Antigenotoxic and antioxidant potential of medicinal mushrooms (Immune Assist) against DNA damage induced by free radicals-an *in vitro* study

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ABSTRACT

Immune Assist (IA) is produced from extract of six species of medical mushrooms: *Agaricus blazei - Cordyceps sinensis - Grifola frondosa - Ganoderma lucidum - Coriolus versicolor - Lentinula edodes*. The genoprotective potential of IA was evaluated for the first time. Significant antigenotoxic effects were detected in human peripheral blood cells against H_2O_2 induced DNA damage, in the pretreatment and in the posttreatment. The most efficient concentration of IA in pretreatment was 500 μg/mL, while in posttreatment it was the concentration of 250 μg/mL. Kinetics of attenuation of H_2O_2 induced DNA damage in posttreatment with the optimal concentration of IA showed significant decrease in the number of damaged cells at all time periods (15–60 min), reaching the greatest reduction after 15 and 45 min. Remarkable ·OH scavenging properties and moderate reducing power, together with the modest DPPH scavenging activity, could be responsible for the great attenuation of DNA damage after 15 min of exposure to IA, while reduction of DNA damage after 45 min could be the result in additional stimulation of the cell's repair machinery. Our results suggest that IA displayed antigenotoxic and antioxidant properties. A broader investigation of its profile in biological systems is needed.

1. Introduction

"Let food be your medicine and medicine be your food" was a quote of Hippocrates 400 BCE Ever since, a widely accepted belief has been that diet is closely associated with optimal health. Today, an increase in scientific reports support the opinion that diet and food components play a significant role in maintaining good health, but can also contribute to many chronic diseases [1].

Mushrooms have long been valued as tasty (with specific flavor and aroma) and nutritional food for human beings and also have been considered to be a good source of digestible proteins [2,3]. The gross composition of mushrooms is water (90%), and from the dry matter: proteins (2–40%), carbohydrates (1–55%), fat (2–8%), fibers (3–32%), and ash (8–10%) [4]. Polysaccharides, complex carbohydrate polymers, most of which are β-glucan polymers have been the main issue of attention for their medicinal properties [5]. The polysaccharide phytocomplex is considered to be responsible for its pharmacotoxicological effects [6]. Immune Assist, manufactured in the United States, contains over 200 different hetero-polysaccharide and 1.3–1.6 beta glucan immune modulator compounds. They are extracted and purified from six different species of medicinal mushrooms long known for their medicinal properties, which contain beta-glucan compounds similar to lentinan from *Lentinus edodes*; PSK and PSP from *Trametes versicolor*; ganoderan A, B, C and D from *Ganoderma lucidum*; 1–3, 1–6-β-glucans from *Agaricus brasiliensis*; cyclomannans and beta-mannans, and other polysaccharides from hybridized *Cordyceps sinensis*; and grifolan and protein bound 1–3, 1–4-β-glucans from *Grifola frondosa*.

It is documented that polysaccharide extracts of medicinal mushrooms *Agaricus bisporus* and *Agaricus brasiliensis* act as natural antioxidants [7]. Similarly, some studies have shown antioxidant properties of different molecular weight fractions of polysaccharides [8] and crude extract of polysaccharide (LEP) [9,10] of *Lentis edodes*. Scarpari et al. [11] demonstrated that polysaccharides form *Tramates versicolor* can generate an antioxidant response by manipulating gene expression. The polysaccharides from *Ganoderma lucidum* displayed antioxidant activities in a concentration-dependent manner [12], as did polysaccharides

from *Cordyceps sinensis* [13] and *Grifola frondosa* [14].

The presence of free radicals, continuously derived from either essential metabolic processes or external sources, is associated with the appearance of oxidative stress, which is involved in aging and is implicated in the initiation and progress of various diseases and conditions [15]. Hydrogen peroxide (H_2O_2) causes DNA damage through oxygen-radical mechanisms that can generate multiple DNA modifications, such as base damage, sugar damage, and DNA protein crosslinks. Such modifications can ultimately lead to single-strand and double-strands breaks, causing cell cycle arrest or promotion of tumorigenesis as well as initiation of immune response and inflammation [16].

The current study was performed in order to investigate the genoprotective potential of Immune assist on human whole blood cells against oxidative DNA damage in vitro, by utilizing the comet assay, a highly sensitive method for examining DNA damage within cells. Also, the antioxidant capacity of Immune assist was evaluated by application of the following: ferric reducing anti-oxidant power (FRAP), radical scavenger ability (DPPH) and hydroxyl radical scavenging ability assays.

2. Material and methods

2.1. Subjects

Peripheral blood samples were obtained from six healthy females, between the age 20 and 30 years. The participants did not use cigarettes, alcohol, medicaments, or food supplements. Participants gave their consent in accordance with the regulations of the ethical standards of Ethics Committee for Clinical Trials of the Faculty of Pharmacy, University of Belgrade.

2.2. Material

A commercial product Immune assist of Aloha Medicinals, Inc, Carson City, NV, USA/Santa Cruz, CA, USA facility, was tested in the form of capsules which contain a proprietary blend of: Agaricus blazei (extract), Cordyceps sinensis (extract), Trametes versicolor (extract), Ganoderma lucidum (extract), Grifola frondosa (extract), and Lentinula edodes (extract). The powder was manufactured from hot water extract of Mycelium, primordia and fruiting bodies of growing six species of medicinal mushrooms in a controlled climate, solid state fermentation process, which harvests the smaller molecular weight polysaccharides from the raw material. The entire concentration was then flash spray dried to yield the full content of the immune active polysaccharides and heteropolysaccharides from the raw materials. The certificate of a chemical analysis of compounds contained in IA displayed total polysaccharide content 76.84%, 1,3-1,6 Beta-Glucan content 31.18% and Alpha-Glucan content 0.96%. The powder from capsules of IA was dissolved in phosphate-buffered saline (PBS, Fisher Scientific, Pittsburgh, PA), stirred for 30 min at 37 °C, and filtered throughout filter paper. Three concentrations (250 μg/mL, 500 μg/mL and 1000 μg/mL) were made to perform the comet assay analysis. These concentrations corresponded to the ones used in our previous studies of medicinal mushrooms [17,18].

2.3. Evaluation of genotoxic and antigenotoxic effect of IA by comet assay

To evaluate genotoxic and antigenotoxic properties, the comet assay was performed. The samples of peripheral blood (6 μL) were suspended in 0.67% low melting point agarose (Fisher Scientific, Pittsburgh, PA) and spread on microscope slides. The cells were exposed to the following treatments: a) to evaluate the genotoxic effect of IA, the cells were exposed to IA in three chosen concentrations; b) two designs of experiments were conducted in order to estimate the genoprotective effects of IA, pretreatment and posttreatment; in pretreatment the cells were treated to IA, rinsed with PBS, and then exposed to H_2O_2 (ZORKA

Pharma, Šabac, Serbia) (an assessment of IA's action at the prevention level) and in posttreatment the cells were treated with H_2O_2, rinsed with PBS, and then exposed to IA (an assessment of the IA's action at the interventional level). In posttreatment, after exposure to H_2O_2 the cells were rinsed with PBS and left for 30 min at 37 °C. A concentration of 50 μM H_2O_2 was used to induce a consistent level of DNA damage in human peripheral whole blood cells. The cells were treated with IA for 30 min at 37 °C, while treatment with H_2O_2 was conducted for 20 min at 4 °C.

2.4. Kinetics of attenuation DNA damage induced by H2O2 with IA posttreatment by comet assay

To evaluate the kinetics of attenuation of H_2O_2 induced DNA damage the cells were treated with the IA at 37 °C for 4 time periods: 15, 30, 45, and 60 min, after the exposure of the cells to oxidant for 20 min at 4 °C. Cells were treated with the most effective concentration from previous posttreatment test. Simultaneously, for the controls, the cells were exposed to PBS and examined at the same intervals.

2.5. The single cell gel electrophoresis (comet assay)

The viability of cells was checked with the trypan blue exclusion method, and the cell viability was above 90%. The comet assay was performed as described by Singh et al. [19]. Before the treatments, the samples of peripheral blood (6 μL) were suspended in 0.67% low melting point agarose and spread on microscope slides as mentioned above, and coverslips were placed over them to distribute the sample evenly, after which the slides were cooled for 5 min at 4 °C in order for agarose to solidify. After removing the coverslips, samples were treated according to two experimental conditions in order to evaluate the genotoxic and antigenotoxic potential of IA in the range of tested concentrations. Following the treatment, samples were covered with a layer of 0.5% low melting point agarose and re-cooled for 5 min at 4 °C. After removing the coverslips, the slides were left in a pre-cooled lysing solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X100 and 10% dimethylsulfoxide, at pH 10 adjusted using NaOH) for 24 h at 4 °C. The next day, the samples were subjected to electrophoresis. Slides were placed in a horizontal gel electrophoresis tank and covered with cold, fresh electrophoresis solution (10 M NaOH, 200 mM EDTA), which allows DNA to denature before electrophoresis. DNA becomes mobile at precisely defined pH values and strength of the electric field, and the distance that the fragments pass will depend on the degree of damage of the DNA molecule. The samples were left in electrophoresis solution for 30 min, after which electrophoresis was performed under dimmed light at 25 V (1.2 V/ cm). After electrophoresis, the samples were washed twice with neutralizing buffer and once with distilled water, with a time interval of 10 min between washings.

Then, the samples were stained with 50 μL ethidium bromide (20 mg/mL). The samples were observed under Olympus BX 50 microscope (Olympus Optical Co., GmbH, Hamburg, Germany), equipped with a mercury lamp HBO (50 W, 516–560 nm, Zeiss) pigment lens and at magnification of 100 x, 15 min after staining.

2.6. Assessment of the degree of DNA damage

All treatments were repeated on individual samples (n = 6). Duplicate slides were made per treatment, and on each slide 100 randomly selected cells were counted. Under the influence of the electric field, negatively charged fragments of DNA molecules migrate towards the anode by forming a comet tail, while the rest represents the head of the comet. DNA damage was evaluated according to Anderson et al. [20]. Depending on the degree of damage of DNA molecules, the comets are classified into five categories: category A (no damage, < 5%), category B (low level of damage, 5–20%), category C (mean damage level, 20–40%), category D (high damage level, 40–95%) and category E

(total damage, > 95%).

2.7. The ferric reducing anti-oxidant power (FRAP) assay

The ferric reducing power was determined according to the method of Oyaizu [21]. Each concentration of IA (0.062–10 mg/mL) in 50% DMSO (Fisher Scientific, Pittsburgh, PA) deionized water was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 10 mg/mL potassium ferricyanide, and the mixture was incubated at 50 °C for 20 min. Afterwards 2.5 mL of 100 mg/mL trichloroacetic acid was added to the mixture and then centrifuged at 2000 G for 10 min. The upper layer was mixed with 5 mL of deionized water and 1 mL of 1 mg/mL ferric chloride; the absorbance was then measured at 700 nm against a blank. A higher absorbance value indicates higher reducing power. Butylated hydroxytoluene (BHT) was used as standard. The concentrations of the IA and BHT used for the analysis were in range of 0.062–10 mg/mL.

2.8. Determination of DPPH radicals scavenging ability

The scavenging ability on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals was determined according to the method of Shimada et al. [22]. Each concentration of IA (0.039–10 mg/mL) in 50% DMSO was mixed with 1 mL of methanolic solution containing DPPH (Sigma-Aldrich, St. Louis, MO) radicals, resulting in a final concentration of 0.2 mM DPPH. The mixture was shaken vigorously and left for 30 min in the dark. The absorbance was then measured at 517 nm against a blank. The scavenging ability was calculated as follows:

Scavenging ability (%) = [(ΔA_{517} of control - ΔA_{517} of sample)/ ΔA_{517} of control] x 100.

IC_{50} value (mg/mL) shows the effective concentration at which 50% of DPPH radicals are inhibited and was obtained by interpolation from linear regression analysis. Trolox was used as standard for comparison. The concentrations of IA and Trolox used for the analysis were in range of 0.039, 0.078, 0.156, 0.625, 1.25, 2.5, 5.0 and 10.0 mg/mL.

2.9. Determination of hydroxyl radical scavenging activity

The effect of hydroxyl radical was assayed using the 2- deoxyribose oxidation method described by Chung et al. [23]. 2-Deoxyribose is oxidized by the hydroxyl radical that is formed by the Fenton reaction and degraded to malondialdehyde. The reaction mixture contains 0.45 mL of 0.2 M sodium phosphate (pH 7.6), 0.15 mL of 10 mM 2- deoxyribose, 0.15 mL of 10 mM $FeSO_4$-EDTA, 0.15 mL of 10 mM hydrogen peroxide, 0.525 mL of distilled water, and 0.075 mL (0.062–2 mg/mL) of IA solution in a tube. The reaction was started by the addition of hydrogen peroxide. After incubation at 37 °C for 1 h, the reaction was stopped by adding 0.75 mL of 2.8% (w/v) trichloroacetic acid and 0.75 mL of 1.0% (w/v) of thiobarbituric acid. The mixture was boiled for 10 min., cooled in ice, and then measured at 535 nm. The reaction mixture not containing test sample was used as the control. Trolox (0.0078–2 mg/mL) was used as standard anti-oxidant. The scavenging activity on hydroxyl radicals was expressed as follows:

IC value (mg/mL) is the effective concentration by which 50% of 2- deoxyribose is degraded and was obtained by interpolation from linear regression analysis. Trolox was used as standard for comparison. The concentrations of IA and Trolox used for the analysis were in range of 0.007, 0.015, 0.031, 0.062, 0.125, 0.250, 0.500, 1.00, and 2.00 mg/mL.

2.10. Statistical analysis

In comet assay experiments the data were analyzed for statistical significance using analysis of variance (one-way ANOVA) with Tukey's post-hoc test for comparisons of different treatments vs. respective controls. Data were expressed as mean ± standard error of the mean (SEM), for the sum of cells with DNA damage (comet categories
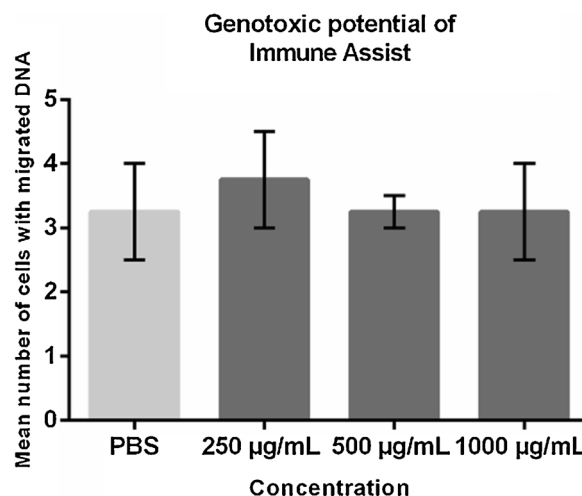

Fig. 1. The evaluation of the genotoxic effects of IA at 3 tested concentrations after 30 min of incubation at 37 °C. Bars represent mean number of cells with DNA damage ± SEM, for n = 6. (by one-way ANOVA with Tukey's post-hoc test).

B + C + D + E), with n = 6. A difference at p < 0.05 was considered statistically significant. Otherwise, IC50 was determined for FRAP, DPPH and ·OH scavenging ability. GraphPad Prism (6.0) statistical software (GraphPad Software Inc, La Jolla, CA, USA) was used for the analysis.

3. Results

3.1. Genotoxic properties of IA

In the assessment of the genotoxic potential of IA, our results showed that the tested range of concentrations applied to agarose embedded blood cells did not increase DNA migration in comparison to control (i.e., the PBS) (Fig. 1). The IA in all tested concentrations showed a lack of genotoxic effect.

3.2. Antigenotoxic properties of IA

In order to determine the antigenotoxic potential of the IA extract, peripheral blood cells were treated with IA extract in the two experimental protocols, pretreatment and posttreatment, assessing IA efficiency on a prevention and intervention level.

Fig. 2 (a) shows that pretreatment application of IA significantly decreases the number of cells with H_2O_2 induced DNA damage at all tested concentrations vs control (i.e., the H_2O_2) (p < 0.05) while a concentration of 500 µg/mL exhibited the most prominent attenuation of DNA damage by H_2O_2. Posttreatment conditions are presented in Fig. 2 (b). All tested concentrations significantly attenuate the number of cells with DNA damage vs control (i.e., H_2O_2 + PBS) (p < 0.05), the most effective concentration being 250 µg/mL.

IA has led to a significant reduction in the number of damaged cells in both protocols, displaying remarkable activity on prevention and intervention level against the H_2O_2 that causes DNA damage through the production of a hydroxyl radical (·OH).

Based on the results of posttreatment, a concentration of 250 µg/mL was taken as the most efficient in action on the intervention level in order for us to investigate a time course of attenuation of DNA damage cells. Fig. 3 represents results of the attenuation of H_2O_2 induced DNA damage of cells in 4 time periods: 15, 30, 45 and 60 min, both in cells treated with IA and control. Our data showed that there was significant attenuation of H_2O_2 induced damage at time points 15, 30, 45 and 60 min, in untreated cells, as well as in cells exposed to IA. Also, significant difference between cells exposed to IA and untreated cells were
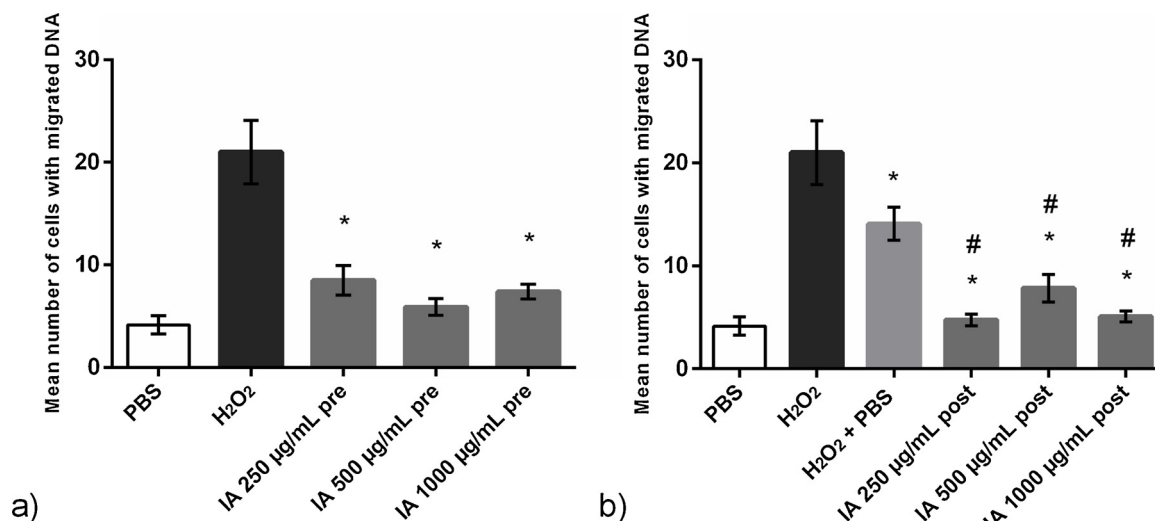
Fig. 2. Evaluation of antigenotoxic properties of IA against the DNA damage induced by H_2O_2 in (a) pretreatment and (b) posttreatment. Bars represent mean number of cells with DNA damage $\pm$ SEM, for n = 6. *p < 0.05 vs. H_2O_2 treated cells, # p < 0.05 vs. H_2O_2 + PBS (by one-way ANOVA with Tukey's post-hoc test).

seen at 15 min, 30 min and 45 min. The greatest reductions were observed at 15 min and 45 min after the exposure of the cells to IA.

### 3.3. Determination of ferric reducing power of IA

Fig. 4 represents the reducing power of IA. IA in the tested concentrations (0.062–10 mg/mL) displayed a concentration-dependent moderate reducing power capacity in comparison to BHT, as a standard. The ferric reduction power in the concentration range of 2 mg/mL had absorbance at 700 nm, that was 0.18.

3.4. Determination of DPPH radical scavenging

Effects of IA are presented in Table 1. IA in tested concentrations had shown a modest free radical scavenging activity, compared to Trolox, a synthetic antioxidant. IA concetration of 2 mg/mL showed 5.22%, while IA concetration of 10 mg/mL showed 11.59% reduction of DPPH.

3.5. Determination of hydroxyl radical scavenging activity of IA

Fig. 5 shows the hydroxyl radical scavenging effect determined by the 2-deoxyribose oxidation method. The scavenging effect of IA on the hydroxyl radical showed a 58% of scavenger activity with
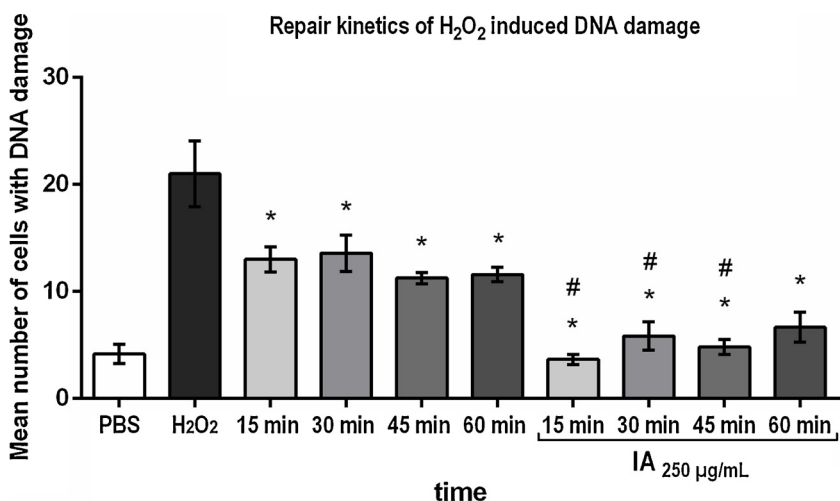
IC = 0.20 mg/mL, while Trolox as standard showed 70% of scavenger efficiency and IC_{50} = 0.023. IA displayed excellent hydroxyl radical scavenging activity.

4. Discussion

Basic objectives of a controlled diet are to maintain well being, improve homeostasis and create the conditions for disease risk reduction. In principle, the aim of food supplementation is to detect beneficial interactions and mechanisms of action between the presence and absence of food components at the cellular level and further, to investigate health benefits for the whole organism [24].

Compared to other macromolecules in the cell, DNA is a sensitive molecule that can be damaged by endogenous and environmental factors. Persistent or repeated DNA damage leads to genome instability that is a prerequisite for the development of cancer [25]. The comet assay, a sensitive and rapid technique for quantifying and analyzing DNA damage in individual cells, was used to evaluate genoprotective potential of IA on human whole blood cells.

Numerous foods contain natural chemicals that damage DNA and are able to produce changes on genomic material [26]. Assessment of genotoxic effects of IA on the human whole blood cells was performed and no genotoxic effect was determined using comet assay.

Otherwise, reactive oxygen species such as superoxide and

Fig. 3. The time course of attenuation of DNA damage cells exposed to H_2O_2 and afterwards incubated for 15, 30, 45, and 60 min - without any treatment and with 250 mg/mL of the IA. Bars represent mean number of cells with DNA damage $\pm$ SEM, for n = 6. *p < 0.05 vs. H_2O_2 treated cells; #p < 0.05 15, 30 and 45 min with IA vs. respective time points in untreated cells (by one-way ANOVA with Tukey's post-hoc test).
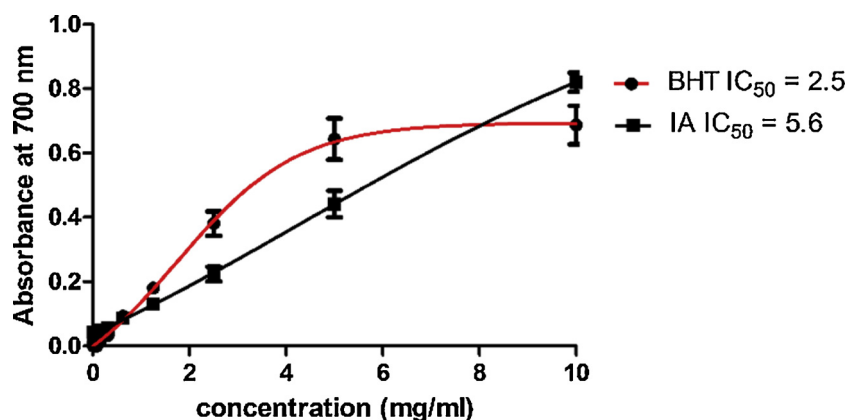
Fig. 4. The ferric reducing power of IA compared to BHT.

Table 1
DPPH scavenging ability of *IA* compared to Trolox, expressed as % inhibition (n = 3).

Percentage inhibition %		
Amount (mg/mL)	IA	Trolox
0.062	1.33 ± 0.16	18.99 ± 0.29
0.125	2.08 ± 0.22	42.15 ± 0.22
0.25	2.50 ± 0.15	90.12 ± 0.30
0.5	2.99 ± 0.22	96.00 ± 0.04
1	3.67 ± 0.15	95.96 ± 0.08
2	5.22 ± 0.22	96.25 ± 0.21
2.5	6.56 ± 0.25	96.50 ± 0.25
5.0	9.55 ± 0.22	96.49 ± 0.23
10.0	11.59 ± 0.60	96.49 ± 0.23

hydrogen peroxide are continually produced during cellular metabolic processes [27]. The genoprotective ability of IA was determined in our current work. The potential of IA to attenuate H_2O_2 induced DNA damage in whole blood cells was detected in both experimental protocols, pretreatment and posttreatment, representing efficiency of IA at the prevention and intervention level. Under the prevention model, the IA may act by increasing the antioxidant capacity of cells or via scavenging activity, making the cell genome more resistant to oxidative damage [28]. The pretreatment with IA resulted in a significant ability to attenuate the number of cells with DNA damage induced by H_2O_2, where 500 µg/mL concentration was the most efficient. Our previous study has shown that *A. blazei* (component of IA), in the same concentration range, displayed moderate ability to decrease the number of cells with H_2O_2 induced DNA damage in pretreatment [18]. Posttreatment results, where the cells were treated with IA after exposure to the oxidant, showed statistically significant decrease of cells with DNA damage in all

tested concentrations vs. control (H_2O_2 + PBS), resulting in marked interventional activity of IA. Regarding the interventional ability of IA, its efficiency might be a result of synergistic action of mechanisms for DNA damage reduction: free radical scavenging, enhancement of the cell antioxidant capacity, and cell's DNA repair stimulation.

It has been accepted that antioxidant properties of mushrooms can be recognized at different stages of the oxidation process and by different mechanisms [29]. Regarding the antioxidant properties of IA, in the current study the reducing power, DPPH and ·OH scavenging abilities were estimated. The ferric reducing power of IA in tested concentrations (0.062–10 mg/mL) displayed concentration-dependent moderate reducing power capacity in comparison to standard. It should be mentioned that in our previous study *A. blazei* has shown also moderate ferric reduction power [18], but *A. blazei* displayed higher ferric reduction power in comparison to IA. In this work we used a hot water extract from growing six species of mushrooms, while results with *A. blazei* were obtained from a dry extract. Possible explanation could be that the antioxidant activity of natural compounds is associated with their structure. Therefore, the structure of IA compounds could disturb some of their antioxidant properties [30]. Further, IA at tested concentrations showed modest DPPH scavenging activity, compared to synthetic antioxidant. IA of 2 mg/mL showed 5.22% inhibition of DPPH. Comparing our DPPH results to the results from *Agaricus* alone, we found an increase in IA scavenger activity vs. *Agaricus* (5.22% vs. 3% respectively). Otherwise, IA displayed exceptional ·OH scavenging activity, showing 58% of scavenger activity, while the standard (control) showed 70% scavenging activity, where IA exhibited almost the same % of ·OH scavenging as *A. blazei* in our previous work (IA IC_{50} = 0.20 mg/mL vs. *A. blazei* IC_{50} = 0.196 mg/mL) [18]. Since hydroxyl radical is the most reactive chemical species among all ROS, known to induce oxidative lesions in DNA, the food components with
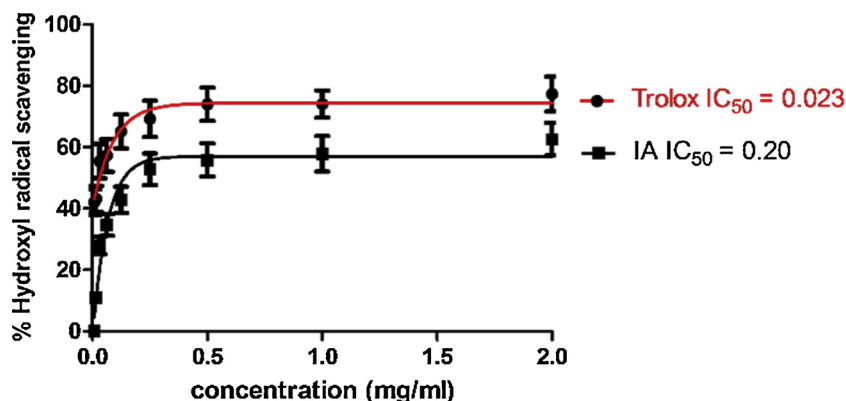

Fig. 5. Hydroxyl radical scavenging activity of IA in a range of concentrations (0.007–2 mg/mL) compared to Trolox.

·OH scavenging ability are of great interest in living systems.

Next, we investigated kinetic of attenuation of H_2O_2 induced DNA damage in the time period of 1 h, in cells which were treated with the optimal concentration of IA and compared with untreated cells (exposed only to PBS). The results showed significant decreases in levels of DNA damage in cells at the 15, 30, 45 and 60 min time periods, both in untreated cells and in cells exposed to IA compared to H_2O_2. Also, we detected significant differences in the number of DNA damaged cells when we compared 15, 30 and 45 min time points in untreated cells vs. cells exposed to IA. The greatest reduction was observed at 15 min and 45 min after the exposure of the cells to IA. Strong scavenger ability of IA, that can act rapidly, could be the explanation of such prominent IA effect at the time point of 15 min. Strong reduction of number of cells with DNA damage at the time period of 45 min, could be result of IA stimulation of the repair capacity of cells, since we detected that the repair capacity of cells exposed to IA was more efficient than in untreated cells. This finding is in concordance with previous studies, which showed that significant repair of DNA damage occurred from 30 min to 1 h after the exposure to the oxidative agent [31]. In comparison to our previous research it should be noted that IA displayed significant attenuation at 60 min after the induction of H_2O_2 DNA damage, while *A. blazei* didn't reach statistically significant attenuation at that point of the investigated time period [18].

The focus of recent research was to assess the genoprotective potential of IA, discovering remarkable potential in reduction of ·OH radicals, as well as, a significant action on prevention and intervention level versus the effects of oxidative damage to DNA structure. Current findings remain a significant challenge, and thus an active area of future research of IA properties on in vivo systems.

5. Conclusion

This preliminary study showed antigenotoxic effects of IA against H_2O_2 induced DNA damage on whole blood cells in pretreatment and posttreatment experiment. Also, IA showed excellent ·OH scavenging properties. Mechanisms underlying mentioned properties should be further evaluated in in vivo studies.

Transparency document

The Transparency document associated with this article can be found in the online version.

Declaration of Competing Interest

None.

Acknowledgments

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