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Review

Mushroom β-glucans as anticancer and therapeutic agents: a focus on their mechanism of action

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β-glucans are biologically active polysaccharides derived from natural sources, especially mushrooms, renowned for their immunomodulatory, antitumor, and anti-inflammatory properties. These substances can be ingested without safety concerns either as dietary supplements or as regular components of daily nutrition. Chemoprevention is a crucial approach in reducing cancer development and tumor progression including the utilization of natural substances to prevent, and even reverse the process of cancer development. β-glucans, especially those from mushrooms, have been recognized as effective chemopreventive and adjuvant agents when combined with standard chemotherapy. They appear to operate through various mechanisms and may exhibit additive or synergistic effects. While *in vitro* and animal (*in vivo*) studies have consistently demonstrated their medical potential, clinical investigations into the precise mechanisms of action remain limited. Therefore, understanding the specific molecular changes that drive cancer development and progression, and developing anticancer and adjuvant chemotherapeutic drugs, which improve cancer treatment is still a main challenge in this field. Therefore, this review intends to summarise recent research findings on the anticancer effects of selected mushroom β-glucans, covering a range of preclinical (*in vitro* and *in vivo*) and several clinical studies, with a focus on their chemopreventive and chemotherapeutic potential. We also discuss their potential molecular mechanisms of action and provide a brief overview of the classification, structural characteristics, sources, and origin of β-glucans obtained from various edible mushrooms.

Keywords: mushrooms, β-glucans, anticancer effects, therapeutic potential

1 Introduction

Mushrooms are a popular and nutritious dietary choice, known for their low-fat, cholesterol-reducing, and low-sodium attributes. They are abundant in health-promoting compounds, with β-glucan being a notable substance. β-glucans are polysaccharides primarily found in the fruiting bodies of mushrooms. They are unique and distinct from those in oats and barley (Zhu et al., 2015), and their positive health effects have been extensively documented over the last twenty years. In particular, β-glucans from edible mushrooms have attracted considerable interest in nutrition, medicine, and biotechnology due to their strong immuno-boosting, antioxidant, and anticancer properties. Chemoprevention is a crucial approach in reducing cancer development and tumor progression, with extensive research spanning several decades. This term includes the utilization of synthetic or natural compounds to hinder, arrest, or even reverse the process of carcinogenesis, even at a premalignant stage. This approach is receiving growing interest from both the scientific community and the general population (Sporn & Suh, 2002). Commonly, chemopreventive agents fall into two main categories, each with distinct mechanisms and targets in carcinogenesis. The first group includes blocking agents that directly inactivate carcinogens, and stimulate antioxidant enzyme activity (Landis-Piwowar & Iyer, 2014), while the second group comprises suppressing agents that inhibit the promotion and progression of cancer development by interfering with key processes and signalling pathways related to cancer cell division, growth, cell cycle regulation, and apoptosis pathways (George et al., 2021). In this regard, numerous studies have indicated the chemopreventive

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and chemotherapeutic potential of mushroom β-glucans, primarily due to their immunomodulatory, growth inhibitory, pro-apoptotic, DNA damaging or cell cycle blocking effects. Many *in vitro* and animal studies investigating cytotoxic, antiproliferative or tumor inhibitory properties of various edible mushroom β-glucans on different cancer cells have failed to clarify the precise molecular mechanism of action. Hence, this review intends to summarise recent research findings on the antitumor activities of selected mushroom β-glucans, covering a range of preclinical models and several clinical studies. The primary focus is on their potential for both chemoprevention and/or chemotherapeutic applications. Moreover, the structural features, sources, and origin, as well as classification of β-glucans present in various edible mushrooms have been included in this contribution.

2 Classification, chemical structure and sources of mushroom β-glucans

Mushrooms are well-known for being rich sources of carbohydrate and fibre, making up the majority of their dry mass. The carbohydrate content may vary from 50% to over 90%, while the fibre content ranges from 4% to 20% (dry weight basis) in some cultivated species like *Agaricus* (*A.*) *bisporus*, *Pleurotus* (*P.*) *ostreatus*, *P. eryngii*, *Auricularia* (*A.*) *auricula-judae*, *Lentinula* (*L.*) *edodes* (Vetter, 2019). Mushroom carbohydrates are not only abundant but also diverse in their structure. They can be categorised into three groups:

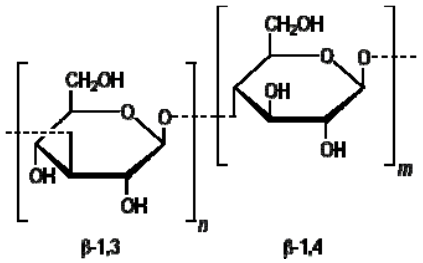
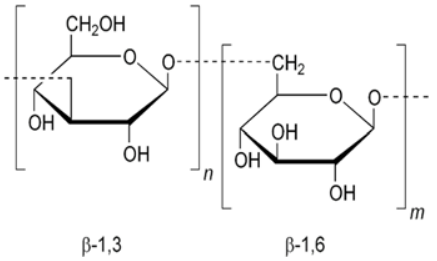
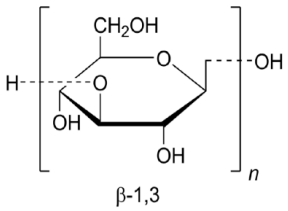
1. monosaccharides, consisting of 5- and 6-carbon sugars (such as glucose, arabinose and fructose molecules) with sugar derivatives (e.g. mannitol);
2. oligosaccharides (such as trehalose and melezitose);
3. polysaccharides, including chitin, hemicellulose, glycogen, glucans, mannans, xylans, and galactans (Vetter, 2023), which are mainly present in some edible mushroom cultivars (exceed more than 90%) (Leong et al., 2021).

Among the extensively examined mushroom-derived polysaccharides, β-glucans represent a category of dietary fibres or water-soluble polysaccharides mainly comprised of a backbone of D-glucopyranose units linked by β-(1,3) glycosidic bonds (Morales et al., 2019). They are integral parts of cell walls and aleurone layers in various organisms. They naturally occur in microorganisms (bacteria, fungi, yeast), mushrooms, lichens, algae, as well as in cereals (especially oats and barley) (Kaur et al., 2020)which, when incorporated in food, is renowned for its ability to alter functional characteristics such as viscosity, rheology, texture, and sensory properties of the food product. The functional properties of β-glucans are directly linked to their origin/source, molecular weight, and structural features. The molecular weight and structural/conformational features are in turn influenced by method of extraction and modification of the β-glucan. For example, whereas physical modification techniques influence only the spatial structures, modification by chemical agents, enzyme hydrolysis, mechanical treatment, and irradiation affect both spatial conformation and primary structures of β-glucan. Consequently, β-glucan can be modified (via one or more of the aforementioned techniques. The source of extraction affects the size, structures, and arrangements of β-glucans. Mushrooms and yeast are sources of water-insoluble β-glucans, which have β-(1,3) glycosidic bonds along with β-(1,6) branching. In contrast, water-soluble β-glucans from cereals are primarily composed of β-(1,3) and β-(1,4) glycosidic linkages. Bacterial and algal β-glucans are characterized by a straight or linear structure, where glucose monomers are connected through β-(1,3) glycosidic bonds (Mudgil, 2017). The β-glucan content fluctuates according to the source, specific type, strain, growth location, and environmental conditions. A detailed description of β-glucan content in different food sources and their chemical structures is shown in Table 1.

The biological effects of β-glucans depend on a range of factors, including their primary composition, branching extent, charge, solubility, molecular structure, and size, particularly in an aqueous environment (Zhu et al., 2015). Several bioactive β-glucans have been extracted from various types of macrofungi species, including lentinan, krestin, grifolan, schizophyllan, and pleuran (Mirończuk-Chodakowska et al., 2021). Lentinan, initially obtained from the cell walls of *L. edodes* in 1970, is a 1,3-1,6-β-glucan, characterized by a central glucose backbone consisting of five glucose molecules, with two glucose side chains connected to the central chain via a β-(1,6) glycosidic bond (Chihara et al., 1970). The structure of schizophyllan is unpartially altered. This β-glucan is derived from the mushroom *Schizophyllum* (*S.*) *commune* and consists of three glucose molecules linked by β-(1,3) glycosidic bonds, with one glucose side chain attached to the main chain via a β-(1,6) glycosidic bond (Mohammadi et al., 2018).

Grifolan, obtained from *Grifola* (G.) *frondosa*, comprises a primary chain of three glucose units and one side chain attached via a β-(1,6) glycosidic linkage. Pleuran, isolated from *P. ostreatus*, consists of a primary chain of four glucose molecules connected by β-(1,3) glycosidic bonds. Additionally, it contains side chains linked to the primary chain through β-(1,6) glycosidic bonds.

Table 1 A summary of representative β-glucans in various food sources

Representative structure of β-glucan backbone	Food source		β-glucan content	References
 β-1,3 β-1,4	higher plant	oats	2.2–5.82%	Herrera et al., 2016
		barley grain	2.5–11.3%	Izydorczyk & Dexter, 2008
		rye	1.2–2.9%	Lazaridou et al., 2007
		wheat	0.4–1.4%	Rakszegi et al., 2014
		rice	0.04–0.88%	Lazaridou et al., 2007
 β-1,3 β-1,6	yeast	*Saccharomyces cerevisiae*	5–7%	Aimanianda et al., 2009
	mushroom	*Gyrophora esculenta*, *Lentinus edodes*, *Ganoderma lucidum*, *Flammulina velutipes*	4.71–46.20%	Lee & Kim, 2005
		Pleurotus ostreatus	22–56%	Golian et al., 2022
	seaweed	*Durvillaea antarctica*	5–33%	Bobadilla et al., 2013
 β-1,3	microalgae	*Euglena gracilis*	≥90%	Barsanti et al., 2001

This specific structure gives it unique properties and bioactivities, making it a valuable component in the food and pharmaceutical industries, often used as a dietary supplement for a long-term use under the trade name Imunoglukan P4H® (Jesenak et al., 2013)

These macrofungal β-glucans have demonstrated a broad spectrum of health advantages, encompassing immunomodulatory, anticancer, antioxidant, antiviral, immune-boosting, and antidiabetic effects (Jesenak et al., 2013; Zi et al., 2020). They are not only valuable as disease-preventing agents but also serve as essential components of anticancer or anti-inflammatory therapy.

3 Anticancer activities and mechanism of action of mushroom β-glucans

Mushroom β-glucans are not only popular as nutritional supplements in many countries, including Slovakia, but they also exhibit remarkable disease-fighting and preventive properties. They provide an extensive array of health-advancing benefits, including immunomodulatory, antidiabetic, antihyperglycemic, antioxidant, antihyperlipidemic, antiatherogenic, anticholesterolemic antiproliferative, anticancer, anti-aging, antiviral, neuroprotective, anti-inflammatory, cardiovascular protective, and radioprotective activities (Afiati et al., 2019; Murphy et al., 2022; Tripodi et al., 2022).

While extensive research has been conducted on mushroom β-glucans in cancer, their precise molecular mechanisms of action remain poorly understood. They are thought to exert their anticancer effects through DNA damage and modification, immunomodulation, activation of inflammatory cells, initiation of apoptosis, cell cycle regulation, and signalling pathway modulation (Schwartz & Hadar, 2014). In the following sections, we provide a brief overview of the antitumor activities of selected edible mushroom β-glucans in preclinical and clinical studies, focusing on their molecular mechanisms and potential as chemopreventive and chemotherapeutic agents.

3.1 Preclinical (in vitro and in vivo) studies

Mushrooms β-glucans have shown their efficiency to directly suppress cell proliferation, inhibit cancer cell proliferation, and induce cytotoxicity in different cancer cell lines and animal tumor models, including breast, neuroblastoma, colorectal, cervical/ovarian, prostate, liver, skin, gastric, bladder, and stomach cancers (Ayeka, 2018; Filiz et al., 2021). One of their notable anticancer mechanisms involves immune system modulation, where

β-glucans moderate inflammatory reactions in animal models of tumors by decreasing the concentrations of pro-inflammatory cytokines, chemokines, and adhesion molecules (Lavi et al., 2006). Although β-glucans recognize numerous membrane receptors, including Dectin-1, the CR3 receptor, and Toll-like receptors (TLRs), which are present on the membrane of myeloid cells such as macrophages, monocytes, granulocytes, and natural killer (NK) cells, as well as dendritic cells, it is not surprising that their anticancer activities are mainly attributed to their modulatory effects and the release of cytokines, nitric oxide, arachidonic acid, and other metabolites within the tumor microenvironment (Schwartz & Hadar, 2014; Vetvicka, et al., 2021). In this regard, studies have unveiled the antiproliferative, cytotoxic, and immunomodulatory effects of β-glucans derived from *Ganoderma* (*G.*) *lucidum* in Lewis lung carcinoma bearing mice (C57BL/6) (at a dose of 10 $mg.kg^{-1}.day^{-1}$) when administered alongside radiation therapy. These effects were achieved by increasing macrophage and T-lymphocyte infiltration in the local tumour (Chen et al., 2014). Activated macrophages resulting from *A. blazei* and *G. lucidum* treatments were found to induce cytokines (such as Tumor Necrosis Factor, TNF-α and Interleukin 8, IL-8) and nitric oxide production in several *in vitro* studies (Sorimachi et al., 2001; Berovic et al., 2003). The antitumor and immunomodulatory activity of β-glucans derived from the mushroom *Flammulina velutipes* was revealed in sarcoma SC-180 cells, whereas these effects were not observed in *in vitro* conditions (Leung et al., 1997). Furthermore, it has been uncovered that the activation of the β-glucan receptor (dectin-1) may induce macrophage M1 polarization, leading to atherogenic effects through the NF-κB-autophagy-dependent pathway (Li et al., 2019). Dectin-1, also known as a non-Toll-like receptor (TLR), appears to play a central role in antifungal immunity (Kimberg & Brown, 2008). Dectin-1 has been extensively studied due to its high affinity for β-1,3-glucans present in fungal cell walls. It is notably expressed on dendritic cells, neutrophils, and macrophages of the immune system, where it plays a crucial role in host defense against fungi and in preventing tumor development (del Fresno et al., 2013). While TLRs are localized on the cell surface or in intracellular compartments, such as the endoplasmic reticulum, endosome, lysosome, or endolysosome, to stimulate innate immune responses for pathogen recognition and anti-fungal activity by regulating the activation of antigen-presenting cells and key cytokines (Duan et al., 2022). It has been widely recognized that Dectin-1 expressed by dendritic cells and macrophages significantly contributes to the activation of natural killer (NK) cells through the interferon regulatory factor 5 (IRF5) pathway, facilitating effective recognition and killing of tumor cells (Chiba et al., 2014). However, recent observations have indicated that these β-glucan receptors, like Dectin-1, are also found to be expressed in cancer cells, suggesting their possible role as an attractive target for experimental therapeutics in neoplastic transformation. Therefore, additional studies in this field are warranted (Seifert et al., 2015; Liu et al., 2023).

Similar immunomodulatory findings of β-glucans were reported in a study by Wang et al. (2015). *G. lucidum* or *Antrodia camphorata* polysaccharides were shown to significantly increase Interleukin 12 (IL-12) and Interferon Gamma (IFN-γ) mRNA expression, reduce transforming growth factor-β (TGF-β) production, Prostaglandin-Endoperoxide Synthase 2 (COX-2), Interleukin 6 (IL-6), Interleukin 10 (IL-10) mRNA expression, and decrease the proportion of M2 macrophages in tumor-bearing mice, resulting in a reduction of immune inhibitory effects in the tumor environment. Furthermore, in research conducted by (Hong et al., 2003)antagonism of growth factor receptors, antibody-dependent cell-mediated cytotoxicity, β-glucans showed promise as potential candidates for immunomodulation and combined cancer immunotherapy.

The induction of the cell apoptosis is another targeted mechanism for effective cancer treatment. In a study by Filiz et al. (2021), β-glucans derived from *L. edodes* exhibited antiproliferative activity and cytotoxic effects on neuroblastoma cell line (SH-SY5Y) by activating the apoptosis pathway via elevating the caspase 3, Poly(ADP-Ribose) Polymerase 1 (PARP) and BCL2 Associated X, Apoptosis Regulator (BAX) protein levels, decreasing the BCL2 Apoptosis Regulator (Bcl-2) protein (at a concentration of 125 $\mu g.ml^{-1}$ for 24 hours). Pro-apoptotic activity of glucans present in the mushroom *P. ostreatus* on human colorectal cancer cells (HT-29) were also proven in another *in vitro* study by Lavi et al. (2006), who observed that treatment with 0.05–0.5% (w/v) glucan fractions resulted in the inhibition of cell proliferation, mediated through the upregulation of pro-apoptotic molecules like BAX and cytosolic cytochrome *c*. The pro-apoptotic impact of β-glucan (at concentrations below 10 $\mu g.ml^{-1}$ for a period of 24 hours) derived from *A. blazei* was further substantiated in human ovarian cancer cells (HRA). In this context, the β-glucan inhibited cell proliferation and stimulated the relocation of BAX from the cytoplasm to the mitochondria, the release of cytochrome c, and the consequent activation of caspase 9. Moreover, there is evidence indicating that β-glucans enhance p38 mitogen-activated protein kinase (MAPK) activity to restrain HRA cell proliferation and improve the apoptosis pathways. This study also showed that β-glucan extracts (at a concentration of 500 $\mu g.ml^{-1}$ for a duration of 21 days) inhibited pulmonary metastasis of 3LL cells

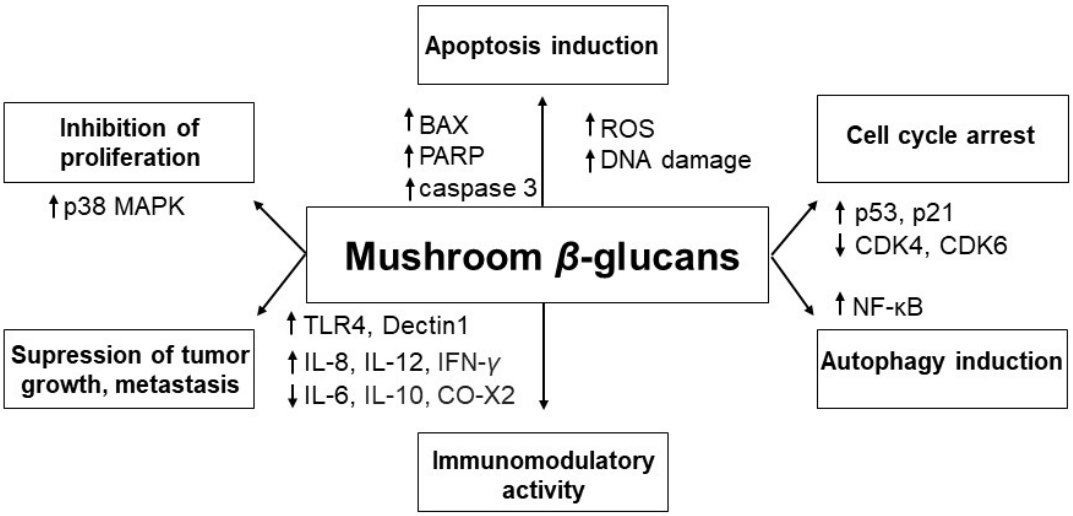


Figure 1 Anticancer effects of mushroom β-glucans with potential molecular targets (an arrow pointing upwards signifies an increase in gene expression, while a downward arrow denotes a decrease in gene expression)

and hindered peritoneal disseminated metastasis of HRA cells *in vivo* (Kobayashi et al., 2005).

Aberrant cell cycle advancement is a frequently observed in a diverse array of human malignancies. The anticancer and antiproliferative effects of β-glucans extracted from *P. ostreatus* on human breast cancer cells (MCF-7) were achieved by inducing G0/G1 cell cycle arrest through the upregulation of the expression of Tumor Protein P53 (p53) and Cyclin Dependent Kinase Inhibitor 1A (p21). Additionally, cell cycle arrest of HT-29 cells was induced at G0/G1 by upregulating the expression of p21 with a dose of 1.0 mg.ml^{-1} of mushroom extract for 24 hours (Jedinak & Sliva, 2008). These alterations were notably associated with the suppression of cyclin/cyclin-dependent kinase (CDK) complexes, particularly CDK4 and CDK6, and the inhibition of phosphorylation in the retinoblastoma Rb protein, which is a key modulator of the G1/S cell cycle checkpoint. In a recent study conducted by Vetvicka et al. (2021), various new molecular targets of β-glucans derived from edible mushrooms were highlighted. These encompass inhibitors of MAPK protein kinase signalling pathways, Nuclear factor kappa (NF-κB) protein kinases, DNA polymerases, activation of natural killer (NK) cells, dectin-1, and Hypoxia Inducible Factor 1 (HIF) factor. Overall, these findings suggest that β-glucans can modulate multiple signalling pathways and molecular targets to exert their antitumor effects. The anticancer activities of mushroom β-glucans and their potential molecular targets are outlined in Figure 1.

3.2 Clinical studies

β-glucan therapy has demonstrated its effectiveness in human clinical studies against a wide array of cancers, including gastric, ovarian, colorectal, esophageal, pancreatic, bladder, breast, liver, lung, bone, cervical, and brain cancer (Sima et al., 2019). Numerous mushroom β-glucans have undergone phase I, II, and III clinical trials and are commonly and effectively employed, primarily in Japan, China, Korea, notably as immunoceuticals. Various species of medicinal mushrooms including *G. frondosa*, *G. lucidum*, *L. edodes*, *Trametes versicolor*, *Phellinus linteus*, *A. blazei*, *Inonotus obliquus* have been studied in numerous clinical trials, demonstrating their anticancer activities with non-toxic and tolerable effects (Wasser, 2014). Although some mushroom β-glucans are already commercialised as nutritional supplements (such as Sizofiran derived from *S. commune*; AndoSan from *A. blazei* Murill; Maitake/grifolan from *G. frondosa*; Shiitake/lentinan derived from *L. edodes*), insufficient data exists for comparing how these products affect individuals in cancer treatment, including details regarding their administration method, dosage, as well as timing of use (Therkelsen et al., 2016; Steimbach et al., 2021). On numerous occasions, mushroom extracts and pure β-glucans were employed as adjunct treatments in conjunction with standard chemotherapy or radiotherapy across a diverse range of cancer types. These interventions not only enhanced treatment effectiveness but also contributed to an overall improvement in patients' quality of life (Vetvicka et al., 2021).

Among the mushroom β-glucans, lentinan is one of the most studied in terms of its anticancer and chemotherapeutic-adjuvant activities. Interestingly, over 9,474 cases of lentinan-assisted cancer treatment have been reported in China. Lentinan is approved as an adjunctive therapy for lung, colorectal, hepatic, gastric, ovarian, breast, pancreatic, nasopharyngeal, duodenal, cervical, and other cancers. Comprehensive

investigations have unveiled the potential mechanisms of lentinan, which encompass the activation of immune cells via various signaling pathways, including TLR4/Dectin1-MAPK, and Syk-PKC-NFκB pathways (Zhang et al., 2019). Moreover, many clinical studies have reported better efficacy in cancer treatment when lentinan was used as an additional drug in conjunction with standard chemotherapeutic agents (fluoropyrimidine/paclitaxel/cisplatin) specifically regarding patient survival in individuals with advanced gastric cancer. The group administered with lentinan exhibited significantly improved one-year, two-year, and five-year survival rates compared to the group treated with chemotherapy alone (91.3% vs 59.4%, 45.7% vs 32.7%, 10.0% vs 0%, respectively) (Ina et al., 2011). Lentinan has also demonstrated its potential as a prognostic predictor, improving the general conditions, symptoms, signs, and overall patients' quality of life with advanced cancers, including pancreatic, colorectal, esophageal, and urothelial bladder cancer (Wang et al., 2012; Sun et al., 2015).

Grifolan has also shown significant antitumor and antimetastatic properties in various clinical trials, and it is well-tolerated with no signs of toxicity or adverse effects (Alonso et al., 2018). It has been demonstrated the potential to improve symptoms or even cause regression in liver, lung and breast cancer patients (58.3%, 62.5% and 68.8%, respectively). The anticancer effects are attributed to its capacity to increase the activity of immune-competent cells (1.2–1.4 times) in comparison to the chemotherapy alone (Kodama et al., 2002; Deng et al., 2009).

Sizofiran combined with irradiation or chemotherapy has also shown therapeutic and anticancer effects in several clinical studies. Sizofiran exhibited anticancer activity in various cancer types, including ovarian, cervical, stomach, lung, head, and neck (Chaichian et al., 2020). For instance, a study conducted by Noda et al. (1992) discovered that the group treated with sizofiran exhibited a higher complete response rate among stage II and III patients with cervical cancer (at a dose of 40 mg and twice at 20 mg per week) compared to the control group, receiving only radiotherapy (91.0% vs. 79.1%; 77.9% vs. 61.2%, respectively). Its antitumor effects are primarily attributed to immune stimulatory activity.

While pleuran has been studied for its health beneficial impacts, particularly in improving the cellular immune system and addressing conditions like respiratory tract infections or metabolic disorders (Piska et al., 2017), there is an insufficient amount of clinical cancer evidence. Recently, a study by (Spacek et al., 2022) suggested potential anticancer effects of pleuran through immunomodulatory actions and significant increases in antitumor cellular immunity in patients with endocrine-dependent breast cancer during extended-term administration (for 12 months). These results also indicate its potential as an important prognostic marker for patients in remission during prolonged treatment.

In summary, additional research and clinical investigations are required to further our understanding of the anticancer properties of mushroom β-glucans, particularly their specific molecular mechanisms of action.

4 Conclusion

In the modern landscape of drug development, combination therapies have emerged as viable approaches for the treatment of a wide array of diseases. Specifically, in the context of cancer management, there is a recognized need to combine conventional chemotherapy with natural adjuvant agents for enhanced efficacy. β-glucans extracted from edible mushrooms are acknowledged as significant therapeutic compounds with chemopreventive, immunomodulatory, and antitumor properties mediated by diverse molecular mechanisms. Furthermore, they are generally well-tolerated, safe, and associated with a minimal risk of causing substantial adverse effects. In this regard, mushroom β-glucans exhibit a synergistic effect with targeted antitumor drugs, potentially improving the overall quality of life for cancer patients However, additional research and clinical studies are required to establish the optimal route of administration and dosage for mushroom β-glucans and gain a more profound understanding of their molecular mechanisms of action in cancer cells.

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