Yeast cell wall polysaccharides as antioxidants and antimutagens: Can they fight cancer?[#]

Minireview

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Polysaccharides represent the major part of the yeast cell wall dry weight and build the skeletal carcass defining cell wall stability and cell morphology (β-D-glucans) or constitute amorphous matrix and cell surface fibrous material (mannans and mannoproteins). It is known that yeast cell wall β-D-glucans reveal immunomodulating properties, which allows for their application in anti-infective and antitumor therapy. Recent data also suggest that polysaccharides reveal antioxidant activity that can result in their protective function as antioxidants, antimutagens, and antigenotoxic agents. The paper provides a review of our continuing research involving water-soluble derivatives of β-D-glucan isolated from the baker's yeast *Saccharomyces serevisiae* and of a glucomannan isolated from the industrial yeast *Candida utilis*. The results are confronted with the available literature data. The derivatives of β-D-glucan demonstrated potent inhibitory effect on lipid peroxidation comparable to that of the known antioxidants and exerted DNA protection from oxidative damage. The free radical scavenging activity was confirmed by spin-trap electron paramagnetic resonance. Antimutagenic and antigenotoxic activity of the yeast polysaccharides was demonstrated using yeast, bacterial, and algal models. The derivatives of β-D-glucan exerted potent enhancement of tumor necrosis factor α (TNF-α) released from murine macrophages and revealed synergistic effect with cyclophosphamide in the treatment of Lewis lung carcinoma and two types of lymphosarcoma in murine models. The results indicate significant protective antioxidant, antimutagenic, and antigenotoxic activities of the yeast polysaccharides and imply their potential application in anticancer prevention/therapy.

Keywords: *Glucan, glucomannan, yeast polysaccharide, antioxidant, antimutagen, anticancer*

Throughout the centuries natural compounds have been widely used for prevention and treatment of cancer. However, in the past century such treatment has been generally undermined by classic medicine. At present, due to the established harmful side-effects of synthetic anticancer agents, a growing number of oncologic patients again opt for the use of natural compounds (sometimes termed "alternative" or "complementary" medicine) or at least for a combination therapy that uses advantages of a synergistic action of conventional and "alternative" cancer therapy.

Such combined therapy has become increasingly popular and earned certain recognition by conventional medicine due to its decreased occurrence of side-effects caused by cytostatic drugs. Hence, natural compounds have been recognized as chemopreventive substances and their mechanism of action has been systematically investigated [1]. The suggested scheme of possible mechanisms of cancer chemoprevention [1] includes primary, secondary, and tertiary prevention

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Abbreviations: AAPH – 2,2-azo-bis(2-amidinopropane)-dihydrochloride; CMG – carboxymethylated (1→3)-β-D-glucan; CPA – cyclophosphamide; DMPO – 5,5-dimethylpyrroline-*N*-oxide; DNA – deoxyribonucleic acid; EPR – electron paramagnetic resonance; GM – glucomannan; LS – lymphosarcoma; ROS – reactive oxygen species; SEG – sulfoethylated (1→3)-β-D-glucan; TNF-α – tumor necrosis factor α; UVA – ultraviolet radiation of the type A.

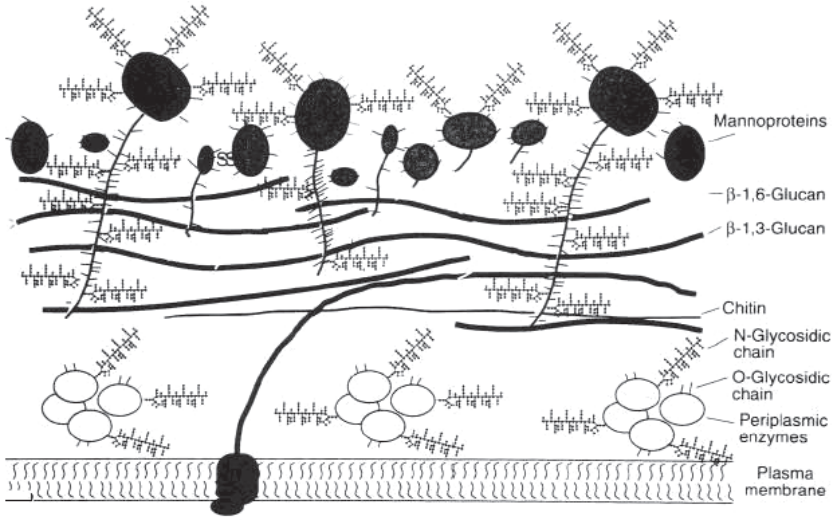

Fig. 1. Composition and structure of the yeast cell wall. Reproduced with permission from [13].

mechanisms of mutagenesis and carcinogenesis. In contrast to the chemoprevention of cardiovascular diseases that has become a common practice, chemoprevention of cancer has not yet been widely accepted nor applied. Like other drugs to be administered to humans, chemopreventive agents should meet certain general demands such as i) reasonably low cost; ii) feasibility of use, regarding sufficient availability, storage conditions, and administration routes, taking into consideration that these agents may need to be applied for long periods of time; iii) efficacy and iv) safety [2]. Taking into consideration these requirements, natural substances that can be isolated from renewable sources and do not have adverse effects, present a welcome supplement to conventional medicines. Natural compounds can attenuate harmful effects of radiotherapy and chemotherapy and at the same time potentiate their pharmacological action [3]. One of the groups of natural compounds that has recently attracted increased interest of researchers and clinicians are fungal polysaccharides [4, 5]. Along with the macroscopic fungi, or mushrooms, yeasts also represent a source of valuable polysaccharides contained in their cell walls.

The yeast cell wall is a thick envelope (100 to 200 nm) representing 15–25% of the dry mass of the cell [6]. The major components of cell wall are polysaccharides (up to 90%), mainly β-D-glucans and α-D-mannans with a minor amount of chitin that constitutes only about 1–2% of the polysaccharides and is located predominantly in the bud scars. Glucans representing 50-60% of all cell wall polysaccharides are principal structural components that play role of a skeletal carcass defining rigidity and stability of the cell and its morphological shape [6, 7, 8]. On the other hand, mannans are linked to proteins forming mannoproteins that are mainly localized at the external surface of the wall and act as a filter for large molecular mass materials [9]. Mannoproteins also participate in cell-cell recognition and determine immunological specificity of yeast strains with oligomannoside side chains of the mannan molecules playing role of immunochemical determinants (epitopes) responsible for the antibody specificity [10, 11, 12]. A schematic depiction of the cell wall of the baker's yeast *Saccharomyces cerevisiae* is provided in Fig. 1.

Besides having distinct structural and physiological functions in the yeast organism, both β-D-glucans and α-D-mannans have been shown to reveal immunomodulating and other beneficial biological effects when applied in various systems in vitro and in vivo. However the systematic study of such properties of the yeast polysaccharides has not yet been carried out and the mechanisms of some of these activities still remain unclear .

In this paper we present an overview of the established antioxidant, antimutagenic, and antitumor activities of the yeast cell wall polysaccharides and summarize our results obtained with the prepared water-soluble derivatives of β-D-glucan isolated from the cell walls of baker's yeast *Saccharomyces cerevisiae* and glucomannan (GM) from the industrial yeast *Candida utilis*. The data obtained are critically compared with the results available to date in the literature.

Antioxidant activity of the yeast polysaccharides. Yeast cell wall polysaccharides, β-D-glucans and α-D-mannans, have been previously demonstrated to reveal antioxidant properties [14, 15]. However, no systematic study of the antioxidant properties of these polysaccharides in relation to their potential antimutagenic/anticarcinogenic properties have been carried out as yet and polysaccharides are not even included in the roster of natural antioxidants [16]. Since it is now generally recognized that oxidative damage caused by reactive oxygen species (ROS) and other free radicals is related to development of many chronic diseases including cancer,

cardiovascular and neurodegenerative diseases that account for major portion of deaths today [17], we have extensively investigated antioxidant and the related antimutagenic/antigenotoxic and anticancer properties of two cell wall polysaccharides isolated from the industrial yeast strains – *Saccharomyces cerevisiae* and *Candida utilis*.

Contrary e.g. to flavonoids, lignins or other natural compounds containing aromatic moieties in their structures, antioxidants of the polysaccharidic nature have been studied only scarcely [18, 19]. Therefore, we have undertaken an in-depth investigation of the antioxidant properties of yeast cell wall polysaccharides. Our results provided evidence for pronounced protective effect of water-soluble carboxymethylated yeast (1→3)-β-D-glucan (CMG) against lipid peroxidation in phosphatidylcholine liposomes, which serve as a model of cell membrane, induced by hydroxyl radicals and microwave irradiation. The extent of lipid peroxidation and damage was quantified as Klein peroxidation index [20], as well as based on the amount of the liposomized fluorescein that leaked as a result of lipid membrane disruption [21]. Comparison of the antioxidant capacities of CMG with that of the established antioxidants showed that it had lower antioxidant activity than α-tocopherol, which is known to be incorporated into the lipid bilayer, however higher than that that of the water-soluble antioxidant D-mannitol [22]. In a recent paper, we have demonstrated that besides rendering protection against lipid peroxidation caused by hydroxyl radicals, CMG also efficiently inhibited peroxidation induced by ultraviolet radiation (UVA), which is known to produce mainly singlet oxygen, $^1O_2$. This capability was lower than that of α-tocopherol. Nevertheless, it was higher than that of hyaluronic acid that is used as a component of sunscreens designed to protect skin against UVA [23].

It is necessary to mention that the precise mechanism, by which yeast polysaccharides exert their antioxidant/free radical scavenging activity is not known yet. Tsiapali et al. suggested that polysaccharides possessed antioxidant properties due to extraction by free radicals of the anomeric hydrogens of the polysaccharide molecules [14]. The authors, using a model of inhibition of 2,2-azo-bis(2-amidinopropane)-dihydrochloride (AAPH)-induced degradation of β-phycoerythrin fluorescence, observed that the tested polysaccharides (α-D-mannan and various water-soluble neutral or charged α- and β-D-glucans) possessed weak free radical scavenging activity that was nevertheless higher than that of their constituent monosaccharides, mannose or glucose. Published in the same year, a paper by Križková et al. describes antioxidant activity of several yeast cell-wall and extracellular α-D-mannans established using a luminol-dependent photochemical method [15]. However, all above mentioned papers only presumed radical-scavenging activity of the polysaccharides as an explanation for the observed polysaccharide-mediated effects.

The first direct proof of the free-radical scavenging activity of yeast polysaccharides was obtained by Kogan et al. by demonstrating efficient scavenging of reactive hydroxyl radicals by CMG established by means of spin-trap electron paramagnetic resonance (EPR) techniques [24]. The experiments involved a study of the scavenging activity of CMG towards the radicals formed in the thermally initiated decomposition of potassium persulfate, hydrogen peroxide, or AAPH in aqueous solutions. In the absence of glucan, high intensity spectra of generated free radicals in the form of their adducts with 5,5-dimethylpyrroline-*N*-oxide (DMPO) were observed. Addition of CMG resulted in concentration-dependent substantial decrease of spectral intensities of adducts as a result of competition of CMG in the scavenging of reactive radicals formed. It was demonstrated that CMG was a more potent radical scavenger than D-mannitol [24]. Fig. 2 demonstrates comparative reduction of the resonance signal of DMPO-radical adduct observed at increased concentrations of CMG and D-mannitol.

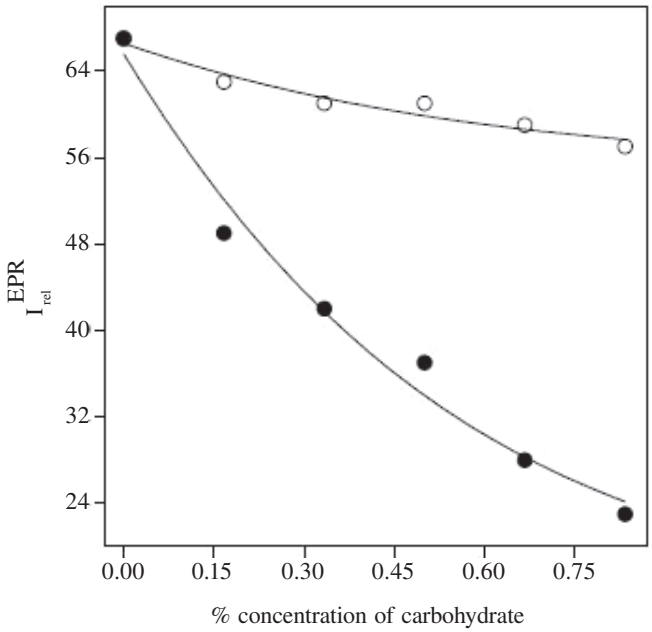

Fig. 2. Relative EPR intensities of ˙DMPO-OH adducts observed after irradiation of DMPO and H_2O_2 at various initial concentrations of D-mannitol (○) and CMG (●) [24].

In the same publication, we corroborated the established antioxidant/radical-scavenging activity of CMG by demonstrating that its administration resulted in suppression of the oxidation-related parameter in the animals with experimentally induced adjuvant arthritis (level of plasma carbonyls), whereas other arthritic parameters not associated with oxidative damage (body mass and activity of plasmatic *N*-acetylglucosaminidase) were not affected [24].

These data supported our earlier observations that CMG and another water-soluble derivative of yeast β-D-glucan, sulfoethyl glucan (SEG), were able to significantly reduce occurrence of DNA strand breaks induced by free radicals, as well as to suppress incidence of DNA lesions caused by ap-

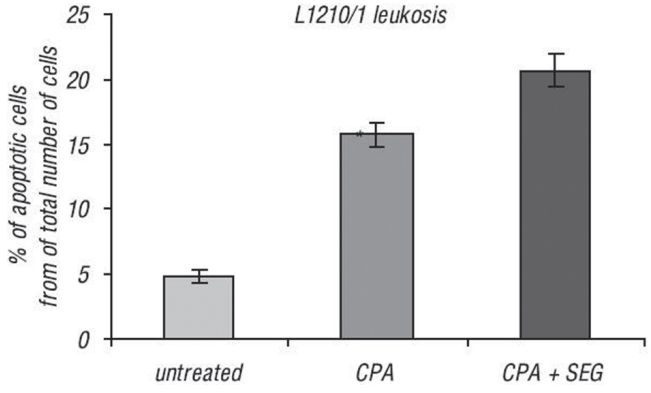

Fig. 3. Enhancement of apoptosis of leukemic cells at the joint administration of cyclophosphamide and sulfoethyl glucan [42].

plication of photosensitized Methylene Blue dye in hamster V79 lung cells [25]. Thus, it seems well established now that water-soluble yeast polysaccharides possess antioxidant and free-radical scavenging activity that may underlie their various protective effects.

Antimutagenic and antigenotoxic activities of the yeast polysaccharides. There is only limited number of reports that describe antimutagenic or antigenotoxic activity of polysaccharides and the majority of the investigated compounds include plant polysaccharides [26], polysaccharide extracts from the fruiting bodies of mushrooms [27], or derivatives of chitin/chitosan prepared from the crustacean shells [28, 29]. Thus, our study of the antimutagenic activity of yeast polysaccharides belongs to the pioneering in this area.

In the early paper stemming from our research group, it was demonstrated that SEG revealed protective effects against mutagenicity induced by potassium bichromate in mice. It has been suggested that the protective effect of SEG can be associated with formation of Cr(VI) ion complexes with sulfoethyl groups and/or by scavenger activity of SEG towards the produced hydroxyl radicals [30]. Later, antimutagenic effect of CMG and glucomannan from *C. utilis* against the clastogenic activity of cyclophosphamide (CPA) was demonstrated using the micronucleation assay in the polychromatic erythrocytes of mouse bone marrow [31, 32]. CPA, an alkylating agent related to the nitrogen mustards, is an efficient cytostatic, immunosuppressant, and anticancer agent acting as it cross-links DNA in actively multiplying cells, however its application is accompanied by many adverse side effects [33, 34]. It is therefore a valuable observation that yeast polysaccharides are able to suppress harmful mutagenic activity of CPA, and, as we have demonstrated in our later publications (see below), there was a synergistic effect of yeast polysaccharides at their combined application with CPA against certain tumor models, which allowed to reduce the applied dosage of CPA, which also contributed to a reduction of its side effects.

Due to the observed pronounced antioxidant properties, derivatives of yeast β-D-glucan as well as yeast glucomannan significantly reduced mutagenicity of the diagnostic mutagens/carcinogens sodium azide, 2-aminofluorene, and 9-aminoacridine in *Salmonella/*microsome assay (Ames test) and in *S. cerevisiae* toxicity/mutagenicity assay using ofloxacin, which is known to be DNA-gyrase inhibitor and producer of ROS [35–37]. It should be noticed that such antimutagenic activity of yeast polysaccharides has been previously described only in one publication [15]. Both types of yeast polysaccharides (water-soluble derivatives of (1→3)-β-D-glucan and glucomannan) also revealed bioprotective effect against methyl methanesulfonate induced genotoxicity in excision repair-deficient algal strain *Chlamydomonas reinhardtii* [35, 36]. GM also elicited anticlastogenic effect against *N*-nitroso-*N'*-methyl urea and maleic hydrazide induced chromosomal aberrations in *Vicia faba* and *Vicia sativa* plants [36, 37], while SEG exhibited statistically significant activity in reducing the damage to choloplast DNA of the flagellate *Euglena gracilis* induced by ofloxacin and acridine orange [38] and markedly reduced the mutagenic effect of ethyl methanesulfonate in the sex-linked recessive lethal assay in *Drosophila melanogaster* [39].

Anticancer activity of the yeast polysaccharides. Having in mind the observed antioxidant and antimutagenic/antigenotoxic activity including DNA protection against oxidative damage, the yeast polysaccharides were subsequently evaluated in several antitumor/anticancer assays. In the first paper of the series, we have demonstrated that the efficiency of chemotherapy of Lewis lung carcinoma with CPA was augmented by administration of CMG. It was found that while CPA showed 57% growth inhibition of the intramuscular tumor implants in comparison with the control group, its combined administration with CMG led to 75–90% inhibition. Similarly, increased inhibition of occurrence of lung metastases (up to 92–94%) was observed using the combined application of the two compounds. The stimulatory effect of CMG was not associated with the changed cellularity of peripheral blood, but was rather due to the obviously increased concentration of the intracellular inhibitor of cysteine proteases – stefin A and cystatin C in tumor tissue [40].

Later, we demonstrated that another β-D-glucan derivative – SEG – enhanced therapeutic effect of cyclophosphamide in mice with the implanted CPA-susceptible and CPA-resistant variants of murine lymphosarcoma (LS), and inhibition of tumor growth was correlated with modulation of the levels of cysteine proteases (cathepsins B and L) and aspartyl protease (cathepsin D) in tumor tissues. Interestingly, application of SEG alone also resulted in increased content of the cathepsins as well as induced tumor regression, which was more pronounced with the susceptible type of LS [41]. Using a model of murine leukosis, it was demonstrated that combined application of CPA and SEG resulted in the enhanced apoptosis of the leukemic cells and was accompanied by a substantial increase of the activity of cysteine proteases cathepsins B and L, in tumor tissues. The results also indicated that at addition of SEG, therapeutic effect of a one-half

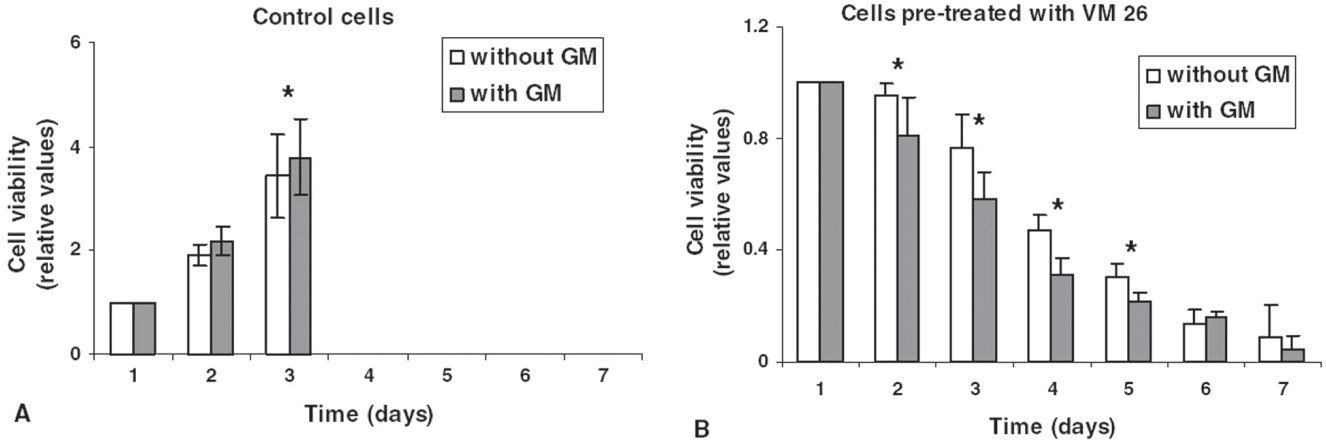

Fig. 4. Cell-revitalization assay using GM. Administration of GM led to enhancement in the proliferation of the untreated mouse leukemia cells (A), while the cells treated in the first phase with drug VM 26 revealed in the presence of GM a statistically significant suppression of viability (B) [37].

reduced dose of CPA was equal or higher than that of the full dose, which permits to use reduced dosage of rather toxic CPA [42]. Fig. 3 illustrates increased apoptosis of the leukemic cells at the combined application of CPA and SEG.

SEG and GM were also evaluated in a so-called cell-revitalization assay that allows to determine whether mouse leukemia cells damaged by exposure to a cytostatic drug (teniposide, VM 26) and subsequently co-cultivated with the yeast polysaccharide are subjected to an accelerated cell death, or are able to recover the cell growth during 1–7 days cultivation. In this way, the cell-revitalization assay assesses the cytotoxic/cytostatic potential of the relevant antineoplastic drug in the presence of bioactive natural compound [43]. We observed that if the non-treated with cytostatic drug control leukemic cells were incubated with a polysaccharide alone, the enhancement of the cell proliferation was recorded already on the second day and became statistically significant on the third day. When leukemic cells were pre-treated with VM 26 and then cultivated in the presence of the yeast polysaccharides, a significantly increased cytotoxic effect was observed (Fig. 4). After removal of the cytostatic drug, cessation of the revitalization of the drug-treated cells confirmed that the administration VM 26 and GM or SEG was more successful and therefore might be worth consideration for a more efficient anticancer therapy [35, 37, 39].

It is currently generally recognized that β-D-glucans mediate their protective and immunopotentiating effect by binding to specific sites (receptors) on monocytes/macrophages and granulocytes triggering a cascade of immunological events. Among the elicited effects are: bone marrow colony stimulating activity leading to augmented production of monocytes and granulocytes, increased antibody titers, boosted cytokine release (including interleukins IL-1, IL-2, IL-6, and tumor necrosis factor TNF-α), prostaglandin E_2 production, activation of alternative complement pathway, and release of lysosomal enzymes [44, 45]. It is now established that β-D-glucan receptors include CR3 [46], lactosylceramide [47], scavenger receptors [48], and Dectin-1 [49]. In agreement with one of the established roles of β-D-glucan in modulation of the innate immunity – stimulation of the number and activity of the immunocompetent cells – we have demonstrated that CMG exerted a potent macrophage-stimulating effect resulting in a dramatic increase of the released TNF-α from the treated murine macrophages in comparison to the control [50]. The eliciting effect was concentration and time-dependent, increasing (although not proportionally) with the increased concentration of the applied polysaccharide and declining with the prolongation of the cultivation period (Table 1) contributing to an overall antitumor/anticancer activity of yeast β-D-glucan.

Glucans isolated from edible mushrooms have been traditionally used mainly in oriental folk anticancer therapy. Even at present times, clinical practice in Japan, China, Korea, and other Asian countries continues to rely on mushroom-derived preparations. Medicinal effects have been demonstrated for many traditionally used mushrooms [51], including extracts of species from genera *Auricularia*, *Flammulina*, *Ganoderma*, *Grifola*, *Hericium*, *Lentinus* (*Lentinula*), *Pleurotus*, *Trametes* (*Coriolus*), *Schizophyllum*, and *Tremella* [4]. Recently, several reviews have been published describing versatile biological including anticancer activities of fungal β-D-glucans

Table 1. Stimulation of release of TNF-α from the murine macrophages by CMG applied in three concentrations: 12.5 μg/ml, 25.0 μg/ml, and 50.0 μg/ml.

Concentration of CMG (mg/ml)	TNF-a release (pg/ml)		
	3 h	6 h	24 h
12.5	1390.4 ± 56.6	1322.8 ± 56.4	1145.2 ± 64.9
25.0	1929.0 ± 136.1	1585.4 ± 69.4	1328.1 ± 84.1
50.0	2653.8 ± 42.6	2504.9 ± 63.1	2114.9 ± 108.6

[5, 52, 53, 54, 55, 56]. Several fungal β-D-glucan preparations have been tested in clinical trials and have shown significant efficacy against various human cancers: lentinan from *Lentinus edodes*, schizophyllan from *Schizophyllum commune*, PSK (Krestin) from *Coriolus versicolor*, and grifolan from *Grifola frondosa* [53]. Despite the fact that there are no data available in literature on the anticancer activity of other yeast polysaccharides, our observation of their antioxidant and antimutagenic activity and the results of *in vitro* and *in vivo* animal studies, can indicate that yeast cell wall polysaccharides could be considered as another suitable natural product for anticancer therapy in combination with the conventional antineoplastic drugs.

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