccines for pigs do not provide complete protection against PRV, and currently, no antiviral therapies are available for managing PRV infections. RGP (Shangxi Ci yuan Biotech) prevents PRV/XJ5 infection by inhibiting virus adsorption and entry, suppressing viral replication, and lowering PRV/XJ5-induced ROS levels and lipid oxidation while also enhancing SOD levels in PK-15 cells [62]. RGPs from DRR can alleviate oxidative stress in IPEC-1 cells by regulating the Nrf2/Keap1 pathway, reducing oxidative damage markers (LDH leakage and MDA levels), enhancing antioxidant enzymes (SOD and CAT), restoring mitochondrial membrane potential, and suppressing caspase-3 activation [19]. RGP (Shangxi Ci yuan Biotech.) enhanced NK cell activation in C57BL/6 mice by increasing circulating NK cell numbers and proliferation in various tissues, promoting TLR-4-dependent interferon-gamma production and CD69 expression, which improved cytotoxic activity against Yac-1 cells and inhibited CT26 tumor growth in the lung [63]. RGPs from RRP exhibited effects that reduce anxiety, and the underlying mechanisms may be associated with the inhibition of MSG-induced reductions in β -synuclein, DJ-1, peroxiredoxin-2, peroxiredoxin-6, DDAH-1, and iron-sulfur proteins within the hippocampus of rats [64].

It is noteworthy that the applications of RGOs/RGPs in food and pharmaceutical fields necessitate rigorous safety evaluation as a prerequisite consideration. RGOs demonstrate multiple beneficial health effects, including hepatoprotective, hypoglycemic, and neuroprotective properties, along with protective effects against gut barrier damage. RGPs demonstrate therapeutic potential in anti-aging, immune modulation, cancer treatment, and diabetes management. The dose-dependent protective effects without toxicity thresholds suggest RGOs/RGPs wide therapeutic window and organ-protective potential. However, most existing evidence comes from animal models, which may not fully predict human safety profiles.

Comprehensive safety evaluations in human subjects remain limited, with insufficient data on potential side effects or long-term toxicity. Further research is urgently needed to establish a clear safety profile and ensure safe clinical applications of RGPs.

5. Structure-activity relationship

Extensive research has established that the pharmacological efficacy of saccharides is intrinsically linked to their structural characteristics. This structure-activity relationship is particularly relevant in RG, where the dynamic transformations of saccharides during processing (from saccharide degradation to polysaccharide accumulation) directly modulate bioactivity. The primary structure (monosaccharide sequence/branching), higher-order conformation (helical/random coil arrangements), and MW distribution—all altered through drying or steaming—collectively determine the immunomodulatory, hematopoietic, and metabolic regulation properties of different processed forms. The structure-activity relationship of RGOs/RGPs and their various bioactivities are shown in Tables. Systematic classification of these structural and bioactive modifications provides: (1) a chemical rationale for the differential efficacy between FRR, DRR, RRP and (2) a theoretical framework for targeted saccharides screening in herbal processing. Raffinose family oligosaccharides—a class of non-digestible prebiotics—demonstrate multifaceted health-promoting effects, including antioxidative capacity, intestinal microbiota modulation to maintain homeostasis, tissue repair enhancement, immune potentiation, anticancer activity and so on [65]. RGOs from RG—typically composed of stachyose, manninotriose, sucrose, and raffinose in varying ratios—exhibit multimodal antidiabetic and hepatoprotective effects. They regulate glucose metabolism by suppressing hepatic gluconeogenesis ($\downarrow$ glucose-6-phosphatase), enhancing glycogen synthesis, and inhibiting intestinal α -glucosidase. RGOs also mitigate diabetic complications via HPA axis modulation, improving neuroendocrine-immune dysfunction and reducing oxidative stress ($\uparrow$ GSH/SOD, $\downarrow$ MDA). In liver disorders, they attenuate fibrosis and hepatocellular damage while inducing p53-mediated apoptosis in carcinoma cells. These actions stem from their unique oligosaccharide profile and multi-target mechanisms. Building on this carbohydrate structure-activity relationship, RGPs exhibit molecular-weight-dependent bioactivities. The high-molecular-weight RGPs (12.1×10^4 – 193.7×10^4 Da) exhibit structure-dependent bioactivities, where their monosaccharide composition determines specific pharmacological effects: (1) Glucose- and galactose-dominated RGPs demonstrate multifaceted activities including anti-inflammatory, antitumor, hematinic, and immunomodulatory effects [2,9]; (2) Glucose-xylose-based RGP primarily shows antioxidant capacity [18]; (3) particularly contribute to hematinic and immunomodulatory properties [2]. Medium-molecular-weight RGPs (1.19×10^4 – 6.35×10^4 Da) demonstrate multiple bioactive properties, including antioxidant, hypoglycemic, anti-inflammatory, and immunomodulatory activities. Structural analysis reveals that: (1) Glucose- and galactose-enriched RGPs demonstrate predominant antioxidant capacity [19]; (2) RGPs containing $\rightarrow 5$)- α -L-Araf-(1 $\rightarrow$ and $\rightarrow 3,5$)- α -L-Araf-(1 $\rightarrow$ linkages in their main chain exhibit potent hypoglycemic and anti-inflammatory effects [28]; (3) galactose and arabinose moieties are crucial for immunomodulatory activity [29]; and (4) glucose-galactose dominant RGPs show significant

hypoglycemic effects^[40]. Low-molecular-weight RGP with MWs ranging from 2.03×10^3 to 6.2×10^3 Da, composed primarily of glucose, galactose, and mannose, exhibit significant anti-inflammatory and anti-cancer activities. A specific structural variant of these RGPs, characterized by a galactose backbone featuring 1,4- α -D-Galp, 1,6- α -D-Galp, and 1,3,4,6- β -D-Galp linkages, demonstrates notable anti-aging effects^[22-23,45]. Furthermore, another structural type containing arabinose-rich backbones with $\rightarrow 5$)- α -L-Araf-(1 $\rightarrow$, $\rightarrow 3$)- α -L-Araf-(1 $\rightarrow$, $\rightarrow 2,3,5$)- α -L-Araf-(1 $\rightarrow$, and $\rightarrow 2,5$)- α -L-Araf-(1 $\rightarrow$ linkages exhibits hypoglycemic activity^[25].

RGPs containing high levels of glucose and galactose exhibit anti-aging activities by modulating the IIS pathway, enhancing the antioxidant enzyme system, restoring the intestinal microbiota, and regulating the expression of key genes (such as *age-1*, *skn-1*, *daf-16*, *gst-4*, *gcs-1*, *ctl-1*, and *sod-3*). They also influence choline, lactate, and citrate levels in the TCA cycle^[22-23,45]. RGPs mainly containing galactose, arabinose, and/or galacturonic acid exhibited immunomodulatory activities by increasing the secretion of IL-2, IL-4, IL-10, IL-1 β , IL-8, TNF- α , IFN- γ , enhancing the activities of LZM, SOD, AKP, ACP, TP, GSH, CAT, GSH-Px, promoting the proliferation of T and B lymphocytes, activating DCs, and modulating the gut microbiota^[2,29].

6. Discussion

RGOs could regulate glucose metabolism through a regulatory impact on glucose metabolism, regulating the hyperactivity of the HPA axis, regulating the dysfunction of the NIM network, inhibiting intestinal α -glucosidase activity, and hepatic gluconeogenesis. However, the synergistic interaction of stachyose with other compounds may enhance its hypoglycemic effects. Li et al.^[66] reported that the combined administration of genistein and stachyose led to a more significant reduction in abnormal weight gain, liver fat accumulation in mice fed a high-fructose diet through lowering the expression of fatty acid synthetase and restoring normal lipid metabolism, and reduce liver damage. The combination of catalpol and stachyose from FRR, particularly in a 1:1 ratio, demonstrated effective regulation of blood glucose levels, lipid profiles, and both hepatic and renal function^[67]. Additionally, RGPs with galactose, glucose, mannose, arabinose, and/or rhamnose exhibited hypoglycemic activities^[27,40]. RGPs containing a high proportion of arabinose and minor amounts of glucose and/or xylose exhibited hypoglycemic and anti-inflammatory activities^[28]. In the future, it is essential to consider the synergistic effects of RGPs and RGOs and the efficacy of combinations with oligosaccharides and other substances, such as phenylethanoid glycosides and iridoid glycosides.

Beyond metabolic regulation, raffinose family oligosaccharides act as effective prebiotics, enhancing gut health by inhibiting pathogenic adhesion while fostering beneficial microbes like *Bifidobacteria* and *Lactobacilli*. Their fermentation yields immune-modulating metabolites and influences neurological functions, including cognition and mood^[13,65]. Raffinose family oligosaccharides are also linked to broader health benefits, such as reduced cancer risk, improved cardiovascular health, enhanced immunity, and anti-obesity effects. Polysaccharides are key bioactive components in RG, playing critical pharmacological roles. RGPs exhibit diverse tonic effects, including anti-aging, immunomodulatory,

antioxidant, and anti-fatigue activities, alongside therapeutic properties such as anti-inflammatory, anti-fibrotic, and anti-cancer actions. Furthermore, RGPs have been engineered into nano-adjuvants to potentiate immune responses, enhance bioavailability, and improve therapeutic efficacy. With rising demand for preventive healthcare, RGOs/RGPs hold significant potential as functional additives in food, medicinal, and cosmetic applications. RGOs/RGPs' multi-target effects and dual food-medicine status—historically documented in the 16th-century *Bencao Gangmu* and recently confirmed by China's 2024 National Health Commission list—position them as prime candidates for functional food development, with significant potential for nutritional and therapeutic applications. This means that the development and application of RG in food will receive widespread attention, entering a rapid growth phase with a significant increase in usage. The author also has some insights into development.

Firstly, the FRR is well-suited for processing various food products, including candied or preserved fruits, jams, canned fruits, beverages, and other ready-to-eat items. Additionally, FRR is an excellent source of prebiotics, especially stachyose, which can promote health. There is considerable potential to investigate its broader pharmacological effects and mechanisms. Its interactions with other molecules influence its pharmacological actions, opening avenues for developing pharmaceutical products and functional foods [68-71].

Secondly, polysaccharide content in FRR and DRR is low, but when processed into RRP, the content and composition of polysaccharides change. While the degradation of polysaccharides during processing is well-documented, the observed increase in content is surprising. Without a catalyst, synthesizing polysaccharides from monosaccharides and oligosaccharides is challenging. Therefore, understanding the mechanisms behind the transformation of functional polysaccharides during processing is crucial, as this transformation may lead to changes in the functionality of RGPs. However, the specific impact of this process on the structure of polysaccharides and their subsequent functions remains unclear. Thus, clarifying the mechanisms responsible for the increase in active polysaccharides in RRP is essential for optimizing the processing methods of this herb. Investigating these mechanisms will guide and refine preparation methods, supporting the efficient production of RRP with high levels of bioactive components and enhanced therapeutic effects, ultimately improving processing efficiency and conserving manpower, financial, and material resources.

Thirdly, the structure of polysaccharides in DRR and the bioactivities of polysaccharides derived from RRP have been extensively studied and reported. However, there is limited research on the specific structures of polysaccharides from RRP, and in-depth investigations into their active mechanisms and structure-activity relationships—particularly regarding their tonifying effects—are lacking. Currently, polysaccharides are primarily extracted using hot or boiling water, and there is a deficiency in innovative, efficient, and environmentally friendly extraction technologies. Future advancements should prioritize the development of efficient and environmentally friendly extraction technologies for RGPs, including subcritical water extraction, ionic liquid-assisted extraction, deep eutectic solvent-water systems, and ionic

liquid-based selective extraction, as well as acid and enzyme-assisted extraction. In contrast, microbial fermentation, biochemical methods (such as enzymatic, acid, and hydrothermal degradation), and chemical synthesis could be developed to obtain the production of RGOs [13,72–76]. Additionally, the decolorization process presents certain challenges, as the polysaccharides often appear in a blackish state. This raises several important questions: Is it necessary to thoroughly remove these black substances? How do they interact with polysaccharides—through intermolecular forces or binding states? What is their contribution to the overall efficacy? Do they exhibit a synergistic effect with polysaccharides (i.e., $1 + 1 > 2$), or do they reduce the activity of the polysaccharides? These issues are commonly encountered by researchers studying polysaccharides in traditional Chinese medicine, particularly in various herbal medicines such as *Polygonatum*, *Codonopsis*, *Astragalus*, and *Ginseng*. Therefore, investigating how these black substances interact with polysaccharides to exert their tonic effects jointly is a topic worthy of in-depth exploration [77].

Finally, the 2020 edition of the “Chinese Pharmacopoeia” states that RRP functions to “nourish the blood, enrich yin, boost essence, and fill the marrow”. However, the contribution rate of polysaccharides, one of the main components of RRP, to this function requires further investigation. Therefore, further investigation into the functions of polysaccharides from RRP in nourishing blood, enriching yin, and benefiting essence and marrow is of great significance. Further investigation into the functions of polysaccharides from RRP is of great significance, as they are involved in nourishing blood (enhancing erythropoiesis and improving blood circulation), enriching yin (regulating endocrine function and providing antioxidant effects), benefiting essence (enhancing reproductive health and supporting overall vitality), and filling marrow (promoting hematopoiesis and supporting nervous system health).

7. Conclusions

From the above review, we can conclude that RGOs with stachyose as the main oligosaccharide mainly come from FRR/DRR and exhibit hypoglycemic and hepatoprotective effects, making it a preferred ingredient for the development of hypoglycemic drugs and health foods. RGOs exhibit a range of beneficial activities, including anti-aging and immunomodulatory effects, suppression of busulfan-induced aplastic anemia, improvement of osteoporosis, alleviation of colitis, antioxidant, anti-inflammatory, antiviral, and anti-cancer properties. These effects can, directly and indirectly, support the principles of “nourishing Yin, supplementing blood, boosting essence, and filling the marrow” as associated with *Rehmanniae Radix Preparata*.

Acknowledgments

This research was supported by Henan Provincial Joint Fund for Science and Technology Research and Development (Advantageous Discipline Cultivation Project) (232301420101, 242301420117), Key Scientific Research Projects of Colleges and Universities in Henan Province (23A230006), Development on the Whole Industry Chain of Yu-Yao (2024YYXT-KFKT-08), Ministry of Science and Technology (MOST 111-2221-E-005-026-MY3, MOST 111-2221-E-005-009, and NSTC 113-2221-E-005-008-MY3).

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Reference

- [1] Y. Huang, C.M. Jiang, Y.L. Hu, X.J. Zhao, C. Shi, Y. Yu, C. Liu, Y. Tao, H.R. Pan, Y.B. Feng, J.G. Liu, Y. Wu, D. Wang, Immunoenhancement effect of *Rehmannia glutinosa* polysaccharide on lymphocyte proliferation and dendritic cell, Carbohydr. Polym. 96 (2013) 516–521. <https://doi.org/10.1016/j.carbpol.2013.04.018>.
- [2] Y.Y. Qian, L. Wang, Z.Q. Zhang, X.X. Li, C.F. Niu, X. Li, E.J. Ning, B.J. Ma, Physical-chemical properties of heteropolysaccharides from different processed forms of *Rehmanniae Radix*, Process. Biochem. 121 (2022) 481–492. <https://doi.org/10.1016/j.procbio.2022.07.032>.
- [3] Chinese Pharmacopoeia Commission, Pharmacopoeia of the People's Republic of China, China Medical Science Press, Beijing, China, 2020.
- [4] R.X. Zhang, M.X. Li, Z.P. Jia, *Rehmannia glutinosa*: review of botany, chemistry and pharmacology, J. Ethnopharmacol. 117 (2008) 199–214. <https://doi.org/10.1016/j.jep.2008.02.018>.
- [5] Y.H. Jia, C.N. Wen, M. Liu, R. Y, Y.Y. Qian, B.J. Ma, L. Wang, Study on physicochemical properties of polysaccharides and glycosides from *Rehmannia glutinosa* under different processing conditions, Food Nutr. Chem. 2 (2024) 168. <https://doi.org/10.18686/fnc.v2i2.168>.
- [6] M.M. Li, H.J. Jiang, Y.L. Hao, K.Q. Du, H.L. Du, C. Ma, H. Tu, Y. He, A systematic review on botany, processing, application, phytochemistry and pharmacological action of *Radix Rehmanniae*, J. Ethnopharmacol. 285 (2022) 114820. <https://doi.org/10.1016/j.jep.2021.114820>.
- [7] R.X. Zhang, J.H. Zhou, Z.P. Jia, Y.X. Zhang, G.M. Gu, Hypoglycemic effect of *Rehmannia glutinosa* oligosaccharide in hyperglycemic and alloxan-induced diabetic rats and its mechanism, J. Ethnopharmacol. 90 (2004) 39–43. <https://doi.org/10.1016/j.jep.2003.09.018>.
- [8] Y.Y. Qian, L. Wang, C.N. Wen, Y. Zhou, X. Li, L.X. Zhang, B.J. Ma, Purification, physicochemical property and antioxidant activity analysis of oligosaccharides from the fresh roots of *Rehmannia glutinosa* Libosch, Nat. Prod. Res. Dev. 33 (2021) 1470–1477. <https://link.cnki.net/doi/10.16333/j.1001-6880.2021.9.004>.
- [9] M.K. Lu, C.C. Chang, C.H. Chao, Y.C. Hsu, Structural changes, and anti-inflammatory, anti-cancer potential of polysaccharides from multiple processing of *Rehmannia glutinosa*, Int. J. Biol. Macromol. 206 (2022) 621–632. <https://doi.org/10.1016/j.ijbiomac.2022.02.112>.
- [10] W. Tan, K.Q. Yu, Y.Y. Liu, M.Z. Ouyang, M.H. Yan, R. Luo, X.S. Zhao, Anti-fatigue activity of polysaccharides extract from *Radix Rehmanniae Preparata*, Int. J. Biol. Macromol. 50 (2012) 59–62. <https://doi.org/10.1016/j.ijbiomac.2011.09.019>.
- [11] J.C. Feng, Z.L. Cai, X.P. Zhang, Y.Y. Chen, X.L. Chang, X.F. Wang, C.B. Qin, X. Yan, X. Ma, J.X. Zhang, G.X. Nie, The effects of oral *Rehmannia glutinosa* polysaccharide administration on immune responses, antioxidant activity and resistance against *Aeromonas hydrophila* in the common carp, *Cyprinus carpio* L, Front. Immunol. 11 (2020) 904. <https://doi.org/10.3389/fimmu.2020.00904>.
- [12] R.X. Zhang, J. Zhou, M.X. Li, H.G. Ma, J.G. Qiu, X.H. Luo, Z.P. Jia, Ameliorating effect and potential mechanism of *Rehmannia glutinosa* oligosaccharides on the impaired glucose metabolism in chronic stress rats fed with high-fat diet, Phytomedicine. 21 (2014) 607–614. <https://doi.org/10.1016/j.phymed.2013.11.008>.
- [13] R.J. Zhang, Y. Zhao, Y.F. Sun, X.S. Lu, X.B. Yang, Isolation, characterization, and hepatoprotective effects of the raffinose family oligosaccharides from *Rehmannia glutinosa* Libosch, J. Agric. Food Chem. 61 (2013) 7786–7793. <https://doi.org/10.1021/jf4018492>.

- [14] X. Li, R. Gui, X.F. Wang, E.J. Ning, L.X. Zhang, Y. Fan, L. Chen, L.Q. Yu, J. Zhu, Z.N. Li, L. Wei, W. Wang, Z.H. Li, Y. Wei, X.B. Wang, Oligosaccharides isolated from *Rehmannia glutinosa* protect LPS-induced intestinal inflammation and barrier injury in mice, *Front. Nutr.* 10 (2023) 1139006. <https://doi.org/10.3389/fnut.2023.1139006>.
- [15] W.R. Zong, K.L. Cheong, D.T. Wu, J. Li, J. Zhao, S.P. Li, Preparation and purification of raffinose family oligosaccharides from *Rehmannia glutinosa* Libosch by fast protein liquid chromatography coupled with refractive index detection, *Sep. Purif. Technol.* 138 (2014) 98-103. <https://doi.org/10.1016/j.seppur.2014.10.001>.
- [16] J.J. Yang, C. Xue, L.H. Zhang, N. Meng, J. Yang, Y. Cui, Y.F. Wang, X. Chai, Exploration on the mechanism of “black as lacquer and sweetness as candy” based on the reactions of “crosslinking coloring and Maillard” and “oligosaccharide hydrolysis” during the processing of *Radix Rehmanniae*, *Arab. J. Chem.* 17 (2024) 105917. <https://doi.org/10.1016/j.arabjc.2024.105917>.
- [17] C. Wu, J.F. Shan, J.C. Feng, J.L. Wang, C.B. Qin, G.X. Nie, C.L. Ding, Effects of dietary *Radix Rehmanniae Preparata* polysaccharides on the growth performance, immune response and disease resistance of *Luciobarbus capito*, *Fish. Shellfish. Immun.* 89 (2019) 641-646. <https://doi.org/10.1016/j.fsi.2019.04.027>.
- [18] C.Y. Liu, T.T. Zhang, P. Zhao, S.M. Liu, X.Y. Li, Y.F. Yuan, Purification and structural analysis of a novel polysaccharide from *Rehmannia Radix Praeparata*, *Chem. Biol. Drug. Des.* 102 (2023) 514-522. <https://doi.org/10.1111/cbdd.14261>.
- [19] H. Ren, Z.Y. Li, R. Gao, T.X. Zhao, D. Luo, Z.H. Yu, S. Zhang, C. Qi, Y.Q. Wang, H.Z. Qiao, Y.M. Cui, L.P. Gan, P. Wang, J.R. Wang, Structural characteristics of *Rehmannia glutinosa* polysaccharides treated using different decolorization processes and their antioxidant effects in intestinal epithelial cells, *Foods* 11 (2022) 3449. <https://doi.org/10.3390/foods11213449>.
- [20] W.T. Chang, Y.H. Choi, R. Van der Heijden, M.S. Lee, M.K. Lin, H.W. Kong, H.K. Kim, R. Verpoorte, T. Hankemeier, J. Van der Greef, M. Wang, Traditional processing strongly affects metabolite composition by hydrolysis in *Rehmannia glutinosa* roots. *Chem. Pharm. Bull.* 59 (2011) 546-552. <https://doi.org/10.1248/cpb.59.546>.
- [21] Z.F. Hu, X.P. Wang, H.L. Guo, J.Z. Chen, Determination of oligosaccharide contents in different processed *Radix Rehmannia Praeparata* by HPLC-ELSD, *Lishizhen Med. Mater. Med. Res.* 24 (2013) 877-879. <https://doi.org/10.7501/j.issn.0253-2670.2016.07.012>.
- [22] Y.Y. Yuan, N.X. Kang, Y.H. Lu, X.Z. Miao, X. Zhang, Y.G. Liu, P. Tan, The effects of polysaccharides from *Rehmannia glutinosa* on *Caenorhabditis elegans*, *Pharmacogn. Mag.* 15 (2019). http://dx.doi.org/10.4103/pm.pm_618_18.
- [23] Y.M. Yue, L.Y. Liang, H. Zhang, C.Q. Li, M.T. Zhao, M.R. Yang, X. Cao, L.Y. Zhong, J.Y. Du, R.X. Shi, R.F. Liang, J.M. Yu, H. Zhao, Y. Q. Zhang, Z.P. Shu, Antioxidant and anti-aging effects of purified *Rehmannia glutinosa* polysaccharide in *Caenorhabditis elegans*, *Process Biochem.* 137 (2024) 41-53. <https://doi.org/10.1016/j.procbio.2023.12.009>.
- [24] M. Tomoda, H. Miyamoto, N. Shimizu, R. Gonda, N. Ohara, Characterization of two polysaccharides having activity on the reticuloendothelial system from the root of *Rehmannia glutinosa*, *Chem. Pharm. Bull.* 42 (1994) 625-629. <https://doi.org/10.1248/cpb.42.625>.
- [25] H. Zhang, Y.M. Yue, Q. Zhang, L.Y. Liang, C.Q. Li, Y. Chen, W. Li, M.M. Peng, M.R. Yang, M.T. Zhao, X. Cao, L.Y. Zhong, J.Y. Du, Y. Wang, X. Zhou, Z.P. Shu, Structural characterization and anti-inflammatory effects of an arabinan isolated from *Rehmannia glutinosa* Libosch, *Carbohydr. Polym.* 303 (2023) 120441. <https://doi.org/10.1016/j.carbpol.2022.120441>.
- [26] Z.C. Wang, Y. Zheng, Z.R. Lai, X.L. Hu, L. Wang, X.Q. Wang, Z.T. Li, M.J. Gao, Y.H. Yang, Q. Wang, N. Li, Effect of monosaccharide composition and proportion on the bioactivity of polysaccharides: A review, *Int. J. Biol. Macromol.* 254 (2024) 127955. <https://doi.org/10.1016/j.ijbiomac.2023.127955>.
- [27] H.E. Chen, X.Y. Liu, M.X. Xie, X.T. Zhong, C.Y. Yan, M.H. Xian, S.M. Wang, Two polysaccharides from *Rehmannia glutinosa*: isolation, structural characterization, and hypoglycemic activities, *RSC Adv.* 13 (2023) 30190-30201. <https://doi.org/10.1039/D3RA05677E>.
- [28] M.X. Xie, J.L. Cai, X.T. Zhong, J.Y. Liang, J.Y. Liang, S.W. Liang, M.H. Xian, C.Y. Yan, S.M. Wang, Extraction and structural profiling of polysaccharides from *Rehmannia glutinosa* and anti-inflammatory action via the NF-κB/IκBα pathway, *Ind. Crop. Prod.* 208 (2024) 117874. <https://doi.org/10.1016/j.indcrop.2023.117874>.
- [29] Y. Zhou, S.C. Wang, W.S. Feng, Z.L. Zhang, H.W. Li, Structural characterization and immunomodulatory activities of two polysaccharides from *Rehmanniae Radix Praeparata*, *Int. J. Biol. Macromol.* 186 (2021). 385-395. <https://doi.org/10.1016/j.ijbiomac.2021.06.100>.

- [30] B.L. Tardy, B.D. Mattos, C.G. Otoni, M. Beaumont, J. Majoinen, T. Kämäräinen, O.J. Rojas, Deconstruction and reassembly of renewable polymers and biocolloids into next generation structured materials, *Chem. Rev.* 121 (2021) 14088-14188. <https://doi.org/10.1021/acs.chemrev.0c01333>.
- [31] H.J. Yuan, S.Y. Lin, G.J. Zhang, J.M. Jin, X.B. Jia, J.J. Chen, L. Feng, New thoughts on material basis and mechanism of Yin-tonifying traditional Chinese medicine in treatment of diabetes mellitus and its complications, *Chin. J. Chin. Mater. Med.* 45 (2020) 531-538. <https://doi.org/10.19540/j.cnki.cjcmm.20190826.502>.
- [32] C. Ning, Y.H. Jiao, J.Q. Wang, W.W. Li, J.Q. Zhou, Y.C. Lee, D.L. Ma, C.H. Leung, R.G. Zhu, H.M.D. Wang, Recent advances in the managements of type 2 diabetes mellitus and natural hypoglycemic substances, *Food Sci. Hum. Well.* 11 (2022). 1121-1133. <https://doi.org/10.1016/j.fshw.2022.04.004>.
- [33] X.L. Ji, J.H. Guo, T.Z. Cao, T.T. Zhang, Y.Q. Liu, Y.Z. Yan, Review on mechanisms and structure-activity relationship of hypoglycemic effects of polysaccharides from natural resources, *Food Sci. Hum. Well.* 12 (2023) 1969-1980. <https://doi.org/10.1016/j.fshw.2023.03.017>.
- [34] H.T. Wang, H.L. Li, Y.T. Hou, P.J. Zhang, M.Q. Tan, Plant polysaccharides: Sources, structures, and antidiabetic effects, *Curr. Opin. Food Sci.* 51 (2023) 101013. <https://doi.org/10.1016/j.cofs.2023.101013>.
- [35] T.W. Lau, D.S. Sahota, C.H. Lau, C.M. Chan, F.C. Lam, Y.Y. Ho, K.P. Fung, C.B.S. Lau, P.C. Leung, An in vivo investigation on the wound-healing effect of two medicinal herbs using an animal model with foot ulcer, *Eur. Surg. Res.* 41 (2008) 15-23. <https://doi.org/10.1159/000122834>.
- [36] C.Y. Chiu, W.H. Hsu, H.K. Liu, S.H. Liu, Y.L. Lin, Prepared *Rehmanniae Radix* oligosaccharide regulates postprandial and diabetic blood glucose in mice, *J. Funct. Foods.* 41 (2018) 210-215. <https://doi.org/10.1016/j.jff.2017.12.031>.
- [37] L. Mobasheri, M. Ahadi, A.B. Namdar, M.S. Alavi, A. Bemidinezhad, S.M.M. Farahi, M. Esmaeilzadeh, N. Nikpasand, E. Einafshar, A. Ghorbani, Pathophysiology of diabetic hepatopathy and molecular mechanisms underlying the hepatoprotective effects of phytochemicals, *Biomed. Pharmacother.* 167 (2023) 115502. <https://doi.org/10.1016/j.biopha.2023.115502>.
- [38] K.S. Ramadan, O.A. Khalil, E.N. Danial, H.S. Alnahdi, N.O. Ayaz, Hypoglycemic and hepatoprotective activity of *Rosmarinus officinalis* extract in diabetic rats, *J. Physiol. Biochem.* 69 (2013) 779-783. <https://doi.org/10.1007/s13105-013-0253-8>.
- [39] J.C. Chao, S.W. Chiang, C.C. Wang, Y.H. Tsai, M.S. Wu, Hot water-extracted *Lycium barb arum* and *Rehmannia glutinosa* inhibit proliferation and induce apoptosis of hepatocellular carcinoma cells, *World J. Gastroentero.* 12 (2006) 4478. <https://dx.doi.org/10.3748/wjg.v12.i28.4478>.
- [40] J. Zhou, G. Xu, J.Y. Yan, K.C. Li, Z.S. Bai, W.N. Cheng, K.X. Huang, *Rehmannia glutinosa* (Gaertn.) DC. polysaccharide ameliorates hyperglycemia, hyperlipemia and vascular inflammation in streptozotocin-induced diabetic mice, *J. Ethnopharmaco.* 164 (2015) 229-238. <https://doi.org/10.1016/j.jep.2015.02.026>.
- [41] W.J. Yue, N. Sun, J. Zhang, W.H. Zhang, Y.S. Wu, X.C. Qu, J.W. Zong, G. Xu, Alleviated diabetic osteoporosis and peripheral neuropathic pain by *Rehmannia glutinosa* Libosch polysaccharide via increasing regulatory T cells, *Int. J. Biol. Macromol.* 277 (2024) 134241. <https://doi.org/10.1016/j.ijbiomac.2024.134241>.
- [42] R.S. Rosen, M.L. Yarmush, Current trends in anti-aging strategies, *Annu. Rev. Biomed. Eng.* 25 (1) (2023) 363-385. <https://doi.org/10.1146/annurev-bioeng-120122-123054>.
- [43] Y.J. Du, Y. Gao, B. Zeng, X.L. Fan, D.Y. Yang, M.Y. Yang, Effects of anti-aging interventions on intestinal microbiota, *Gut Microbes.* 13 (2021) 1994835. <https://doi.org/10.1080/19490976.2021.1994835>.
- [44] Y.N. Wang, J.C. Shi, K. Liu, Y. Wang, Y.J. Xu, Y.F. Liu, Metabolomics and gene expression levels reveal the positive effects of teaseed oil on lifespan and aging process in *Caenorhabditis elegans*, *Food Sci. Hum. Well.* 12 (2023) 1391-1401. <https://doi.org/10.1016/j.fshw.2022.10.032>.
- [45] L.Y. Liang, Y.M. Yue, L.Y. Zhong, Y.F. Liang, R.X. Shi, R.F. Luo, M.T. Zhao, X. Cao, M.R. Yang, J.Y. Du, X.J. Shen, Y. Wang, Z.P. Shu, Anti-aging activities of *Rehmannia glutinosa* Libosch. crude polysaccharide in *Caenorhabditis elegans* based on gut microbiota and metabonomic analysis, *Int. J. Biol. Macromol.* 253 (2023) 127647. <https://doi.org/10.1016/j.ijbiomac.2023.127647>.
- [46] A. Mukherjee, C. Lordan, R.P. Ross, P.D. Cotter, Gut microbes from the phylogenetically diverse genus *Eubacterium* and their various contributions to gut health, *Gut Microbes* 12 (2020) 1802866. <https://doi.org/10.1080/19490976.2020.1802866>.

- [47] H.M. Liu, Y. Wang, W.P. Zhang, W.C. Sun, X.X. Ji, S.A. Zhang, K. Qiao, Lentinan extends lifespan and increases oxidative stress resistance through DAF-16 and SKN-1 pathways in *Caenorhabditis elegans*, *Int. J. Biol. Macromol.* 202 (2022) 286-295. <https://doi.org/10.1016/j.ijbiomac.2022.01.071>.
- [48] X.H. Chen, S. Ge, P. Xiao, Y. L. Liu, Y. Yu, Y. Liu, L.P. Sun, L. Yang, D.Q., Wang, UV-stimulated riboflavin exerts immunosuppressive effects in BALB/c mice and human PBMCs. *Biomed. Pharmacother.* 173 (2024) 116278.
- [49] H.M. Nail, C.C. Chiu, C.H. Leung, M.M. Ahmed, H.M.D. Wang, Exosomal miRNA-mediated intercellular communications and immunomodulatory effects in tumor microenvironments, *J. Biomed. Sci.* 30 (2023) 69. <https://doi.org/10.1186/s12929-023-00964-w>.
- [50] Z.F. Sui, L. Li, B. Liu, T.M. Gu, Z.L. Zhao, C.F. Liu, R.Y. Yang, Optimum conditions for *Radix Rehmanniae* polysaccharides by RSM and its antioxidant and immunity activity in UVB mice, *Carbohydr. Polym.* 92 (2013) 283-288. <https://doi.org/10.1016/j.carbpol.2012.08.087>.
- [51] X.Y. Shi, C. Wu, H. Ma, J.S. Liu, C.S. Fu, R.Y. Zhou, Y. Jiang, R.Q. Zhang, Exploring the effects of astragalus polysaccharide food supplementation on growth performance, immunity, antioxidant capacity, and intestinal microflora in Chinese mitten crabs, *Aquacult. Rep.* 34 (2024) 101908. <https://doi.org/10.1016/j.aqrep.2023.101908>.
- [52] X. Liu, D.D. Bian, J.J. Jiang, S. Tang, X. Lu, D.Z. Zhang, Q.N. Liu, B.P. Tang, L.S. Dai, Transcriptomic analyses provide new insights into immune response of the *Procambarus clarkii* intestines to *Aeromonas hydrophila* challenge, *Aquacult. Rep.* 36 (2024) 102103. <https://doi.org/10.1016/j.aqrep.2024.102103>.
- [53] Y.H. Wang, M. Kwak, P.C.W. Lee, J.O. Jin, *Rehmannia glutinosa* polysaccharide promoted activation of human dendritic cells, *Int. J. Biol. Macromol.* 116 (2018) 232-238. <https://doi.org/10.1016/j.ijbiomac.2018.04.144>.
- [54] M. Kwak, K.J. Yu, P.C.W. Lee, J.O. Jin, *Rehmannia glutinosa* polysaccharide functions as a mucosal adjuvant to induce dendritic cell activation in mediastinal lymph node, *Int. J. Biol. Macromol.* 120 (2018) 1618-1623. <https://doi.org/10.1016/j.ijbiomac.2018.09.187>.
- [55] Y.H. Jia, Z.Q. Zhang, S.M. Zhang, X. Ma, Y. Ruan, B.J. Ma, L. Wang, Effects of polysaccharide from hot-compressed steamed *Rehmannia glutinosa* on the immune system and gut microbiota in an immunosuppressed mice model, *Int. Immunopharmacol.* 142 (2024) 113202. <https://doi.org/10.1016/j.intimp.2024.113202>.
- [56] Y. Huang, L. Nan, C.W. Xiao, Q.A. Ji, K. Li, Q. Wei, Y. Liu, G.L. Bao, Optimum preparation method for self-assembled pegylation nano-adjuvant based on *Rehmannia glutinosa* polysaccharide and its immunological effect on macrophages, *Int. J. Biol. Macromol.* 14 (2019) 9361-9375. <https://doi.org/10.2147/IJN.S221398>.
- [57] Y. Huang, T. Qin, Y.F. Huang, Z.G. Liu, R.N. Bo, Y.L. Hu, J.G. Liu, Y. Wu, D.Y. Wang, *Rehmannia glutinosa* polysaccharide liposome as a novel strategy for stimulating an efficient immune response and their effects on dendritic cells, *Int. J. Nanomed.* 11 (2016) 6795-6808. <https://doi.org/10.2147/IJN.S119108>.
- [58] Y. Huang, Z.G. Liu, R. Bo, J. Xing, L. Luo, S.S. Zhen, Y.L. Niu, Y.L. Hu, J.G. Liu, Y. Wu, D.Y. Wang, The enhanced immune response of PCV-2 vaccine using *Rehmannia glutinosa* polysaccharide liposome as an adjuvant, *Int. J. Biol. Macromol.* 86 (2016) 929-936. <https://doi.org/10.1016/j.ijbiomac.2016.02.003>.
- [59] H. Lv, H.P. Jia, W.J. Cai, R.J. Cao, C.Y. Xue, N. Dong, *Rehmannia glutinosa* polysaccharides attenuates colitis via reshaping gut microbiota and short-chain fatty acid production, *J. Sci. Food Agr.* 103 (2023) 3926-3938. <https://doi.org/10.1002/jsfa.12326>.
- [60] N.A. Liu, J.Q. Liu, Y. Liu, Q. Zhu, D.D. Zheng, F. Li, L.Z. Meng, M. Qiu, *Rehmannia glutinosa* polysaccharide regulates bone marrow microenvironment via HIF-1 α /NF- κ B signaling pathway in aplastic anemia mice, *An. Acad. Bras. Cienc.* 95 (2023) e20220672. <https://doi.org/10.1590/0001-3765202320220672>.
- [61] L. Ou, W.Q. Kang, J.H. Zhang, Z.Y. Liang, M. Li, F. Gao, L. Chen, Effects of *Rehmannia glutinosa* polysaccharides on bone tissue structure and skeletal muscle atrophy in rats with disuse, *Acta Cir. Bras.* 36 (2021) e360403. <https://doi.org/10.1590/ACB360403>.
- [62] C.C. Huan, J.T. Yao, X.T. Wang, H.Y. Zhang, X.B. Wang, L.Y. Jiang, S. Gao, *Rehmannia glutinosa* polysaccharide exerts antiviral activity against pseudorabies virus and antioxidant activity, *Int. J. Biol. Macromol.* 274 (2024) 133455. <https://doi.org/10.1016/j.ijbiomac.2024.133455>.

- [63] L. Xu, W. Zhang, L. Zeng, J.O. Jin, *Rehmannia glutinosa* polysaccharide induced an anti-cancer effect by activating natural killer cells, *Int. J. Biol. Macromol.* 105 (2017) 680-685. <https://doi.org/10.1016/j.ijbiomac.2017.07.090>.
- [64] Y. Cui, C.L. Rong, J.M. Wang, C. Cui, L. Wang, Z.Y. Feng, B. Niu, Mechanism-based anti-anxiety effects of polysaccharides extracted from shudihuang (*Radix Rehmanniae Preparata*) by two-dimensional electrophoresis analysis in rat hippocampus proteins, *J. Tradit. Chin. Med.* 33 (2013) 524-530. [https://doi.org/10.1016/S0254-6272\(13\)60159-4](https://doi.org/10.1016/S0254-6272(13)60159-4).
- [65] F. Kanwal, D. Ren, W. Kanwal, M.Y. Ding, J.Q. Su, X.Y. Shang. The potential role of nondigestible raffinose family oligosaccharides as prebiotics, *Glycobiology* 33 (2023) 274-288. <https://doi.org/10.1093/glycob/cwad015>.
- [66] W.F. Li, Z. Li, X. Han, D. Huang, Y.L. Lu, X.B. Yang, Enhancing the hepatic protective effect of genistein by oral administration with stachyose in mice with chronic high fructose diet consumption, *Food Funct.* 7 (2016) 2420-2430. <https://doi.org/10.1039/C6FO00038J>.
- [67] X.F. Chen, D.Q. Liao, Z.X. Qin, X.E. Li, Synergistic interactions of catalpol and stachyose in STZ-HFD induced diabetic mice: Synergism in regulation of blood glucose, lipids, and hepatic and renal function, *Chin. Herb. Med.* 11 (2019) 70-77. <http://dx.doi.org/10.1016/j.chmed.2018.05.006>.
- [68] H.M.D. Wang, C.C. Chen, P. Huynh, J.S. Chang, Exploring the potential of using algae in cosmetics, *Bioresour. Technol.* 184 (2015) 355-362. <https://doi.org/10.1016/j.biortech.2014.12.001>.
- [69] H.M.D. Wang, X.C. Li, D.J. Lee, J.S. Chang, Potential biomedical applications of marine algae, *Bioresour. Technol.* 244 (2017) 1407-1415. <https://doi.org/10.1016/j.biortech.2017.05.198>.
- [70] C. Wang, J.Y. Bai, B.T. Wang, L.L. Yu, F.W. Tian, J.X. Zhao, H. Zhang, H.Y. Suo, W. Chen, Q.X. Zhai, Stachyose modulates gut microbiota and alleviates DSS-induced ulcerative colitis in mice, *Food Sci. Hum. Well.* 12 (2023) 2211-2220. <https://doi.org/10.1016/j.fshw.2023.03.041>.
- [71] Y.X. Hu, X.Z. Liu, X.N. Wu, Z.X. Zhang, D.R. Wu, C.X. Chen, W.J. Su, L.Y. Zhang, J. Li, H.M.D. Wang, Several natural phytochemicals from Chinese traditional fermented food-pickled *Raphanus sativus* L.: Purification and characterization, *Food Chem. X* 15 (2022) 100390. <https://doi.org/10.1016/j.fochx.2022.100390>.
- [72] P.P. Yue, Y.J. Hu, R. Tian, J. Bian, F. Peng, Hydrothermal pretreatment for the production of oligosaccharides: A review, *Bioresour. Technol.* 343 (2022) 126075. <https://doi.org/10.1016/j.biortech.2021.126075>.
- [73] T.J. Boltje, T. Buskas, G.J. Boons, Opportunities and challenges in synthetic oligosaccharide and glycoconjugate research, *Nat. Chem.* 1 (2009) 611-622. <https://doi.org/10.1038/nchem.399>.
- [74] X.T. Ta, B.T. Wang, J.Y. Bai, J.L. Yu, H.Y. Chen, C. Wang, The source, extraction, purification, physiological function, and application of stachyose in the food industry, *Food Chem.* 416(15) (2024) 140791. <https://doi.org/10.1016/j.foodchem.2024.140791>.
- [75] L.X. Zheng, Y. Liu, S.J. Tang, W.C. Zhang, K.L. Cheong, Preparation methods, biological activities, and potential applications of marine algae oligosaccharides: A review, *Food Sci. Hum. Well.* 12 (2023) 359-370. <https://doi.org/10.1016/j.fshw.2022.07.038>.
- [76] Z.W. Zhao, L. Wang, Y. Ruan, C.N. Wen, M.H. Ge, Y.Y. Qian, B.J. Ma, Physicochemical properties and biological activities of polysaccharides from the peel of *Dioscorea opposita* Thunb. extracted by four different methods, *Food Sci. Hum. Well.* 12 (2023) 130-139. <https://doi.org/10.1016/j.fshw.2022.07.031>.
- [77] W.C. Lien, X.R. Zhou, Y.J. Liang, C.T.S. Ching, C.Y. Wang, F.I. Lu, H.C. Chang, F.H. Lin, H.M.D. Wang, Therapeutic potential of nanoceria pretreatment in preventing the development of urological chronic pelvic pain syndrome: Immunomodulation via reactive oxygen species scavenging and SerpinB2 downregulation, *Bioeng. Transl. Med.* 8(1) (2023) e10346. <https://doi.org/10.1002/btm2.10346>.
- [78] N.X. Kang, Y.G. Luan, Y. Jiang, W.H. Chang, Y.J. Liu, Z. J. Su, Y.G. Liu, P. Tan, Neuroprotective effects of oligosaccharides in *Rehmanniae Radix* on transgenic *caenorhabditis elegans* models for alzheimers disease, *Front. Pharmacol.* 13 (2022) 878631. <https://doi.org/10.3389/fphar.2022.878631>.
- [79] P.S. Wu, S.J. Wu, Y.H. Tsai, Y.H. Liu, J.C.J. Chao, Hot water extracted *Lycium barbarum* and *Rehmannia glutinosa* inhibit liver inflammation and fibrosis in rats, *Am. J. Chinese Med.* 39 (2011) 1173-1191. <https://doi.org/10.1142/S0192415X11009482>.