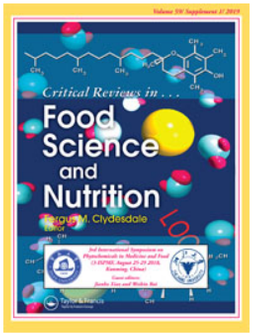

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REVIEW

Recent developments in *Hericium erinaceus* polysaccharides: extraction, purification, structural characteristics and biological activities

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ABSTRACT

Hericium erinaceus (*H. erinaceus*), an edible mushroom with medicinal value, has a long history of usage in China and other oriental countries. Polysaccharide is supposed to be one of the major bioactive compounds in *H. erinaceus*, which possesses immunomodulating, anti-cancer, antioxidant, gastroprotection and intestinal health promotion, neuroprotective, hepatoprotective, antihpyerglycemic and hypolipidemic activities. In this review, the current advancements on extraction, purification, structural characteristics and biological activities of polysaccharide from different sources (fruiting body, mycelium and culture broth) of *H. erinaceus* were summarized. Among these aspects, summaries of the structural characteristics focused on the purified polysaccharides. Meanwhile, comparisons on the structural characteristics among the purified polysaccharides obtained from above three sources were made. Moreover, their biological activities were introduced on the basis of *in vivo* and *in vitro* experiments, and some possible action mechanisms were listed. Furthermore, the structure-activity relationship of the polysaccharide was discussed. New perspectives for the future work of *Hericium erinaceus* polysaccharide were also proposed.

HIGHLIGHTS

- Extraction, purification, structural characteristics and biological activities of *Hericium erinaceus* polysaccharide (HEP) were summarized.
- Structural characteristics of the purified polysaccharides from different sources (fruiting body, mycelium and culture broth) of *Hericium erinaceus* were summarized and compared.
- Structure-activity relationship of HEP was discussed, and new perspectives for the future work of this polysaccharide were proposed.

KEYWORDS

Hericium erinaceus; polysaccharide; structure; biological activity; structure-activity relationship

Introduction

Mushrooms are widely consumed as a part of daily diet in many countries for their abundant nutritional ingredients, such as fiber, protein, vitamins and minerals (Kalač 2009). Up to 2004, more than 200 species have been proved to be edible and medicinal fungi among over 12000 mushroom species (Sánchez 2004). Currently, the pharmacological actions of mushroom have attracted much attention (Rathore, Prasad, and Sharma 2017; Wong et al. 2017; Younis 2017). Some mushrooms are considered to be potential sources of prebiotics that have the capacity of improving human health through modulating the gut microbiota (Aida et al. 2009). A few mushrooms like *H. erinaceus* are demonstrated to have anticancer potential (Patel and Goyal 2012).

H. erinaceus, known as an edible and medicinal mushroom, has been commonly used in traditional Chinese medicine and cuisine since the earliest history. Fruiting body, mycelium and culture broth are there different morphologies of *H. erinaceus* (seen in Figure 1). Fruiting body is widely found in the nature and it can be obtained by artificial cultivation. Mycelium is usually produced from *H. erinaceus* strain by submerged fermentation on artificial medium, and the culture broth is collected from the fermented medium of mycelium. In the past few decades, large amounts of metabolites such as erinacines, erinacerins, erinaceolactones, glycoprotein, polysaccharides and sterols have been isolated from *H. erinaceus* fruiting body, and the health-promoting properties of fruiting body have been extensively reviewed (Wang et al. 2014; Khan et al. 2013; Jiang et al. 2014). Recently, bioactivity compounds including polysaccharides from mycelium and culture broth of *H. erinaceus* are received increasing attention (Friedman 2015; Sokol et al. 2015; Thongbai et al. 2015). Some new bioactive metabolites such as diterpenoid (Chen et al. 2018; Rupcic et al. 2018; Tzeng et al. 2018; Zhang et al. 2018), xanthurenate (Lin et al. 2018) and isoflavone (He et al. 2018) have been derived from mycelium and culture broth of *H. erinaceus*. Otherwise, erinacine A-enriched *H. erinaceus* mycelium has

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Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/bfsn.
*Xiao-Yin Wang and Duo-duo Zhang have made equal contribution to this work.

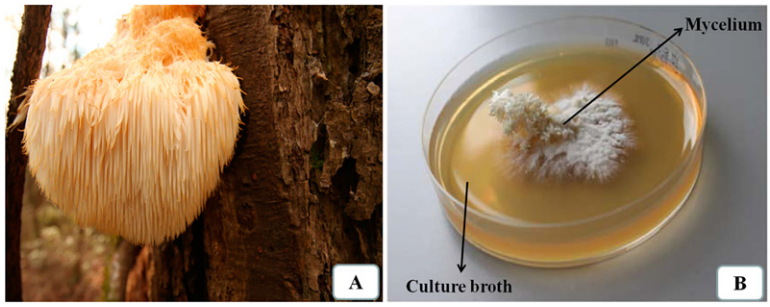

Figure 1. Images of fruiting body, mycelium and culture broth of *H. erinaceus*. A, Fruiting body of *H. erinaceus*, photograph was from the article of Friedman (Friedman 2015); B, Mycelium and culture broth of *H. erinaceus*, reprinted with permission from Springer Nature Thongbai et al. (2015).

been demonstrated to have neurohealth properties (Li, Lee, et al. 2018) and antidepressant-like effect (Chiu et al. 2018).

H. erinaceus polysaccharide (HEP), generally recognized as one major bioactive compound of *H. erinaceus* (He et al. 2017; Wang et al. 2014), can be extracted from fruiting body, mycelium and culture broth. Currently, HEP from the fruiting body has been extensively investigated, whilst the data concerning those from two other sources was scattered. In view of this, summarization on polysaccharides from mycelium and culture broth is necessary. Meanwhile, the only one review taken by He et al. (2017) was mainly concentrate on the advancement of HEP from the fruiting body. Besides, some information on HEP has been partially provided in other reviews (Friedman 2015; Khan et al. 2013; Sokol et al. 2015; Thongbai et al. 2015; Wang et al. 2014). Thus, summarizing the information on HEP from the perspective of source (fruiting body, mycelium and culture broth) is of great value.

On the other hand, the relationship between structure and activity of the polysaccharide has not been disclosed, which might limit its exploitation and utilization. Hence, the aim of the present review is to summarize the recent advances in extraction, purification, structural characteristics and biological activities of HEP from the fruiting body, mycelium and culture broth respectively, and discuss the structure-activity relationship. Particularly, the summary of the structural characteristics is only targeted at the purified polysaccharides.

Extraction, separation and purification of HEP

Factors affecting HEP production in H. Erinaceus

Fruiting body, mycelium and culture broth of *H. erinaceus* are main sources of HEP. Variation of polysaccharide depends greatly on the cultivation condition for fruiting body, mycelium or culture broth. Total polysaccharide content in the fruiting body is increasing during its growing (Li et al. 2015). Meanwhile, polysaccharides from the fruiting body in different maturation stages show differences in chemical composition and macrophage activation (Li et al. 2015; Li et al. 2016). However, a similar structure has been observed in the polysaccharide obtained from the fruiting body in small fungal spine stage, mid-fungal spine stage or mature stage (Li et al. 2016). In terms of mycelium, polysaccharide production is influenced by many factors including medium composition, cultivation temperature, cultivation pH and incubation period (Cui et al. 2010; Malinowska, Krzyczkowski, Łapienis, et al. 2009). Thus, much endeavor has been made to optimize these parameters for improving polysaccharide production. To culture broth, ascorbic acid and uracil play crucial roles in the yield of exopolysaccharides (Lee et al. 2010).

Extraction of HEP

Since the bioactive components including HEP of fruiting body, mycelium and culture broth of *H. erinaceus* always have considerably different structures (Friedman 2015; Lee, Cho, and Hong 2009; Lee et al. 2009), HEPs extracted from these three different sources even with the same method had different structures (Lee, Cho, and Hong 2009; Lee et al. 2009). Moreover, to the same source, HEPs obtained by nearly same methods showed differences in molecular weight, monosaccharide composition, and glycosyl linkage type (Zhang et al. 2006; Li et al. 2016). Furthermore, HEPs from the same source by different methods exhibited different structural features (Yan et al. 2018). Therefore, HEPs from these three sources usually show different structures.

Currently, HEP from the fruiting body is mainly extracted using water and alkali extraction methods (Table 1). Water extraction, the most popular approach, always requires long extraction time, high temperature, high liquid-to-solid ratio or multiple extraction steps to pursue high polysaccharide yield (Xing et al. 2013), resulting in large consumptions of energy and time. Therefore, it is necessary to optimize the extraction conditions. A optimal extraction conditions carried out by Box-Behnken design and response surface methodology for the Selenium (Se)-enriched HEP were: extraction time of 115 min, extraction temperature of 98 °C, liquid-to-solid ratio of 4.0, and extraction number of 4 (Luo and Chen 2010). Moreover, microwave irradiation and enzyme-assisted extraction were applied to improve this isolation process. The findings of Ookushi, Sakamoto, and Azuma (2006) showed that HEP obtained by microwave-assisted extraction (140 °C, 5 min) was almost equivalent to that isolated by hot water extraction (100 °C, 6 h). Obviously, this assistant method has an advantage in economizing time (Ookushi, Sakamoto, and Azuma 2009). Enzyme-assisted extraction was shown to generate an increase of 67.72% in the HEP yield compared with hot water extraction (Zhu, Li, et al. 2014), thereby it was

Table 1. Structural characteristics of the purified polysaccharide from the fruiting body of *H. erinaceus*.

Polysaccharide fraction	Extraction, separation and purification procedure	M_w (kDa)	Monosaccharide composition	Structural feature	Structural characterization method	Reference
FI_0-a-α	Water extraction (100 °C), Con A-AF-Toyopearl 650 column	89	Xyl: Man = 100: 4 (molar ratio)	Xylan-protein complex had β-(1→3) and β-(1→6)-glucan chains	Gel chromatography, GC, IR, NMR (^{1}H, ^{13}C)	Mizuno et al. (1992)
FI_0-a-β		175	Glc: Xyl: Man: Gal = 100: 233: 58: 13 (molar ratio)	Heteroglycan containing β-(1→3) and β-(1→6)-glucan chains		
HEPF3	Water extraction (boiling), Sephacryl S-300 column	19	Fuc: Gal = 1: 4.12 (molar ratio)	α-L-Fuc*p*-(1→, →2,6)-α-D-Gal*p*-(1→ and →6)-α-D-Gal*p*-(1→	HPLC, GC-MS, methylation analysis, NMR (^{1}H, ^{13}C, COSY, TOCSY, NOESY, HMBC, HSQC)	Zhang et al. (2006)
HEPF1	Water extraction (boiling), DEAE-Sepharose fast flow column, Sephacryl S-300 column	19.4	Fuc: Gal: Glc = 1: 4: 1 (molar ratio)	α-L-Fuc*p*-(1→, →2,6)-α-D-Gal*p*-(1→, →6)-α-D-Gal*p*-(1→ and →6)-β-D-Glc*p*-(1→	HPLC, GC-MS, methylation analysis, NMR (^{1}H, ^{13}C, COSY, TOCSY, NOESY, HMBC, HSQC)	Zhang, Sun, et al. (2007)
HEP-S	Water extraction (85 °C), DEAE-sepharose fast flow column, Sephadex-G100 column	18.3	Rha: Fuc: Man: Glc: Gal = 1.47: 0.93: 1.36: 8.68: 4.08 (molar ratio)	α-D-Glc*p*-(1→, →3,4)-α-D-Glc*p*-(1→, →6)-α-D-Gal*p*-(1→, →3,4)-β-D-Man*p*-(1→, →3,6)-α-Rha*p*-(1→ and →2)-β-L-Fuc*p*-(1→	HPGPC, FT-IR, GC, periodate oxidation-smith degradation, NMR (^{1}H, ^{13}C, HMBC, HSQC)	Wu, Zhou, Zhou, Ou, Zhang, et al. (2017)
HEP-W		15.9	Rha: Fuc: Man: Glc: Gal = 0.98: 1.59: 0.89: 5.60: 7.06 (molar ratio)	α-D-Glc*p*-(1→, →3,6)-α-D-Glc*p*-(1→, →2,6)-α-D-Gal*p*-(1→, β-Gal*p*-(1→, →3,4)-β-D-Man*p*, →3)-α-Rha*p* and →2)-β-L-Fuc*p*	HPGPC, GC, periodate oxidation-Smith degradation, FT-IR, NMR (^{1}H, ^{13}C)	Wu, Zhou, Zhou, Ou, and Huang (2017)
HPB-3	Water extraction (100 °C), Sephacryl S-300 column	15	Fuc: Gal: Glc = 5.2: 23.9: 1 (molar ratio)	α-L-Fuc*p*-(1→, →2,6)-α-D-Gal*p*-(1→ and →6)-α-D-Gal*p*-(1→	HPLC, UV, IR, HPAEC, GC-MS, methylation analysis, NMR (^{1}H, ^{13}C, COSY, TOCSY, NOESY, HMBC, HSQC)	Li et al. (2016)
HEPF4	Water extraction (boiling), Sephacryl S-400 and Sephacryl S-300 columns	20.3	Rha: Fuc: Gal: Glc = 0.12: 1.00: 3.27: 0.28 (molar ratio)	Fuc*p*-(1→, →6)-Gal*p*-(1→ and →2,6)-Gal*p*-(1→	HPLC, GC-MS, methylation analysis, NMR (^{1}H, ^{13}C, COSY, TOCSY, NOESY, HMBC, HSQC)	Zhang, Fu, et al. (2012)
HPA	Water extraction (100 °C), DEAE-Sepharose CL-6B column, Sephadex G-100 column	50	Glc: Gal: Fuc = 1: 2.110: 0.423 (molar ratio)	→6)-Glc*p*-(1→, →6)-Gal*p*-(1→ and Gal*p*-(1→	GC, IR, methylation analysis, GC-MS, periodate oxidation-smith degradation, partial acid hydrolysis	Wang, Luo, and Liang (2004)
HPB		30	Glc: Gal = 1: 11.529 (molar ratio)	Glc*p*-(1→, →3)-Glc*p*-(1→, →6)-Glc*p*-(1→, →6)-Gal*p*-(1→ and →3,6)-Glc*p*-(1→		
HEP-1	Water extraction (boiling), DEAE-cellulose column, Sephadex G-200 column	18	Rha: Gal: Glc = 1.19: 3.81: 1.00 (molar ratio)	Rha*p*-(1→, →2,6)-Glc*p*-(1→, →6)-Gal*p*-(1→ and →2,6)-Gal*p*-(1→	HPGPC, TLC, GLC, GC-MS, methylation, partial acid hydrolysis, IR, NMR (^{1}H, ^{13}C)	Jia et al. (2004)
HEP	Water extraction (80 °C), DEAE-52 cellulose column, Sephadex G-200 column	16.18	Glc: Gal: Man: Ara = 51.02: 42.24: 4.5: 2.2 (mass percentage)	NA	FT-IR, GPC, HPLC	Wu, Jiang, et al. (2017)
HEP	Water extraction (80 °C), Sephadex G-100 column	16.15	Glc: Gal: Man: Ara = 50.80: 42.30: 4.58: 2.29 (molar ratio)	NA	FT-IR, GPC, HPLC	Qin, Ren, et al. (2017)
hHEP	Water extraction (80 °C), Sephadex G-100 column, hydroxyethylation	15	Glc: Fru: Gal: Man: Ara= 30.23: 25.30: 2.45: 1.04: 0.18 (molar ratio)	NA	FT-IR, GPC, HPLC	Ren et al. (2017)
HEF-AP Fr II	Water extraction (121 °C), DEAE-cellulose column, Sepharose CL-6B column	13	Glc: Gal: Man = 91.11: 6.09: 2.80 (molar ratio)	Glc*p*-(1→, →3)-Glc*p*-(1→, →6)-Glc*p*-(1→ and →3,6)-Glc*p*-(1→	Methylation, reductive cleavage, acetylation, FT-IR, GC-MS	Lee et al. (2009)
W-1	Water extraction (140 °C), Superdex 75 column	180		Fuc*p*-(1→, Glc*p*-(1→, →3)-Glc*p*-(1→, →6)-Glc*p*-(1→, →3,6)-	SEC, HPAEC, methylation	Ookushi, Sakamoto, and Azuma (2009)

			Fuc: Ara: Rha: Gal: Glc: Xyl: Man = 7.8: 0.4: 1.5: 47.7: 30.9: 0.9: 10.8 (molar ratio)	Glc*p*-(1→, Gal*p*-(1→, →4,6)-Gal*p*-(1→, →6)-Gal*p*-(1→ and →2,6)-Gal*p*-(1→		
W-2-N	Water extraction (140 °C), Superdex 75 column, DEAE-650M column	NA	Fuc: Rha: Gal: Glc: Xyl: Man = 15.8: 0.6: 77.3: 3.4: 0.1: 2.9 (molar ratio)	Fuc*p*-(1→, Glc*p*-(1→, →3)-Glc*p*-(1→, →6)-Glc*p*-(1→, →3,6)-Glc*p*-(1→, Gal*p*-(1→, →4,6)-Gal*p*-(1→, →6)-Gal*p*-(1→ and →2,6)-Gal*p*-(1→		
	Fuc*p*-(1→, Glc*p*-(1→, →3)-Glc*p*-(1→, →6)-Glc*p*-(1→, →3,6)-Glc*p*-(1→, Gal*p*-(1→, →4,6)-Gal*p*-(1→, →6)-Gal*p*-(1→ and →2,6)-Gal*p*-(1→		W-2-A	Fuc: Ara: Rha: Gal: Glc: Xyl: Man = 6.1: 0.7: 0.7: 26.6: 57.6: 2.1: 6.2 (molar ratio)		
WIP	Alkali extraction	NA	Glc: Man: Xyl: Rha = 82.5: 7.4: 7.3: 1.8 (molar ratio)	→3)-Glc*p*-(1→, Glc*p*-(1→, →3,4)-Glc*p*-(1→ and →3,6)-Glc*p*-(1→	Total acid hydrolysis, methylation analysis, FT-IR, FT-Raman, ^{1}H NMR	Wiater et al. (2016)
FIII-2b	5% sodium hydroxide extraction, Toyopearl HW-65F column	30	Glc: Xyl: Gal = 100: 51: 32 (molar ratio)	Glucoxylan having β-l,3- and β-l,6- glucosidic linkages	Gel chromatography, GC, IR, NMR (^{1}H, ^{13}C)	Mizuno et al. (1992)
FII-1	1% ammonium oxalate extraction, DEAE-cellulose column	155	Glc: Xyl: Gal = 100: 179: 17 (molar ratio)	Galactoxyloglucan-protein complex having β-l,3- and β-l,6-glucosidic linkages		
M-1	Microwave irradiation in water (140 °C), Superdex 75 column	180	Fuc: Gal: Glc: Xyl: Man = 1.6: 8.7: 88.1: 0.4: 1.3 (molar ratio)	Fuc*p*-(1→, Glc*p*-(1→, →3)-Glc*p*-(1→, →6)-Glc*p*-(1→, →3,6)-Glc*p*-(1→, Gal*p*-(1→, →4,6)-Gal*p*-(1→, →6)-Gal*p*-(1→ and →2,6)-Gal*p*-(1→	SEC, HPAEC, methylation	Ookushi, Sakamoto, and Azuma (2009)
M-2-N		NA	Fuc: Gal: Glc: Xyl: Man = 1.1: 8.6: 88.1: 0.4: 1.7 (molar ratio)	Fuc*p*-(1→, Glc*p*-(1→, →3)-Glc*p*-(1→, →6)-Glc*p*-(1→, →3,6)-Glc*p*-(1→ and Gal*p*-(1→,		
M-2-A			Fuc: Gal: Glc: Man = 1.5: 10.8: 85.5: 2.2 (molar ratio)	Fuc*p*-(1→, Glc*p*-(1→, →3)-Glc*p*-(1→, →6)-Glc*p*-(1→, →3,6)-Glc*p*-(1→, →6)-Gal*p*-(1→ and →2,6)-Gal*p*-(1→		
HE	Water extraction (121 °C), DEAE cellulose column, Sepharose CL-6B column	13	Glc	1,3-branched-β-1,6-glucan	Methylation, reductive cleavage, acetylation, FT-IR, GC-MS	Yang et al. (2016)
HEPF5	Water extraction (boiling), DEAE-sepharose column, Sephacryl S-500 column	423	Glc	Glc*p*-(1→ and →3,4)-Glc*p*-(1→	IR, HPAEC, HPLC, GC-MS, methylation analysis, NMR (^{1}H, ^{13}C, COSY, TOCSY, NOESY, HMBC, HSQC)	Zhang et al. (2011)
HEP3	Alkali extraction, DEAE-cellulose column	>1000	Glc	Glc*p*-(1→, →3)-Glc*p*-(1→ and →3,6)-Glc*p*-(1→	HPGPC, TLC, methylation analysis, GC-MS, periodate oxidation, smith degradation, IR, NMR (^{1}H, ^{13}C, DEPT)	Dong, Jia, and Fang (2006)

NA: information was not available.

adopted to HEP preparation by some investigators (Qin, Zhang, et al. 2017; Zhu, Chen, et al. 2014).

Alkali is an effective solvent in solubilizing glucans so that HEP obtained by alkaline extraction from the fruiting body contained high α- or β-glucan contents (Wiater et al. 2016; Yan et al. 2018). Moreover, after water extraction, many polysaccharides especially β-glucans still remain in the water-insoluble residues of the fruiting body owing to its multi-layered cell-wall structure. In this case, some investigators have made much effort to acquire β-glucans from the water-insoluble residues by sodium hydroxide (Dong, Jia, and Fang 2006; Ookushi, Sakamoto, and Azuma 2008). For improving the extractability of β-glucans, Ookushi, Sakamoto, and Azuma (2008) have also conducted a combination method of enzymatic extraction and microwave irradiation to extract β-glucans from the water-insoluble residues. Furthermore, Yan et al. (2018) suggested that HEP extracted with citric acid revealed stronger *in vitro* antioxidant and antihyperglycemic activities. In addition, a sequential processes method using green solvents has been carried out for isolating the polysaccharide (Parada et al. 2015). It exerted a good application prospect in reducing the toxicity and environmental pollution resulted from conventional extraction solvents.

HEP from the mycelium is also frequently extracted by water extraction method (Cui et al. 2014; Wang, Gao, et al. 2015; Wang et al. 2017; Wang, Konishi, et al. 2015; Wang, Gao, et al. 2015; Zhang et al. 2017), while that from the culture broth can be obtained by ethanol precipitation (Lee, Cho, and Hong 2009; Yang, Park, and Song 2003), acetone precipitation (Park et al. 2002) and water extraction (Shang et al. 2015; Shang, Song, Wang, et al. 2014; Wang, Hu, Su, et al. 2001). Enzyme-assisted extraction means has also been applied to acquire the polysaccharide from the mycelium. Qin, Zhang, et al. (2017) used combinative enzyme solutions (cellulose, pectinase and papain in a ratio of 2:1:1) to extract polysaccharides from the mycelium, and determined the optimal extraction protocols by Box-Behnken design as follows: extraction time of 79 min, extraction temperature of 50 °C, pH value of 5.7 and liquid-to-solid ratio of 33.4 mL/g.

Separation and purification of HEP

After extraction, the crude HEP is mostly obtained by ethanol precipitation. Since this kind of polysaccharide usually contains proteins, pigments and small molecule materials, further separation and purification procedures are needed. They can be summarized as follows: the precipitated crude polysaccharide is received washing with ethanol, hyperfiltration through ultrafiltration membrane (Shang, Song, Wang, et al. 2014; Wang et al. 2017; Wang, Gao, et al. 2015) and dialysis against water to remove low-molecular-weight compounds (Jia et al. 2004; Lee et al. 2009; Li et al. 2016; Wang, Luo, and Liang 2004; Wong et al. 2015). Sevag method (Jia et al. 2004; Shang, Song, Wang, et al. 2014; Wang, Luo, and Liang 2004; Wu, Zhou, Zhou, Ou, and Huang 2017; Wu, Zhou, Zhou, Ou, Zhang, et al. 2017; Zhang et al. 2017) or trichloroacetic acid approach (Zhu, Chen, et al. 2014) is conducted for the removal of floating protein. Decoloration is performed by using 30% H_2O_2 solution (Wang, Luo, and Liang 2004). The resulting polysaccharide is then mainly purified by different column chromatographies including ion-exchange chromatography using DEAE-cellulose (Dong, Jia, and Fang 2006; Jia et al. 2004; Lee, Cho, and Hong 2009; Lee et al. 2009; Zhang et al. 2017), DEAE-Sephadex (Wang, Gao, et al. 2015), DEAE-Sepharose fast flow (Cui et al. 2014; Li et al. 2016; Wu, Zhou, Zhou, Ou, and Huang 2017; Wu, Zhou, Zhou, Ou, Zhang, et al. 2017), DEAE-Sepharose CL-6B (Wang, Luo, and Liang 2004) and Toyopearl DEAE-650 (Ookushi, Sakamoto, and Azuma 2009) columns, gel permeation chromatography with Sephadex (Park et al. 2002; Qin et al. 2017; Wang, Luo, and Liang 2004), Superdex (Li et al. 2016; Ookushi, Sakamoto, and Azuma 2009), Sephacryl (Zhang, Fu, et al. 2012; Zhang, Sun, et al. 2007; Zhang et al. 2006; Zhang et al. 2011) and Sepharose (Lee, Cho, and Hong 2009; Lee et al. 2009; Shang, Song, Wang, et al. 2014; Yang et al. 2016) columns, and affinity chromatography using Con A-AF-Toyopearl 650 affinity column (Mizuno et al. 1992). During these chromatographic purification processes, phenol-sulfuric acid method is widely used to monitor the eluted fractions (Cui et al. 2014; Dong, Jia, and Fang 2006; Jia et al. 2004; Ookushi, Sakamoto, and Azuma 2009; Wu, Zhou, Zhou, Ou, and Huang 2017; Wu, Zhou, Zhou, Ou, Zhang, et al. 2017; Zhang, Fu, et al. 2012; Zhang, Sun, et al. 2007; Zhang et al. 2006).

Moreover, the crude polysaccharide obtained by ethanol precipitation can be successively sub-fractionated by gradual ethanol precipitation at appropriate final ethanol concentrations (Zhang, Lv, et al. 2012; Zhu, Chen, et al. 2014; Wang, Yin, Nie, et al. 2018). Otherwise, Ren et al. (2018) have applied an ultrafiltration method to acquire a homogeneous polysaccharide fraction from the dry powder of fermented *H. erinaceus* mycelium.

Structural characteristics of the purified HEP

Structural characterization method

Molecule weight (M_w), monosaccharide composition, glycosyl linkage type, structures of backbone and branched chain, and conformation of the purified polysaccharide from *H. erinaceus* are assessed by a combination of chemical analysis, chromatography technology and spectrum scanning (Table 1 and Table 2). Homogeneity and M_w are mostly measured using gel filtration (Mizuno et al. 1992), high performance liquid chromatography (HPLC) (Li et al. 2016; Zhang, Fu, et al. 2012; Zhang, Sun, et al. 2007; Zhang et al. 2006), high performance gel permeation chromatography (HPGPC) (Dong, Jia, and Fang 2006; Wu, Zhou, Zhou, Ou, and Huang 2017) and size exclusion chromatography (SEC) (Ookushi, Sakamoto, and Azuma 2009) technologies. After being completely hydrolyzed with trifluoroacetic acid (Jia et al. 2004; Qin, Zhang, et al. 2017; Wu, Zhou, Zhou, Ou, and Huang 2017; Zhang, Fu, et al. 2012) or sulfuric acid (Mizuno et al. 1992; Ren et al. 2018; Wang, Yin, Nie, et al. 2018), the hydrolysate is separated and analyzed by HPLC

Table 2. Structural characteristics of the purified polysaccharides from the mycelium and culture broth of *H. erinaceus*.

Polysaccharide fraction	Source	Extraction method	M_w (kDa)	Monosaccharide composition	Structural feature	Structural characterization method	Reference
EP-1	Mycelium	Water extraction (70 °C), hollow-fiber ultrafiltration, DEAE-Sephadex column	3.1	Man: Glc: Gal =6.42: 67.87: 1.00 (molar ratio)	→3)-Glc*p*-(1→, Man*p*-(1→, →3,4)-Glc*p*-(1→, →3,4)-Gal*p*-(1→,	HPLC, GPC, GC, GC-MS, FT-IR, NMR (^{1}H, ^{13}C).	Wang, Gao, et al. (2015; Wang et al. (2017)
HEG-5	Mycelium	Water extraction (4 °C), DEAE-Sepharose fast flow column	14.4	Glc: Rha: Gal: Man= 1.00: 1.09: 2.45: 7.14 (molar ratio)	→4)-*β*-Gal*p* and *β*-Glc*p*-(1→	SDS-PAGE, Native-PAGE, MALDI-TOF-MS, FT-IR, CD, GC	Cui et al. (2014)
HIPS1	Mycelium	Water extraction (80 °C), DEAE-52 cellulose column	NA	Xyl: Man: Gal: Glc =3.02: 4.36: 9.41: 83.21 (mass percentage)	NA	UV, GC, FT-IR	Zhang et al. (2017)
HIPS2				Man: Gal: Glc =23.40: 50.34: 26.27 (mass percentage)			
HECP	Mycelium	Ethanol precipitation, water extraction, ultrafiltration (10 kDa)	86.67	Rha, Ara, Gal, Glc, Xyl and Man	NA	HPGPC, GC	Ren et al. (2018)
HEP-2	Mycelium	Enzyme-assisted extraction, DEAE-52 cellulose column	38.76	Glc: Gal: Man: Fuc: Ara: Xyl: Rha =42.05: 21.31: 13.07: 12.47: 0.96: 8.76: 1.38 (molar ratio)	NA	HPAEC, HPLC.	Qin, Zhang, et al. (2017)
HFCP	Culture broth	Water extraction (80 °C), Sepharose CL-6B column	46.9	Glc	{-1,4-Glc_3-1,6-Glc}$_n$	Methylation, reductive cleavage, acetylation, FT-IR, GC-MS	Shang, Song, Wang, et al. (2014)
HEB-AP Fr I	Culture broth	Ethanol precipitation, DEAE cellulose and Sepharose CL-6B column	46	Man	Man*p*-(1→, →2)-Man*p*-(1→, →3)-Man*p*-(1→, →6)-Man*p*-(1→, →2,6)-Man*p*-(1→	Methylation, reductive cleavage, acetylation, FT-IR, GC-MS	Lee, Cho, and Hong (2009)
Exo-polysaccharide	Culture broth	Acetone precipitation, Sephadex CL-5B column	1000	Glc: Gal: Xyl: Man: Fru =1.5: 1.7: 1.2: 0.6: 0.9 (molar ratio)	NA	GC, HPLC	Park et al. (2002)
EBP	Culture broth	Ethanol precipitation, dialysis	40	Fuc: Rib: Ara: Xyl: Man: Gal: Glc =20.1: 0.2: 1.5: 0.3: 35.4: 34.1: 8.4 (mass percentage)	Galactomannan	GC, HPLC	Yang, Park, and Song (2003)

NA: information was not available.

(Lee et al. 2009), gas-liquid chromatography (GLC) (Dong, Jia, and Fang 2006; Jia et al. 2004), gas chromatography (GC) (Ren et al. 2018; Wang, Luo, and Liang 2004), gas chromatography-mass spectrometer (GC-MS) (Zhang, Sun, et al. 2007; Zhang et al. 2006) or high performance anion-exchange chromatography (HPAEC) (Li et al. 2016; Wang, Yin, Nie, et al. 2018; Zhang, Fu, et al. 2012; Zhang et al. 2011) system to collect the information on monosaccharide composition. Detection of functional group is commonly carried out by infrared radiation (IR) or fourier transform infrared spectroscopy (FT-IR). Combination of methylation analysis and GC-MS technology is effective in identifying the linkage type of glycosyl residues. Some research groups have applied periodate oxidation, smith degradation, reductive cleavage and acetylation approaches to estimate the types of sugar ring and glycosylic bond (Dong, Jia, and Fang 2006; Wang, Luo, and Liang 2004; Wu, Zhou, Zhou, Ou, and Huang 2017; Yang et al. 2016). For determining the compositions of backbone and branched chain, the polysaccharide is firstly cleaved into oligosaccharide fragments through partial acid hydrolysis and then analyzed by methylation analysis or GC instrument (Jia et al. 2004; Wang, Luo, and Liang 2004; Yang et al. 2016). 1D/2D nuclear magnetic resonance (NMR) spectra including ^{1}H, ^{13}C, correlated spectroscopy (COSY), total correlation spectroscopy (TOCSY), nuclear overhauser effect spectroscopy (NOESY), heteronuclear multiple bond correlation (HMBC) and heteronuclear multiple quantum correlation (HMQC) have been widely operated to ascertain the anomeric configuration along with position and linkage order of glycoside residues. Conformation structure can be directly analyzed by circular dichroism (CD) spectrum (Cui et al. 2014), and the conformational characteristics in solution is usually determined through characterizing its Congo red-polysaccharide complexes (Dong, Jia, and Fang 2006; Lee, Cho, and Hong 2009; Lee et al. 2009).

Structural feature of the purified HEP from the fruiting body

Up to now, more than twenty-seven purified polysaccharides gained from the fruiting body have been systematically characterized, as is shown in Table 1. In general, M_w is approximately in the range of 13–1000 kDa. Most of polysaccharides are heteropolysaccharides consisting of two or more kinds of monosaccharides like glucose (Glc), xylose (Xyl), rhamnose (Rha), mannose (Man), fucose (Fuc), galactose (Gal) and arabinose (Ara). Fucogalactan (Zhang et al. 2006), fucoglucogalactan (Zhang, Sun, et al. 2007), rhamnoglucogalactan (Jia et al. 2004) and glucoxylan (Mizuno et al. 1992) are extensively discovered in them. Current researches have shown that the (1→6)-linked α-D-galactopyranosyl backbone is widely found in the heteropolysaccharide (Jia et al. 2004; Li et al. 2016; Zhang, Fu, et al. 2012; Zhang, Sun, et al. 2007; Zhang et al. 2006), and branches are usually composed of α-L-fucopyranose at the *O*-2 position (Li et al. 2016; Zhang, Fu, et al. 2012; Zhang, Sun, et al. 2007; Zhang et al. 2006). A primary structure of the heteropolysaccharide has been predicted by Li et al. (2016), as is revealed in Figure 2. In comparison, a heteropolysaccharide reported by Jia et al. (2004) had a (1→6)-linked α-D-galactopyranosyl backbone with branches that were made up of Rha and Glc attached to *O*-2. Wang, Luo, and Liang (2004) found that a heteropolysaccharide HPA consisted of a (1→6)-linked β-D-galactan backbone, and the sugar residues chain was branched with β-L-fucopyranose and β-D-glucopyranosyl at the *O*-2 position, and a heteropolysaccharide HPB possessed a (1→6)-linked β-D-glucopyranosyl backbone and its branches contained β-D-glucopyranosyl and β-D-galactopyranosyl residues attached to *O*-3. Moreover, a structure with a (1→6)-linked β-D-glucopyranosyl residue as the backbone and with a (1→3)-linked β-D-glucopyranosyl branch has been determined by Lee et al. (2009).

Several glucans composed of only Glc have also been obtained from the fruiting body. α- or β-Glucan can be purified from the water-extract using different chromatographic columns. A α-(1→3)-D-glucan branched with α-D-Glc*p*-(1→ and →3,4)-α-D-Glc*p*-(1→ at the *O*-4 position was purified by DEAE-sepharose and Sephacryl S-500 columns (Zhang et al. 2011), while a purified component primarily consisted of β-1,3-branched-β-1,6-glucan was fractionated through DEAE-cellulose and Sepharose CL-6B columns (Yang et al. 2016). Moreover, glucan separated from the alkali-extract of the water-insoluble residues obtained after water extraction was identified as a β-D-glucan (Dong, Jia, and Fang 2006). Its main chain was composed of (1→3)-linked β-D-glucopyranosyl residues, and it had a single β-D-glucopyranosyl branch substituted at *O*-6 of every third backbone residue, corresponding structure is illustrated in Figure 3.

HEP from the fruiting body not only contains monosaccharide, but also combines with proteins. Mizuno et al. (1992) have discovered a galactoxyloglucan-protein and a xylan-protein from the water-extract and 5% sodium hydroxide extract, respectively. Both of them had β-(1→3) and (1→6) glucosidic linkages based on IR and NMR (^{1}H, ^{13}C) analysis.

Structural features of the purified HEP from mycelium and culture broth

To the purified polysaccharide from mycelium, only heteropolysaccharide and glycoprotein have been reported, while no homopolysaccharide has been recently discovered (Table 2). Most heteropolysaccharides consist of Man, Glc and Gal (Wang, Gao, et al. 2015; Zhang et al. 2017), and the possible structure of a heteropolysaccharide (EP-1) has been established through HPLC, GPC, GC, GC-MS, FT-IR and NMR (^{1}H, ^{13}C) measurements (Wang, Gao, et al. 2015) (seen in Figure 4). EP-1 had a main chain mainly composed of →3)-α-D-Glc*p*-(1→ and →3)-β-D-Glc*p*-(1→ and some side chains which attached to C-4 position, and it was terminated by α-D-Man*p*-(1→. An acidic glycoprotein (HEG-5) with a ratio of protein to polysaccharide approximately as 10:1 (%/%) has been characterized by SDS-PAGE, Native-PAGE, MALDI-TOF-MS, FT-IR, CD and GC approaches

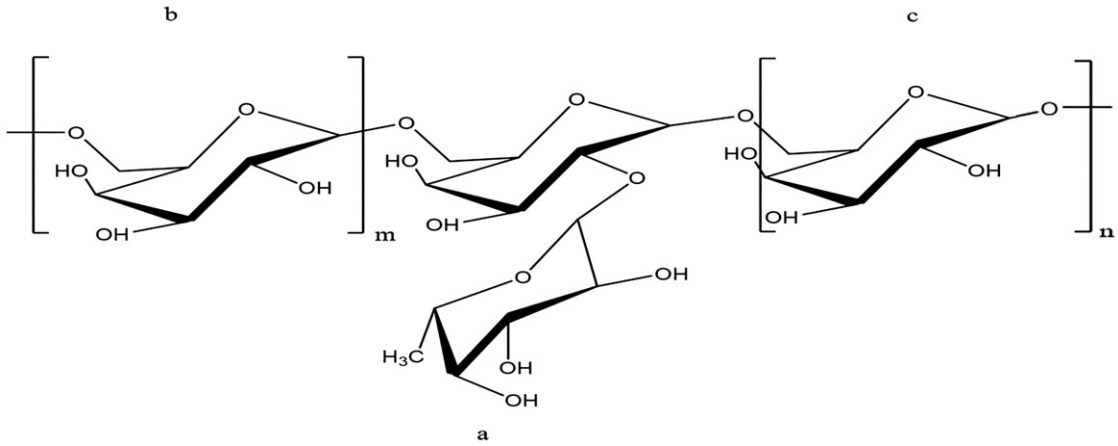

Figure 2. The predicted primary structure of a heteropolysaccharide from the fruiting body by water extraction, from Li et al. (2016). a was α-L-fucopyranose, b was (1→2,6)-linked α-D-galactopyranose and c was (1→6)-linked α-D-galactopyranose; m + n = 3, reprinted with permission from Elsevier (Li et al. 2016).

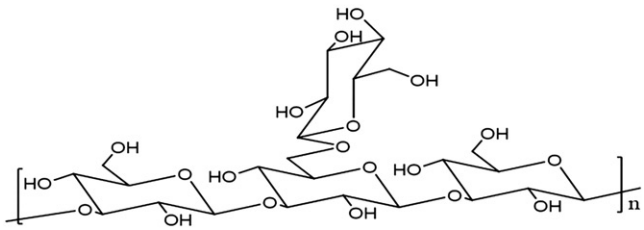

Figure 3. The proposed structure for a β-D-glucan isolated from the fruiting body by alkali extraction, reprinted with permission from Dong, Jia, and Fang (2006).

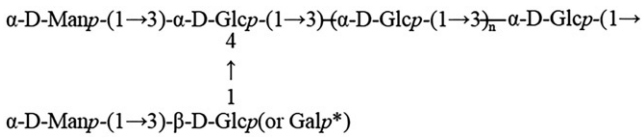

Figure 4. The possible structure of a heteropolysaccharide extracted from the mycelium by ethanol precipitation, from Wang, Yang, et al. (2015). *means only approximately 10% Gal*p* was linked with the main chain, reprinted with permission from Elsevier (Wang et al. 2015).

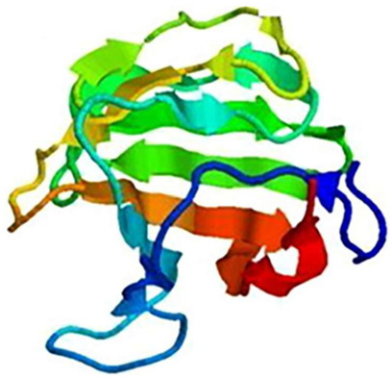

Figure 5. The predicted three-dimensional structure of a glycoprotein obtained by online SWISS-MODEL Workspace, reprinted with permission from Cui et al. (2014).

(Cui et al. 2014), and the relevant structure is shown in Figure 5. The protein and polysaccharide were connected with →4)-β-Gal*p* and β-Glc*p*-(1→ residues.

Heteropolysaccharide, glucan and mannan have been purified from culture broth by several investigators. An exopolysaccharide gained by acetone precipitation and Sephadex CL-5B column chromatography was reported to have a M_w of 1000 kDa and it consisted of Glc, Gal, Xyl, Man and fructose (Fru) in a molar ratio of 1.5: 1.7: 1.2: 0.6: 0.9 (Park et al. 2002), while another obtained by ethanol precipitation and dialysis was ascertained to possess an apparently lower M_w (40 kDa) and was composed of Fuc, ribose (Rib), Ara, Xyl, Man, Gal and Glc with the mass percentage of 20.1: 0.2: 1.5: 0.3: 35.4: 34.1: 8.4 (Yang, Park, and Song 2003). Obviously, different extraction and purification methods produce these distinctions. Similar phenomenon was observed in homopolysaccharides. Shang, Song, Wang, et al. (2014) acquired a α-D-glucan without branch structure through water extraction and Sepharose CL-6B column chromatography, and its repeat structure might be {-1,4-Glc_3-1,6-Glc}$_n$. While, a polysaccharide obtained by ethanol precipitation and DEAE cellulose and Sepharose CL-6B column chromatographies was identified to be a β-1,3-branched-β-1,2-mannan, which had a (1→2)-linked mannopyranosyl backbone with a (1→3)-linked mannopyranosyl side chain (Lee, Cho, and Hong 2009).

Conformation of the purified HEP

The conformational characteristics of the purified HEP from *H. erinaceus* have been preliminarily investigated. A β-1,3-branched-β-1,6-glucan from fruiting body (Lee et al. 2009; Yang et al. 2016) and a β-1,3-branched-β-1,2-mannan from culture broth (Lee, Cho, and Hong 2009) were determined to possess a laminarin-like triple helix conformation structure in NaOH solution. However, a β-D-glucan from fruiting body was reported to have a highly ordered hydrogen-bond dependent conformation structure in water, and this structure was collapsed in strong alkaline solution (Dong, Jia, and Fang 2006). Based on CD spectrum scanning, the secondary structure of a glycoprotein from mycelium was estimated to contain 13.9% of α-helix, 56.5% of β-sheet and 29.6% of random-coil structure (Cui et al. 2014).

Differences in structural features among the purified HEP from three different sources

Various purified polysaccharides with different M_w have been derived from fruiting body, mycelium and culture broth because of diversities in source materials, isolation methods and fractionation protocols (Table 1 and Table 2). Although the purified fractions from these three sources were mostly composed of Gal, Glc and Man, the monosaccharide compositional ratios of them are multifarious. Many glycosyl linkage types have been discovered in the purified HEPs from fruiting body, such as 1-, 1,6-, 1,2,6-, 1,4,6-linked Gal*p*, 1-, 1,2-linked Fuc*p*, 1,3,4-linked Man*p*, 1-, 1,3-, 1,6-, 1,3,6-, 1,3,4-, 1,2,6-linked Glc*p* and 1-, 1,3-linked Rha*p*. In contrast, relatively less glycosyl linkage types including 1,4-, 1,3,4-linked Gal*p*, 1,3-, 1,3,4-, 1,4-, 1,6-linked Glc*p* and 1-, 1,2-, 1,3-, 1,6-, 1,2,6-linked Man*p* have been reported in those from mycelium and culture broth. What's more, diversity of types, sequences and connection sites of glycoside residues resulted in different structures of backbone and branch chain among the purified HEP from these three sources. According to recent results, purified HEP from fruiting body or mycelium of *H. erinaceus* might contain both α- and *β*-configuration (Wang, Yang, et al. 2015; Wu, Zhou, Zhou, Ou, and Huang 2017; Wu, Zhou, Zhou, Ou, Zhang, et al. 2017; Zhang, Sun, et al. 2007), while only one type of configuration (α- or *β*-) has been found in those from culture broth (Lee, Cho, and Hong 2009; Shang, Song, Wang, et al. 2014).

Different structures of the purified polysaccharides from these three sources cause different behaviors on the biological activity. As is shown in Table 3 and Table 4, some HEPs with different structures, obtained from these three sources, have been demonstrated to exhibit different immunomodulatory effects on RAW 264.7 cells (Lee et al. 2009; Wu et al. 2017) and anti-cancer activities on SGC-7901 cells (Yang et al. 2016; Zan et al. 2015). Moreover, the findings of Lee et al. and coworkers showed that both a *β*-1,3-branched-*β*-1,6-glucan from fruiting body and a *β*-1,3-branched-*β*-1,6-mannan from culture broth could improve NO synthesis and the expression of TNF-α and IL-1*β* mediated by RAW264.7 cells (Lee et al. 2009; Lee, Cho, and Hong 2009). However, the former enhanced much more than the latter in the NO synthesis and IL-1*β* expression, which was attributed to their different structural features. The former with M_w of 13 kDa was mainly composed of Glc, and it had Glc*p*-(1→, →6)-Glc*p*-(1→, →3)-Glc*p*-(1→ and →3,6)-Glc*p*-(1→ in a relative molar ratio of 0.22: 1.00: 0.46: 0.21. While, the latter with M_w of 46 kDa only consisted of Man, and it contained Man*p*-(1→, →2)-Man*p*-(1→, →3)-Man*p*-(1→, →6)-Man*p*-(1→ and →2,6)-Man*p*-(1→ in the relative molar ratio of 0.26: 1.00: 0.59: 0.27: 0.40.

Biological activities of HEP

Immunomodulation activity

Numerous *in vitro* and *in vivo* studies have been performed and demonstrated the immunomodulation activity of HEP on splenic lymphocyte, macrophage and dendritic cell, and this action has been employed for the disease resistance against *Aeromonas hydrophila* in grass carp (Gou et al. 2018) (Table 3). Spleen is the largest secondary immune organ which combines the innate and adaptive immune system in the body (Cesta 2006; Mebius and Kraal 2005). HEP from fruiting body and culture broth can activate splenic lymphocytes. In the report described by Sheng et al. (2017), the ConA-stimulated proliferation of splenic lymphocytes was significantly promoted when mice were administered with HEP from fruiting body at a dose of 150 mg/kg body weight. The findings of Wu et al. showed that the polysaccharide from fruiting body could stimulate T and B lymphocytes proliferation and interleukin-2 (IL-2), interleukin-4 (IL-4) and interferon-*γ* (IFN-*γ*) secretions (Wu, Zhou, Zhou, Ou, Zhang, et al. 2017). Similarly, the mitogenic activities of splenocytes were obviously up-regulated as splenic lymphocytes from spleen of BALB/c mice were cultivated with the crude polysaccharide fractions (HE-GE-CP-1, 3 and 5) from submerged culture of *H. erinaceus* in the medium supplemented with Korean ginseng extracts (Kim et al. 2009).

Macrophage is a crucial member of the mononuclear phagocyte system that exhibits multiple functions during immune responses (Guilliams et al. 2014). Recent evidences have shown that HEP possessed macrophage activation activity (Kim, Ra, et al. 2012; Lee, Cho, and Hong 2009; Lee et al. 2009; Li et al. 2015; Ren et al. 2017; Wu, Zhou, Zhou, Ou, and Huang 2017; Wu, Zhou, Zhou, Ou, Zhang, et al. 2017), which mostly appeared as enhancing pinocytic and phagocytic capacities and nitric oxide (NO) and pro-inflammatory cytokines secretions (Lee, Cho, and Hong 2009; Lee et al. 2009; Ren et al. 2017; Wu, Zhou, Zhou, Ou, and Huang 2017; Wu, Zhou, Zhou, Ou, Zhang, et al. 2017). Meanwhile, the mRNA and protein expressions of NOS, interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) were improved (Wu, Zhou, Zhou, Ou, and Huang 2017; Wu, Zhou, Zhou, Ou, Zhang, et al. 2017). Moreover, the action mechanism of HEP from fruiting body in activating RAW 264.7 cells has been proposed, whilst that from mycelium or culture broth was unknown. Wu et al. declared that the macrophage activation activity of a heteropolysaccharide (HEP-W), a ultrapure water elution fraction of the crude polysaccharide from fruiting body, was realized through myeloid differentiation protein 88 (MyD88)/IL-1R-associated kinase (IRAK-1)/TNF receptor associated factor 6 (TRAF-6)/phosphoinositide-3-kinase (PI3K)/protein kinase B (Akt)/mitogen-activated protein kinases (MAPKs) signaling pathways, and toll-like receptor 2 (TLR2) synergized with mannose receptor (MR) to co-regulate the immunomodulatory response in RAW 264.7 cells (Wu, Zhou, Zhou, Ou, and Huang 2017), as is shown in Figure 6. However, the possible mechanism of another eluted with 0.05 M NaCl solution in inducing macrophage immunomodulatory activity was mainly via PI3K/Akt/MAPKs/NF-*κ*B and MyD88/IRAK/TRAF-6/IKKs/IKBs/NF-*κ*B signaling pathways (Figure 7) (Wu, Zhou, Zhou, Ou, Zhang, et al. 2017). This might be explained by their differences in structural features resulted from different elution solvent.

Table 3. Immunomodulation activity of HEP.

Polysaccharide	Source	Main glycosidic bond	Target	Experiment	Action or mechanism	Reference
HEF-AP Fr II	Fruiting body	Glc*p*-(1→, →3)-Glc*p*-(1→, →6)-Glc*p*-(1→ and →3,6)-Glc*p*-(1→	RAW 264.7 cells	*In vitro*	Macrophages activation, NO production (+), IL-1β and TNF-α expressions (+).	Lee et al. (2009)
HP6	Fruiting body	NA	Macrophage cells	*In vitro*	Macrophage activation.	Li et al. (2015)
hHEP	Fruiting body	NA	RAW 264.7 cells	*In vitro*	Macrophage activation, NO, IL-6 and TNF-α production (+), iNOS expression (+).	Ren et al. (2017)
HEP-W	Fruiting body	α-D-Glc*p*-(1→, →3,6)-α-D-Glc*p*-(1→, →2,6)-α-D-Gal*p*-(1→, β-Gal*p*-(1→, →3,4)-β-D-Man*p*-(1→, →3)-α-Rha*p*-(1→ and →2)-β-L-Fuc*p*-(1→	RAW 264.7 cells	*In vitro*	Pinocytic and phagocytic capacity (+), IL-6 and TNF-α production (+), promotes mRNA and protein expression of NOS, IL-6 and TNF-α (+) through MyD88/IRAK-1/TRAF-6/PI3K/AKT/MAPKs signaling pathways.	Wu, Zhou, Zhou, Ou, and Huang (2017)
HEP-S	Fruiting body	α-D-Glc*p*-(1→, →3,4)-α-D-Glc*p*-(1→, →6)-α-D-Gal*p*-(1→, →3,4)-β-D-Man*p*-(1→, →3,6)-α-Rha*p*-(1→ and →2)-β-L-Fuc*p*-(1→	Spleen lymphocytes, RAW 264.7 cells	*In vitro*	Lymphocytes: T and B lymphocytes proliferation (+), IL-2, IL-4 and IFN-γ secretions (+); RAW 264.7 cells: pinocytic and phagocytic capacity (+), ROS production (+), NO, IL-6 and TNF-α production (+), mRNA and protein expression of NOS, IL-6 and TNF-α (+).	Wu, Zhou, Zhou, Ou, Zhang, et al. (2017)
sHEP	Fruiting body	NA	Dendritic cells (DCs)	*In vitro*	Surface expression of MHC-II and CD86 (+), DCs maturation (+), DCs endocytosis (-), IL-12 and IFN-γ production (+), TLR4 activation, phosphorylation of ERK, p38, and JNK (+), nuclear translocation of p-c-Jun, p-CREB, and c-Fos (+), NF-κB signaling activation, IκBα/β and nuclear translocation of p65 and p50 (-).	Qin, Ren, et al. (2017)
HE-PS	Fruiting body	β-1,3-D-glucan	DCs	*In vivo and in vitro*	DCs maturation (+), endocytosis by DC_S (-), IL-12/IFN-γ secretion (+), IL-10 secretion (-).	Sheu et al. (2013)
HEP	Fruiting body	NA	Intestinal mucosal immunity system	*In vivo*	Splenic lymphocyte proliferation (+), serum hemolysin levels (+), macrophage phagocytosis (+), NK cell activity (+), SigA secretion (+), MAPK and AKT cellular signaling pathways (+).	Sheng et al. (2017)
HEP	Fruiting body	NA	Intestinal mucosal immunity system	*In vivo*	Intestinal morphological structure (+), intestinal mucosal IELs (-), goblet cells and mast cells (-), secretion of sIgA, IFN-γ and IL-4 (+).	Wu, Jiang, et al. (2017)
HE-GE-10-CP-II and HE-CP-II	Mycelium	NA	Macrophage, peyer's patch cells	*In vitro*	Lysosomal enzyme activity and IL-12 production of macrophage (+), intestinal immune system modulating activities through Peyer's patches.	Kim, Ra, et al. (2012)
HEB-AP Fr I	Culture broth	Man*p*-(1→, →2)-Man*p*-(1→, →3)-Man*p*-(1→, →6)-Man*p*-(1→, →2,6)-Man*p*-(1→	RAW 264.7	*In vitro*	Macrophages activation, NO production (+), IL-1β and TNF-α expressions (+).	Lee, Cho, and Hong (2009)
Water-soluble Numbers of CD4+ cells, T cells and macrophages (+).	poly- Wang, Hu, Su, et al. (2001)	saccharides	Culture broth	NA	CD4+ cells, T cells and macrophages	*In vivo*

(*continued*)

Table 3. Continued.

Polysaccharide	Source	Main glycosidic bond	Target	Experiment	Action or mechanism	Reference
HE-GE-CP-1, 3 and 5	Culture broth	NA	Macrophage, splenocyte and bone marrow cells	*In vivo* and *in vitro*	Mitogenic activity of splenocytes (+), bone marrow cell proliferation (+), lysosomal phosphatase of macrophage (+).	Kim et al. (2009)
HCMP	Culture broth	NA	Fish	*In vivo*	Bactericidal and lysozyme activities of serum (+), complement C3 and superoxide dismutase activity of serum (+), Total serum protein, albumin and globulin contents (+), immune-related genes (IL-1β and TNF-α) expressions (+), IL-10 expressions (-).	Gou et al. (2018)

(+): improve or promote; (-): inhibit or reduce.
NA: information was not available.

Dendritic cells (DCs) initiate and regulate the highly pathogen-specific adaptive immune responses and are central to the development of immunologic memory and tolerance (Geissmann et al. 2010). HEP from fruiting body was found to induce DCs maturation and suppress DCs endocytosis (Qin, Ren, et al. 2017; Sheu et al. 2013). Meanwhlie, the polysaccharide exposure significantly stimulated DCs to secrete IL-12 and IFN-γ cytokines that promote TH1 responses. Further study has indicated that the selenizing HEP enhanced the MAPK and NF-κB signaling pathways downstream of TLR4 (Qin, Ren, et al. 2017).

Immunomodulatory effect of HEP can be mediated by intestinal immune system as gut tract owns the highest immune activity of body (Hu, Nie, and Xie 2018). HEP from fruiting body could up-regulate the secretion of immunoglobulin A (sIgA) and activate the MAPK and AKT cellular signaling pathways in the intestine of mice (Sheng et al. 2017), as is illustrated in Figure 8. Also, the treatment of it significantly increased the secretion of sIgA, IFN-γ and IL-4 to enhance intestinal mucosal immune functions of Muscovy duck reovirus (MDRV)-infected Muscovy ducklings (Wu, Jiang, et al. 2017). Furthermore, Kim, Ra, et al. (2012) argued that HEP from mycelium produced regulation effect on intestinal immune system through the activation of Peyer's patch cells, which is considered to be the major inductive site for initiating the high affinity secretory IgA immune responses in gastrointestinal tract (Yamamoto et al. 2000).

Anti-cancer activity

Resistances of HEP on colonic, hepatocellular, gastric, cervical and mammary cancers have been manifested in cell and animal models (Table 4). HEPs from fruiting body and mycelium inhibited the proliferation or growth of HepG2 (Lee and Hong 2010), HeLa (Li et al. 2012; Qin, Zhang, et al. 2017) and SGC-7901 cells (Zan et al. 2015), and promoted the apoptosis of SGC-7901 cells (Yang et al. 2016) *in vitro*. After being received the administration of HEPs from fruiting body, ICR/Slc mice implanted with sarcoma 180 cells showed smaller tumor size and lower mortality along with longer survival time (Mizuno et al. 1992). Kim et al. (2009) discovered that the lung metastasis of tumor was notably alleviated when mice inoculated colon 26-M3.1 cells were supplemented with HEPs from culture broth. In a cell experiment, caspase-8/-3-dependent, p53-dependent mitochondrial-mediated and PI3k/Akt signaling pathways involved in the apoptosis and cell cycle arrest of SGC-7901 cells induced by a purified glycoprotein HEG-5 from mycelium (Zan et al. 2015), as is shown in Figure 9.

Recently, chemotherapy using doxorubicin (Dox) is frequently adopted in cancer treatment. HEP is reported to be an enhancer for the anti-cancer capacity of Dox, which may be helpful to reduce the adverse effects from the excessive usage of Dox. Yang et al. (2016) highlighted that 100 μg/mL of the polysaccharide elevated Dox-

Table 4. Anti-cancer activity of HEP.

Polysaccharide	Source	Main glycosidic bond	Target	Experiment	Action or mechanism	Reference
Fl_0-a-α, Fl_0-a-β; FII-1; FIII-2b	Fruiting body	(l,3), (1,6)-β-glucan	ICR/Slc mice implanted with sarcoma 180 cells	*In vivo*	Tumor size (-), survival time (+) and mortality (-) of mice.	Mizuno et al. (1992)
HE	Fruiting body	1,3-branched-β-1,6-glucan	Human hepatocellular carcinoma cell line, HepG2	*In vitro*	Cell growth (-), Dox-mediated apoptotic signaling (+), reducing c-FLIP expression via JNK activation and enhancing intracellular Dox accumulation via the inhibition of NF-κB activity.	Lee and Hong (2010)
HE	Fruiting body	1,3-branched-β-1,6-glucan	Human gastric cancer cells SGC-7901	*In vitro*	Viability (-) and apoptosis (+) of cell, Dox-induced ROS (+), expression of HIF-1α (-).	Yang et al. (2016)
Crude Polysaccharide	Fruiting body	NA	HeLa cells	*In vitro*	Cell proliferation (-).	Li et al. (2012)
HEP-2	Mycelium	NA	HeLa cells	*In vitro*	Cell growth (-).	Qin, Zhang, et al. (2017)
HEG-5	Mycelium	1,4-linked β-Gal*p* and β-linked Glc*p*	Human gastric cancer cells SGC-7901	*In vitro*	Proliferation and colony formation of SGC-7901 cells (-); Caspase-8/-3-dependent, p53-dependent mitochondrial-mediated and PI3k/Akt signaling pathways: expressions of Bcl2, PI3K and AKT1 (-), expressions of Caspase-8, Caspase-3, p53, CDK4, Bax and Bad (+).	Zan et al. (2015)
HE-GE-CP-1, 3, and 5	Culture broth	NA	BALB/c mice inoculated with colon 26-M3.1 carcinoma cells	*In vivo*	Lung metastasis (-).	Kim et al. (2009)

(+): improve or promote; (-): inhibite or reduce.
NA: information was not available.

induced ROS and down-regulated the protein expression of Hypoxia-inducible factor-1α (HIF-1α) in SGC-7901 cells. The study taken by Lee and Hong (2010) implied that this polysaccharide activated the Dox-mediated apoptotic signaling through reducing cellular FLICE-inhibitory protein (c-FLIP) expression via c-Jun N-terminal kinase (JNK) activation and enhancing intracellular Dox accumulation via the inhibition of NF-κB activity in HepG2 cells. Accordingly, combined utilization of this polysaccharide with Dox might be a more effective strategy for cancer therapy.

Antioxidant activity

Lots of *in vitro* experiments have denoted that HEP had scavenging effects on the free radicals including 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydroxyl, ABTS and oxygen radicals (Chen et al. 2015; Cheng et al. 2016; Li et al. 2012; Malinowska, Krzyczkowski, Herold, et al. 2009; Wang et al. 2017; Zhang, Lv, et al. 2012; Yan et al. 2018; Wang, Yin, Zhao, et al. 2018). Its ferrous ion-chelating activity, reducing power and inhibition of lipid peroxidation have also been verified (Li et al. 2012; Malinowska, Krzyczkowski, Herold, et al. 2009; Wang et al. 2017; Wang, Yin, et al. 2018; Zhang, Lv, et al. 2012). Cell studies have shown that the polysaccharide could inhibit the production of reactive oxygen species (ROS), thereby reducing the oxidative stress-induced damage (Cheng et al. 2016; Wang et al. 2017; Wei and Van Griensven 2008).

Numerous evidences insinuated that HEP was capable of elevating the enzymatic and non-enzymatic antioxidant activity and reducing lipid peroxidation *in vivo*. Shang et al. 2014 discovered significant improvements of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities and decrease of malondialdehyde (MDA) level in the serum, liver, and breast muscle when broilers were supplemented with an exo-biopolymer (total sugar content: 775 g/kg) from the submerged fermentation concentrate of *Hericium caput-medusae* (Bull.:Fr.) Pers. In a CCl_4-induced hepatotoxicity model, levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the serum were prominently reduced when mice were treated with HEP. And increases of SOD, GSH-Px and CAT activities and a decline of MDA level in liver of mice were clearly observed (Zhang, Lv, et al. 2012). As for a renal ischemia reperfusion (IR) model, pre-administration of HEP generated remarkable declines of blood urea nitrogen (BUN) and serum creatinine in IR animals. Falls in the activities of antioxidant enzymes (SOD, CAT, GSH-Px and glutathione reductase) and the level of reduced glutathione, and the rise of MDA level in serum and renal of mice were greatly attenuated (Han, Ye, and Wang 2013).

Additionally, the antioxidant activity of HEP may be a momentous mediator to its anti-skin-aging and anti-fatigue activities. Xu et al. (2010) put forward that HEP observably enhanced antioxidant enzymes (SOD, CAT and GSH-Px) activities and decreased MDA and lipofuscin levels in dose-dependent manners in the skin of aged rats. The results in a study of Liu et al. (2015) signified that its strong anti-fatigue

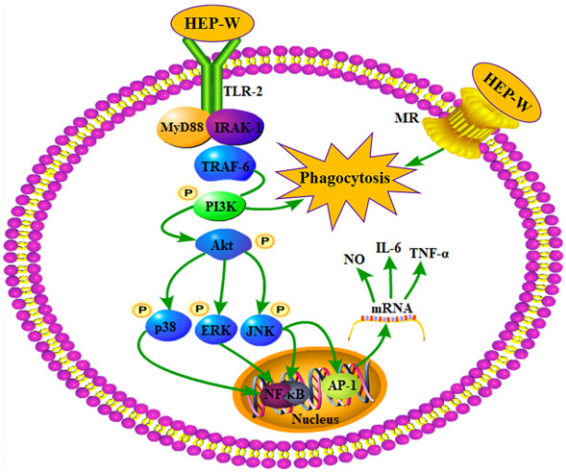

Figure 6. Possible signal transduction pathways involved in macrophage activation by HEP-W obtained by water elution from the water-extract of *H. erinaceus* fruiting body, reprinted with permission from Wu, Zhou, Zhou, Ou, and Huang (2017).

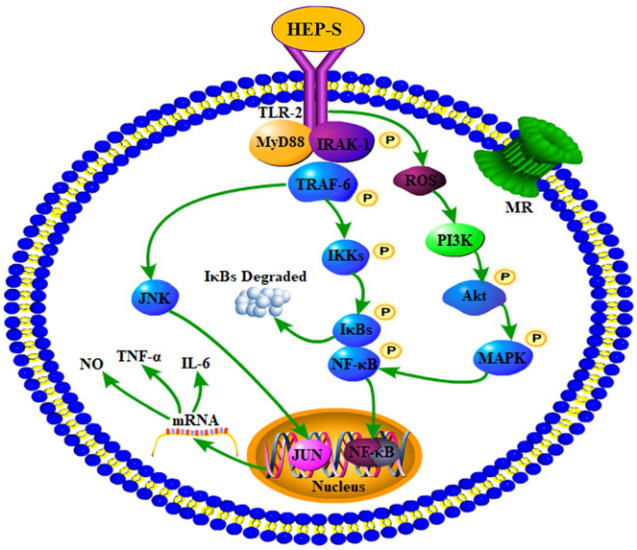

Figure 7. The proposed signal transduction pathways involved in macrophage activation by HEP-S obtained by 0.05 M NaCl elution from the water-extract of *H. erinaceus* fruiting body, reprinted with permission from Wu, Zhou, Zhou, Ou, Zhang, et al. (2017).

activity was achieved by declining blood lactic acid, SUN and MDA content, and increasing tissue glycogen content and antioxidant enzyme activities (SOD and GSH-Px).

To the best of our knowledge, many mushroom polysaccharides usually contain some amounts of polyphenols, which may influence its antioxidant activity. Regarding HEP, Shang et al. argued that the phenolic compounds in it were associated with its antioxidant capacity in broilers (Shang, Song, Jiang, et al. 2014). In our previous study, crude and refined HEPs showed different behaviors in scavenging effects of DPPH· and hydroxyl radicals and Fe^{2+}-chelating ability *in vitro* were mainly caused by their differences in total phenols contents (Wang, Yin, Zhao, et al. 2018). Therefore, the *in vivo* and *in vitro* antioxidant activities of HEP are influenced by its polyphenols. Besides, the findings of Yan et al. (2018) implied that the ABTS radical

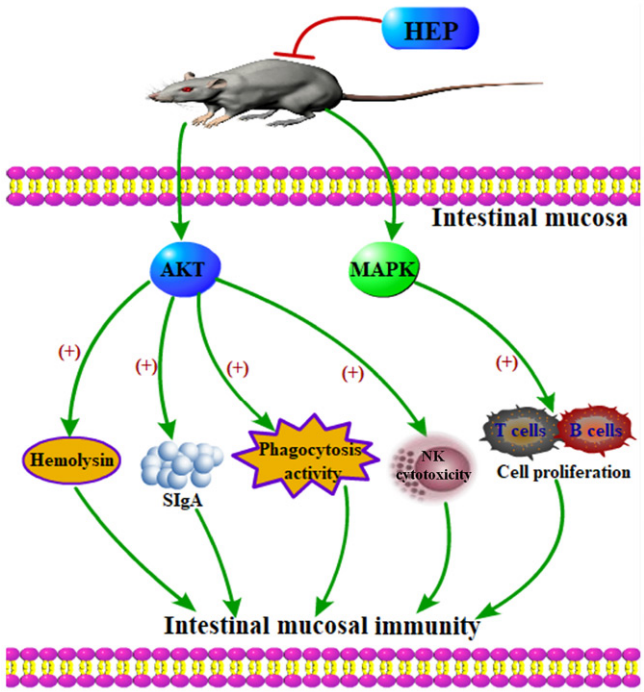

Figure 8. Possible mechanism of HEP obtained by water extraction from *H. erinaceus* fruiting body in immunomodulation on the basis of intestinal immunology, reprinted with permission from Sheng et al. (2017).

scavenging activity *in vitro* of HEP might be positively related to its protein content.

Gastroprotection and intestinal health promotion

H. erinaceus has been widely used in the adjunctive therapy of gastric ulcer in folk consumption or in the past researches (Abdulla et al. 2008; Wong et al. 2013). The gastroprotective activities of polysaccharides from mycelium and fruiting body have been recently demonstrated. Wang, Konishi, et al. (2015) highlighted that HEP was the bioactive compound of mycelium extracts that could dramatically decrease the ulcerated area of ethanol-induced ulcer mice and the viability of MC cells, a precancerous transformation of GES-1 cells. Another work made by them has confirmed that a purified polysaccharide EP-1 from mycelium obviously inhibited the growth of MC cells (Wang, Gao, et al. 2015). The mechanism of EP-1 in preventing H_2O_2-induced apoptotic cell death of GES-1 cells has been further identified to be inhibiting activation of apoptotic cellular signals within mitochondria-dependent apoptotic pathways (Wang et al. 2017). Moreover, in our previous study, HEP from fruiting body was able to reduce ethanol-induced gastric mucosal lesion and pylorus ligation-induced gastric ulcer in rats. In the pylorus ligation-induced model, results suggested that the possible action mechanism seemed to be connected with the regulation of gastric secretion (volume of gastric juice, gastric acid, pepsin and mucus), improvement of anti-inflammatory (TNF-α and IL-1β) and antioxidant status (SOD and GSH-Px activities), as well as increment in the releases of defense factors (NO, prostaglandin E2 and epidermal growth factor) (Wang, Yin, Zhao, et al. 2018).

Otherwise, the study taken by Zhu, Chen, et al. (2014) indicated that two purified HEP prepared from fruiting body and theirs bismuth (Bi^{3+})-complexes had definite inhibition effects on *Helicobacter pylori*, a most prevalent global pathogens of gastrointestinal diseases such as peptic ulcers, gastric marginal zone lymphoma and gastric carcinoma (Thung et al. 2015).

HEP has great influences on improving the intestinal health of animals. Dietary polysaccharides from the submerged fermentation concentrate of *Hericium caput-medusae* (Bull.:Fr.) Pers produced obvious declines in *Escherichia coli* count and pH of cecum, along with significant increases in counts of *Lactobacilli* and *Bifidobacteria* and concentration of propionic acid of cecum in broilers (Shang, Song, Wang, et al. 2014). Our previous works showed that pretreatments of the crude and refined HEPs from fruiting body improved colonic health of mice which appeared as increasing short chain fatty acids production and moisture amounts, and reducing pH values of colonic and cecum contents along with feces (Wang, Yin, Nie, et al. 2018). Interestingly, HEP plays a crucial role in alleviating inflammatory bowel disease (IBD). Chen et al. (2017) proclaimed that HEP extracted from fruiting body had prebiotic effects that restoring cytokine levels such as TNF-γ, IL-10, IL-12, IL-17α, IL-4 and TNF-α to normal, decreasing the expression of NF-κB, TNF-α, and IL-17 and enhancing the settling down of *Bifidobacterium* in the colons of trinitro-benzene-sulfonic acid (TNBS)-induced rats. The results of Ren et al. (2018) revealed that HEP from mycelium could ameliorate dextran sulfate sodium (DSS)-induced colitis in C57BL/6 mice via regulation of oxidative stress, inflammation-related signaling pathways and modulation of gut microbiota composition, the relevant mechanism is shown in Figure 10.

Figure 9. Possible schematic diagram of antitumor mechanism of HEG-5 via the caspase-8/-3 dependent pathway, p53-dependent mitochondrial mediation, and PI3k/Akt regulation in SGC-7901 cells, reprinted with permission from Zan et al. (2015).

Neuroprotective activity

Neuroprotective effect of HEP on rat pheochromocytoma nerve cells (PC12) has recently attracted considerable attention. Enhancements in the growth and differentiation of PC12 cells have been viewed after the cells were cultured with an exo-polysaccharide from culture broth (Park et al. 2002). Meanwhile, HEP from fruiting body exhibited restoration and regeneration capability on PC12 cells after injury induced by amyloid beta (Cheng et al. 2016) and L-glutamic acid (Zhang et al. 2016). An *in vivo* investigation revealed that both HEPs from culinary and medicinal *H. erinaceus* could accelerate sensory functional recovery after peripheral

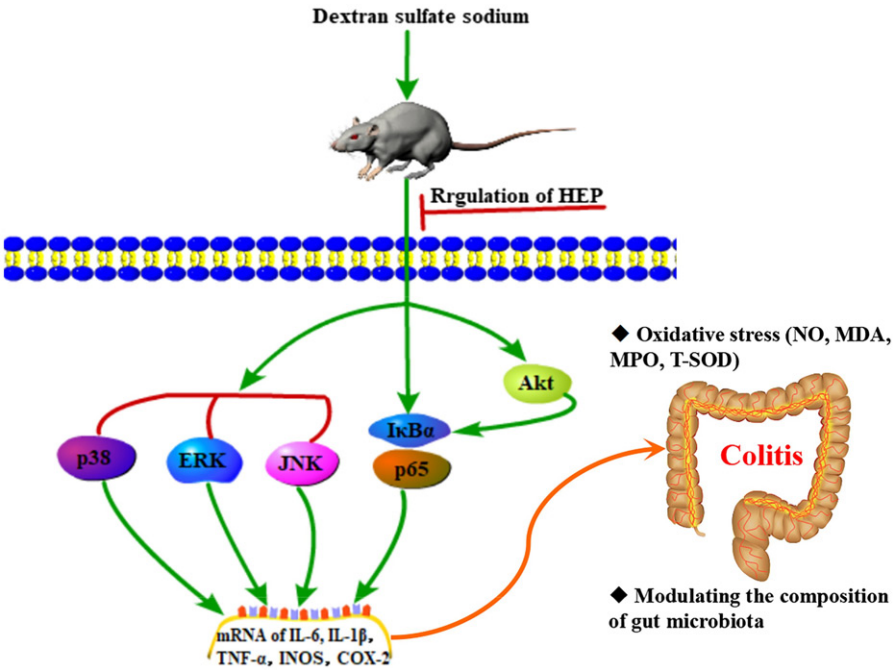

Figure 10. The mechanism of a polysaccharide (M_w: 86.67 kDa) obtained from the mycelium by ethanol extraction and water extraction in inhibiting DSS-induced colitis of C57BL/6 mice, reprinted with permission from Ren et al. (2018).

nerve injury, and this action involved with the activation of AKT/p38 MAPK signaling pathways and restoration of blood-nerve barrier (Wong et al. 2015). Furthermore, HEP from mycelium behaved as a novel substance for treating or preventing Alzheimer's disease in that its neuroprotective activity has been demonstrated in an Alzheimer's disease mouse model induced by the combination of $AlCl_3$ and D-galactose (Zhang et al. 2016).

Hepatoprotective effect

The CCl_4-induced hepatic injury model is frequently applied in the estimation of hepatoprotective activity of HEP *in vivo* (Cui et al. 2016; Zhang, Lv, et al. 2012). The findings of Cui et al. (2016) displayed that extracellular and intracellular polysaccharides from mycelium lowered AST and glutamic pyruvic transaminase activities, and improved blood lipid (cholesterol, triglyceride, and albumin) levels in the serum of mice. Meanwhile, lipid peroxidation and MDA level were largely decreased, and SOD and CAT activities were significantly increased in liver tissues. HEP also showed protective effect against *Salmonella Typhimurium*-induced liver damage in mice (Kim, Moon, et al. 2012). In addition, HEP synergized with Dox might be served as an effective tool for treating drug-resistant human hepatocellular carcinoma (Lee, and Hong 2010).

Antihyperglycaemic and hypolipidemic activities

Like other polysaccharide (Nie et al. 2017), HEP exerted antihyperglycaemic and and hypolipidemic activities *in vivo*. Zhang et al. (2017) found that supplement of mycelium polysaccharide markedly lessened the blood glucose level, suppressed the increments of alkaline phosphatase, ALT, AST, BUN and creatinine levels in serum, improved the antioxidant enzymatic (SOD, GSH-Px and CAT) activities, and attenuated the pathological damage to pancreas, liver and kidney in streptozotocin-induced diabetic mice. Hypolipidemic effect of HEP obtained from the submerged mycelial culture of *H. erinaceus* has been assessed by Yang and the coworkers. In the investigation, a certain dose administration of this polysaccharide substantially declined the plasma total cholesterol, LDL cholesterol, triglyceride, phospholipid, atherogenic index and hepatic HMG-CoA reductase activity, and elevated the plasma HDL cholesterol level in dietary-induced hyperlipidemic rats (Yang, Park, and Song 2003). Additionally, HEP was found to reduce fat deposition and promote cholesterol metabolism in broilers (Shang et al. 2015; Shang, Song, Wang, et al. 2014).

In vitro antihyperglycaemic activity of HEP was proved to have inhibition effect on α-amylase and α-glucosidase activities (Zhang et al. 2017; Yan et al. 2018). The findings of Yan et al. (2018) indicated that HEP extracted with citric acid solution exhibited stronger inhibitory effects on the α-glycosidase and α-amylase activities than that extracted with hot water, 0.9% NaCl or 1.25 M NaOH/0.05% $NaBH_4$.

Other biological activities such as antibacterial action, inhibition activity of HIV-1 reverse transcriptase and the therapeutic potential against human monocytic leukemia of HEP have been pointed out in the previous researches (Kim et al. 2011; Li et al. 2010; Ren et al. 2014).

Structure-activity relationship

A large number of reports have stated that biological activities of mushroom polysaccharides are greatly influenced by the structural features including molecular weight (M_w), chemical composition, branching configuration and conformation (Meng, Liang, and Luo 2016; Ren, Perera, and Hemar 2012; Zhang, Cui, et al. 2007). With respect of HEP, it is feasible to explore the structure-activity relationship thanks to its relatively simple structure. Therefore, the influences of M_w, monosaccharide composition, functional group, glycosyl linkage type, degree of branching and conformation characteristics on some bioactivities of them were discussed as follows.

Influence of M_w

M_w seems to be closely correlated with bioactivity of mushroom polysaccharide (Liu et al. 2010). In the case of HEP, two polysaccharides (FI_0-a and FI_0-b) have been fractionated by DEAE-cellulose chromatography from the hot water extract of fruiting body. FI_0-a (M_w: 118.0 kDa) exhibited 40.3% of inhibition rate on the tumor size of Sarcoma 180 (S-180) in mice, whereas FI_0-b (M_w: 565.0 kDa) generated 63.8% inhibition percentage. Meanwhile, the anti-tumor activities of FI_0-a-α and FI_0-a-β further purified from FI_0-a using Con A-AF-Toyopearl 650 affinity column have been evaluated. The former with M_w of 89 kDa exerted lower inhibition effect on S-180 than the latter which had M_w of 175 kDa (Mizuno et al. 1992). It could be speculated that higher M_w polysaccharides from *H. erinaceus* fruiting body might have stronger anti-tumor activities. However, to polysaccharide-protein from mycelium, an opposite phenomenon appeared. Cui et al. have isolated three glycoprotein (HEG-1, HEG-3 and HEG-5) fractions from mycelium and studied their inhibitory effect on the proliferation of SGC-7901 cells. The results indicated such effects of them were in an order of HEG-1< HEG-3< HEG-5, which was contrary to the M_w order that was HEG-1> HEG-3> HEG-5 (Cui et al. 2014). Therefore, more efforts should be spent to monitor the correlation of anti-tumor action with M_w for HEP. On the other hand, HEP with low M_w from fruiting body exhibited high scavenging ability on DPPH, hydroxyl and ABTS radicals and ferric reducing activity along with strong inhibitory effects on α-glycosidase and α-amylase activities (Yan et al. 2018). And the results of Li, Li, et al. (2018) implied that HEP with immune activity usually had a relatively large M_w, and that with low M_w had strong antioxidant activity. Therefore, the activity of HEP was significantly correlated with its M_w. However, the correlation between M_w and bioactivity of it should be further confirmed.

Influence of monosaccharide composition

HEP mainly composed of Glc is reported to have strong activities. An investigation conducted by Wang, Hu, Su, et al. (2001) implied that HEP extensively consisted of glucose was more effective than the one that was mainly composed of galactose in anti-artificial pulmonary metastatic tumor and immunoenhancement effects in ICR mice. Particularly, β-glucan from *H. erinaceus* is a good source for immunomodulation and anti-cancer. Lee et al. (2009) found that a β-1,3-branched-β-1,6-glucan from fruiting body stimulated NO production and the expressions of IL-1β and TNF-α mediated by activated macrophages. In the researches of Lee et al. and Yang et al., this polysaccharide has been further demonstrated to have anti-cancer activities on hepatocellular carcinoma (Lee et al. 2010) and gastric cancer (Yang et al. 2016). Moreover, a α-D-glucan from the submerged fermentation concentrate of *Hericium caput-medusae* (Bull.:Fr.) Pers greatly affected the performance, gutmicroflora, and cholesterol metabolism of broiler chickens (Shang et al. 2014).

Otherwise, β-1,3-D-glucan content in HEP might play important roles in its bioactivity. Sheu et al. (2013) proposed that the bioactive components of HEP was β-1,3-D-glucan, which exhibited immunomodulatory effects on DCs. Yan et al. (2018) considered that the β-1,3-D-glucan content had certain effects on the *in vitro* antioxidant activity of the HEP.

Influence of functional group

Relationship between functional group and antihyperglycaemic activity of HEP has been preliminarily uncovered in a research of Zhang et al. (2017). In their study, two purified fractions (HIPS1 and HIPS2) were separated from the intracellular polysaccharide (HIPS) of *H. erinaceus*, and HIPS1 exhibited more superior effects in antihyperglycaemia and organic protection than HIPS2 which possible owing to the abundant functional groups (-NH2, -COOH and S = O) of HIPS1. Since immunomodulation activity of HEP was also greatly affected by the functional group, some investigators have applied some chemical methods such as selenylation and hydroxyethylation to modify the purified polysaccharide for enhancing this activity. Qin et al. (2017) acquired nine selenizing polysaccharides with O-Se-O and Se-O-C groups, and found some of them had more apparent actions in inducing DCs maturation than the unmodified polysaccharide. In a study of Ren et al., the FT-IR spectrum illustrated that the hydroxyethylated polysaccharide (hHEP) presented one additional absorption band at 1363 cm^{-1} in comparison with the original polysaccharide HEP, indicating the presence of a symmetrical -CH_2- stretching vibration. Cell experimental results have shown that hHEP exerted higher immunomodulation function than HEP (Ren et al. 2017). Besides, the anti-*Hp* activity of the polysaccharide was related to the containing functional group. Zhu, Chen, et al. (2014) applied chemical modification methods to add Bi^{3+} to the carboxyl group of HEP and found that the Bi^{3+}-polysaccharide complexes expressed stronger anti-*Hp* activity.

Influence of glycosyl linkage type, degree of branching and conformation

Mizuno et al. (1992) thought that β-(1→3) and β-(1→6) glucosidic linkages might be significant for HEP from fruiting body in its anti-cancer activity against Sarcoma 180 cells. This principle has been confirmed by other researches. A β-1,3-branched-β-1,6-glucan which had a backbone consists of (1→6)-linked glucopyranosyl and a branch composed of (1→3)-linked glucopyranosyl exerted macrophage activation, anti-gastric cancer and anti-hepatoma actions (Lee et al. 2009; Lee et al. 2010; Yang et al. 2016). These findings might indicate that a structure with (1→6)-linked glucopyranosyl in the main chain and (1→3)-linked glucopyranosyl in the side chain was important for the anti-cancer and immune-stimulating activities of HEP. Otherwise, a β-1,3-branched-β-1,2-mannan possessed a (1→2)-linked mannopyranosyl main chain and a (1→3)-linked mannopyranosyl side chain could also activate macrophages (Lee, Cho, and Hong 2009).

Polysaccharide with a degree of branching (DB) in the range of 0.2-0.33 is commonly considered as having strong bioactivity (Bae et al. 2013; Miyazaki et al. 1979), which might also be appropriate for HEP. A β-1,3-branched-β-1,6-glucan and a β-1,3-branched-β-1,6-mannan with a DB value of 0.2, obtained from fruiting body and culture broth of *H. erinaceus* respectively, exerted macrophage activation activity (Lee et al. 2009; Lee, Cho, and Hong 2009). Meanwhile, both of them were found to have a triple helical conformation, which was supposed to be a crucial factor for the biological actions of polysaccharides (Falch et al. 2000; Mueller et al. 2000). Furthermore, HEP with this conformation structure could synergize with doxorubicin to resist human hepatocellular carcinoma and gastric cancer (Lee and Hong 2010; Yang et al. 2016). Therefore, the triple helix conformation structure might be an important factor for the immunomodulation and anti-tumor activity of HEP.

Future perspectives

H. erinaceus has attracted considerable attention because it has a variety of biological activities and the properties both as food and medicine. Although fruiting body, mycelium and culture broth of *H. erinaceus* have been applied in some foods or medicines, more human nutritional, clinical and epidemiological studies are recommended for further confirming the practical effects. Up to now, bioactive compounds of the fruiting body have been extensively studied, while fewer researches have been carried out to investigate those of mycelium and culture broth. Since bioactive compounds from these three sources usually have different structures and bioactivities, more attention paid to the bioactive metabolites of mycelium and culture broth is necessary in the future. In this review, HEPs from these three sources have been extensively listed. Neither of structure and bioactivity of HEP showed regularities, so that how to choose *H. erinaceus* sources for acquiring desired HEP is still unknown. However, it is possible to apply fruiting body as the material to get β-glucan with strong

immunomodulation and anti-cancer activities by a suitable means, as several researches have demonstrated that β-glucan from the fruiting body was effective in these two activities (Lee et al. 2009; Lee and Hong 2010; Yang et al. 2016). Alkali extraction method is reported to be a good way for the acquisition of β-glucan from fruiting body of *H. erinaceus*, whether it is also appropriate for mycelium and culture broth is uncertain. And the biological activity of this kind of β-glucan has been rarely investigated.

The current advances on the extraction, separation and purification, structural characteristics and biological activities of the HEP from the fruiting body, mycelium and culture broth were systematically summarized. However, it is difficult to identify the exact structure of HEP due to its structure is affected by a lot of factors. Even though some action mechanisms of HEP have been illustrated, there is a doubt that whether the same mechanism is appropriate for HEP from three different sources or not. Moreover, the structure-activity relationship of HEP was preliminary discussed and some views should be further confirmed. Furthermore, for enhancing the bioactivity of HEP, more efforts should be spent to the structural modification of it. Additionally, TLR-2 and MR receptors were supposed to be two important receptors for HEP in macrophage activation (Figure 7 and Figure 8). While, the connection between HEP and these two receptors remains unknown. As HEPs from fruiting body, mycelium and culture broth have been demonstrated to possess various health-promoting and medicinal properties, their applications in health product and clinical medicine are promising.

Acknowledgments

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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