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# Antimutagenic effect of *Agaricus blazei* Murrill mushroom on the genotoxicity induced by cyclophosphamide

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**Abstract**

*Agaricus blazei* Murrill extracts have previously been shown to have anticarcinogenic and antimutagenic properties. These results suggest that antimutagenic activity, besides the modulation of the immune system, might be involved in the anticarcinogenic action of *A. blazei*. To investigate the possible antimutagenic effect of *A. blazei* in vivo, we evaluated its effect on clastogenicity induced by cyclophosphamide (CP) in mice, using the micronucleus test in bone marrow (MNPCE) and in peripheral blood (MNRET). Male Swiss mice were treated with CP (25 or 50 mg/kg i.p.) or with CP plus mushroom solution at three different temperatures: 4, 21, and 60°C. Aqueous solution of a mixture from various lineages of the mushroom inhibited induction of micronuclei by CP in bone marrow and in peripheral blood of mice. In contrast to the mixture of lineages, a single isolated lineage did not lead to a reduction of CP-induced MN frequencies in either bone marrow or blood cells of mice. The results suggest that under certain circumstances these mushrooms exhibit antimutagenic activities that might contribute to an anticarcinogenic effect. 

*Keywords:* Antimutagenicity; *Agaricus blazei* Murrill; Micronucleus test; Cyclophosphamide

## 1. Introduction

Various mushrooms have a long history of use in folk medicine, and higher Basidiomycetes have become matters of great interest, due to their many-fold nutritional, medicinal, and pharmacological properties. Mushroom extracts are widely sold as nutritional supplements and touted as beneficial for health. However, only a few studies are available on the biological effects of mushroom consumption.

The mushroom Basidiomycete *Agaricus blazei* Murrill, a native mushroom in Brazil, popularly known in Japan as Himematsutake, has been largely produced and consumed as food and tea, due to its medicinal effects, possibly including anticarcinogenic activities. This mushroom has been considered a potent chemopreventive agent, but its mechanisms of action are still unknown. Studies with isolated fractions of the fruit body of *A. blazei* showed that some of them exhibit antimutagenic, anticarcinogenic, and immunostimulative activities [1–3]. The antimutagenic

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effect of *A. blazei* extracts, evaluated by the Ames/*Salmonella*/microsome assay, show that various fractions (IIa, IIb, IIc and IId) of mushroom inhibited the mutagenicity of benzo-(a)pyrene (B(a)P), and that linoleic acid was found to be the main substance associated with such activity [3]. Furthermore, antitumor activity was detected in the fraction III-2b, containing a (1 → 6)β-D-glucan–protein, a complex consisting of protein (43.3%) and carbohydrate (50.2%), with a high content of the amino acids alanine and leucine and a low content of methionine, histidine, and tyrosine [4,5]. This activity was characterized by growth inhibition of sarcoma-180 implanted s.c. in mice, possibly due to the immunostimulating and immunomodulatory properties of the polysaccharide–protein complex of the fraction III-2b [2]. The antitumor activity of polysaccharide–protein complex, including β-D-glucans, from the mushroom *A. blazei*, is possibly due to immunological host-mediated mechanisms involving the action of various immunocompetent cells [6–8]. A selective tumoricidal effect of soluble proteoglucan extracted from *A. blazei* Murrill, mediated via natural killer-cell activation and by the induction of apoptosis was demonstrated by Fujimiya et al. [9]. It seems to suggest that the polysaccharides from *A. blazei* may be important both as a nutritionally functional food and as a prophylaxis against diseases such as cancer [10,11].

To further elucidate the mechanisms involved in the anticarcinogenic activity of *A. blazei*, we then investigated, its possible antimutagenic effect in vivo. The effect of *A. blazei* was tested on the clastogenicity induced by cyclophosphamide (CP) in mice using the micronucleus test (MNT) in bone marrow and peripheral blood cells.

## 2. Materials and methods

### 2.1. Animals

Male Swiss mice aged 7–8 weeks (weighting 35–40 g), obtained from the breeding colony of the Centro de Bioterismo da Universidade de Campinas, SP, Brazil, were used. The animals were housed in plastic cages and were kept in an air-conditioned room (20–25°C) with a 12 h light–dark cycle, at 55 ± 10% humidity, receiving food and water ad libitum.

### 2.2. Chemical

CP, Genuxal, Asta Médica, Lot No. 807034, was dissolved in 0.9% NaCl and administered intraperitoneally (i.p.) injection at final doses of 25 or 50 mg/kg.

### 2.3. Preparation of mushroom solutions

The mushroom *A. blazei* Murrill was obtained from Departamento de Produção Vegetal, Faculdade de Ciências Agronômicas, Botucatu, SP, Brazil. Dry mushrooms were pulverized and aqueous extracts were prepared from the powder (2.5 g in 100 ml) at three different temperatures: 4, 21, and 60°C. The solutions were filtered and used at room temperature. The animals were treated orally by gavage (0.6 ml/day per animal) for 15 consecutive days. The total amount of mushroom extract delivered per animal was about 0.225 g.

### 2.4. Micronucleus test

The micronucleus assay from bone marrow or peripheral blood cells was performed according to the protocol described by MacGregor et al. [12] and by Hayashi et al. [13], respectively. The number of micronucleated cells was counted in 1000 polychromatic erythrocytes (PCEs) and in 1000 reticulocytes (RETs) per animal. The slides were analyzed in a blind test, using a light microscope with a 100× immersion objective for PCEs, and a fluorescent microscope with a combination of a blue excitation (e.g. 488 nm) and a yellow to orange barrier filter (e.g. 515 nm long pass), with a 40× objective, for RETs.

The percentage of reduction in the frequency of MN, was calculated according to Manoharan and Banerjee [14] and Waters et al. [15], by the following formula:

$$\text{reduction (\%)} = \frac{\text{frequency of MN in A} - \text{frequency of MN in B}}{\text{frequency of MN in A} - \text{frequency of MN in C}} \times 100$$

where A is the group treated with CP (positive control); B the group treated with mushroom solutions

plus CP; and C the group treated with 0.9% NaCl (negative control).

## 3. Experimental designs

### 3.1. Experiment 1

#### 3.1.1. MNT with a mixture of lineages from A. blazei

To investigate the protective effect of *A. blazei* solutions (mixture of the lineages AB 96/07, AB 96/09, and AB 97/11) against the clastogenicity induced by cyclophosphamide (25 or 50 mg/kg body weight (b.w.)), the animals were divided into 12 groups of 12 animals each (Fig. 1).

In group 1 (a,b), mice received drinking water (0.6 ml/day by gavage) for 2 weeks, and were i.p.-treated on day 15 with 0.9% NaCl. Groups 2 (a,b), and 3 (a,b) received drinking water (0.6 ml/day by gavage) for 2 weeks, and were treated with CP on day 15 (group 2: 25 mg/kg b.w. i.p.; group 3: 50 mg/kg b.w. i.p.). Groups 4 (a,b)–9 (a,b) received solutions of mushroom (0.6 ml/day by gavage) prepared at three different temperatures: 4°C (groups 4 and 7), 21°C (groups 5 and 8), and 60°C (groups 6 and 9), for the 2 weeks before treatment with CP 25 mg/kg (groups 4–6) or 50 mg/kg (groups 7–9) on day 15. Groups 10 (a,b), 11 (a,b), and 12 (a,b) received only treatments with mushroom solutions during 2 weeks, to investigate a possible effect on spontaneous micronucleus frequencies.

The animals were killed by cervical dislocation, on day 16 (groups 1a–12a) for the evaluation of micronucleated polychromatic erythrocytes (MNPCEs) in bone marrow, then, on day 17 (groups 1b–12b) for the evaluation of micronucleated retyculocytes (MNRETs) in peripheral blood.

### 3.2. Experiment 2

#### 3.2.1. MNT with a single lineage of A. blazei

In order to investigate the protective effects of *A. blazei* (isolated lineage AB 99/26-Jun 17) against the clastogenicity induced by cyclophosphamide, the animals were divided into 8 groups of 20 animals each (Fig. 2).

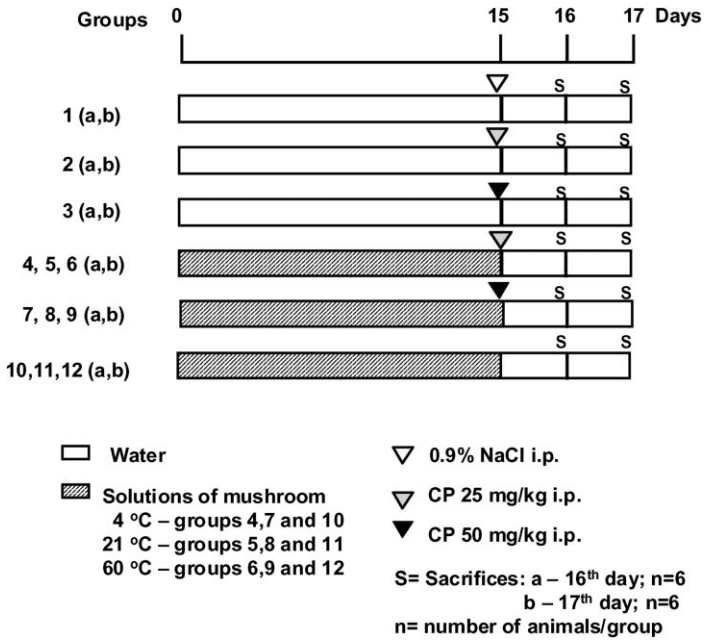


Fig. 1. Experimental design to evaluate the effects of mushroom *A. blazei* Murrill (mixture of different lineages) on the induction of micronuclei by cyclophosphamide (Section 3.1).

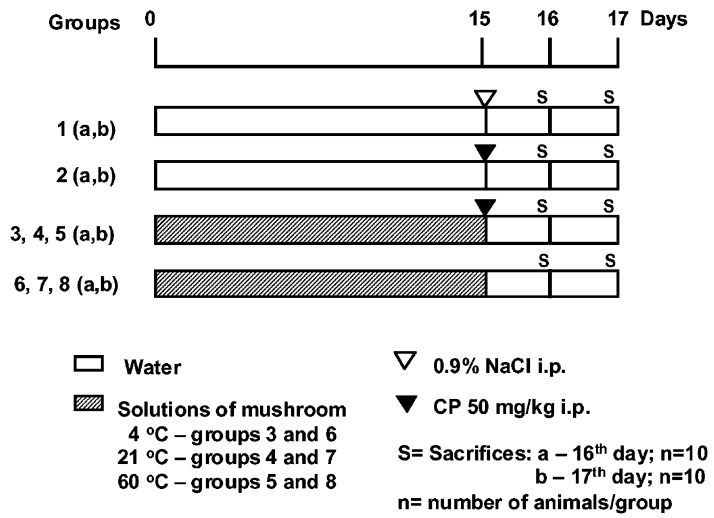


Fig. 2. Experimental design to evaluate the effects of *A. blazei* Murrill (single lineage) on the induction of micronuclei by cyclophosphamide (Section 3.2).

In group 1 (a,b) mice received drinking water (0.6 ml/day by gavage) for 2 weeks before the treatment with 0.9% NaCl (i.p.). Group 2 (a,b) received drinking water (0.6 ml/day by gavage) for 2 weeks, and was treated with CP 50 mg/kg on day 15. Groups 3 (a,b)–8 (a,b) received mushroom solutions (0.6 ml/day by gavage) prepared at three different temperatures: 4 (groups 3 and 6), 21 (groups 4 and 7), and 60°C (groups 5 and 8), during the 2 weeks before the treatment with CP 50 mg/kg. In groups 6 (a,b)–8 (a,b), the mice only received treatments with mushroom solutions, to investigate a possible effect on spontaneous micronucleus frequencies.

The animals were killed by cervical dislocation on day 16 (groups 1a–8a) for the evaluation of micronucleated polychromatic erythrocytes (MNPCEs) in bone marrow, then, on day 17 (groups 1b–8b) for the evaluation of micronucleated retyculocytes (MNRETs) in peripheral blood.

## 4. Statistical analysis

The data from the micronucleus assay statistically analyzed by the Chi-square test [16]. The significance level considered was $P < 0.05$.

## 5. Results

### 5.1. Experiment 1

Table 1 shows the frequencies of MN in PCEs of mice treated with a mixture of lineages from *A. blazei* and with CP (25 or 50 mg/kg). CP alone induced a clear and dose-related increase in MN frequencies. Pre-treatment with *A. blazei* solutions led to a statistically significant reduction in the frequency of MN in PCEs induced by CP in both doses. The reduction was between 30 and 50%, it was not clearly related to the temperature at which the mushroom solutions were prepared. While the solution prepared at 4°C in combination with CP 25 mg/kg had the strongest effect, the solution prepared at 21°C, in combination with CP 50 mg/kg. Taken together, these results suggest that the mushroom *A. blazei*, prepared at three different temperatures, provides protection against the genotoxicity of CP. The results also show that the frequencies of MN in PCE of animals treated only with the solution of mushroom are not different from those of untreated controls.

Table 2 shows the frequencies of MN in RETs of mice treated with a mixture of lineages from *A. blazei* and with CP (25 or 50 mg/kg). CP induced a

Table 1
The effect of treatment with *A. blazei* Murrill (mixture of lineages) on the micronuclei induced by CP in bone marrow cells of mice[a]

| Treatment | Number of analyzed cells | MNPCEs | | Reduction (%) |
|---|---|---|---|---|
| | | Number | % | |
| Water + 0.9% NaCl (negative control) | 6000 | 5 | 0.08 | |
| Water + CP (25 mg/kg) (positive control) | 6000 | 80 | 1.33 | |
| *A. blazei* | | | | |
| Solution 1 + CP (25 mg/kg) | 6000 | 40 | 0.66*** | 53.6 |
| Solution 2 + CP (25 mg/kg) | 6000 | 57 | 0.95* | 30.4 |
| Solution 3 + CP (25 mg/kg) | 6000 | 57 | 0.95* | 30.4 |
| Water + CP (50 mg/kg) (positive control) | 6000 | 135 | 2.25 | |
| *A. blazei* | | | | |
| Solution 1 + CP (50 mg/kg) | 6000 | 68 | 1.13*** | 51.6 |
| Solution 2 + CP (50 mg/kg) | 6000 | 65 | 1.08*** | 53.9 |
| Solution 3 + CP (50 mg/kg) | 4000[b] | 57 | 1.42** | 38.3 |
| Solution 1 | 6000 | 4 | 0.07 | |
| Solution 2 | 6000 | 9 | 0.15 | |
| Solution 3 | 6000 | 8 | 0.13 | |

[a] *A. blazei* solution 1, 4°C; solution 2, 21°C (room temperature); solution 3, 60°C.
[b] Two animals died.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

Table 2
The effect of treatment with *A. blazei* Murrill (mixture of lineages) on the micronuclei induced by CP in peripheral blood cells of mice[a]

| Treatment | Number of analyzed cells | MNRETs | | Reduction (%) |
|---|---|---|---|---|
| | | Number | % | |
| Water + 0.9% NaCl (negative control) | 6000 | 13 | 0.21 | |
| Water + CP (25 mg/kg) (positive control) | 6000 | 85 | 1.42 | |
| *A. blazei* | | | | |
| Solution 1 + CP (25 mg/kg) | 6000 | 34 | 0.57** | 70.3 |
| Solution 2 + CP (25 mg/kg) | 6000 | 33 | 0.55** | 71.9 |
| Solution 3 + CP (25 mg/kg) | 6000 | 57 | 0.95* | 38.8 |
| Water + CP (50 mg/kg) (positive control) | 6000 | 151 | 2.52 | |
| *A. blazei* | | | | |
| Solution 1 + CP (50 mg/kg) | 6000 | 42 | 0.70** | 78.8 |
| Solution 2 + CP (50 mg/kg) | 6000 | 40 | 0.67** | 80.1 |
| Solution 3 + CP (50 mg/kg) | 6000 | 67 | 1.12** | 60.6 |
| Solution 1 | 6000 | 13 | 0.22 | |
| Solution 2 | 5000[b] | 5 | 0.10 | |
| Solution 3 | 6000 | 14 | 0.23 | |

[a] *A. blazei* solution 1, 4°C; solution 2, 21°C (room temperature); solution 3, 60°C.
[b] Two animals died.
* $P < 0.01$.
** $P < 0.001$.

Table 3
The effect of *A. blazei* Murrill (lineage AB 99/26-Jun 17) on the frequencies of micronuclei induced by CP in bone marrow cells of mice[a]

| Treatment | Number of analyzed cells | MNPCEs | |
|---|---|---|---|
| | | Number | % |
| Water + 0.9% NaCl (negative control) | 10000 | 14 | 0.14 |
| Water + CP (50 mg/kg) (positive control) | 10000 | 333 | 3.33 |
| *A. blazei* | | | |
| Solution 1 + CP | 10000 | 350 | 3.50 |
| Solution 2 + CP | 10000 | 328 | 3.28 |
| Solution 3 + CP | 10000 | 313 | 3.13 |
| Solution 1 | 10000 | 17 | 0.17 |
| Solution 2 | 10000 | 15 | 0.15 |
| Solution 3 | 10000 | 13 | 0.13 |

[a] *A. blazei* solution 1, 4°C; solution 2, 21°C (room temperature); solution 3, 60°C.

Table 4
The effect of *A. blazei* Murrill (lineage AB 99/26-Jun 17) on the frequencies of micronuclei induced by CP in peripheral blood cells of mice[a]

| Treatment | Number of analyzed cells | MNRETs | |
|---|---|---|---|
| | | Number | % |
| Water + 0.9% NaCl (negative control) | 10000 | 23 | 0.23 |
| Water + CP (50 mg/kg) (positive control) | 10000 | 286 | 2.86 |
| *A. blazei* | | | |
| Solution 1 + CP | 10000 | 253 | 2.53 |
| Solution 2 + CP | 10000 | 242 | 2.42 |
| Solution 3 + CP | 10000 | 229 | 2.29 |
| Solution 1 | 10000 | 32 | 0.32 |
| Solution 2 | 10000 | 32 | 0.32 |
| Solution 3 | 10000 | 19 | 0.19 |

[a] Positive control. *A. blazei* solution 1, 4°C; solution 2, 21°C (room temperature); solution 3, 60°C.

clear and dose-related increase in MN frequencies. Pre-treatment with *A. blazei* solutions led to a statistically significant reduction in the frequency of MN induced by CP in RETs. The reduction was between 38 and 80%, but was not clearly related to the temperature at which the solution was prepared. In combination with both 25 mg/kg CP or 50 mg/kg, the solution prepared at 21°C led to the greatest reduction. Taken together, these results suggest that the mushroom *A. blazei*, prepared at three different temperatures, provides protection against the genotoxicity of CP. The results also show that the frequencies of MN in RETs of animals treated only with the solution of mushroom are not different from those of untreated controls.

### 5.2. Experiment 2

Tables 3 and 4, respectively, show the frequencies of MN in PCEs and in RETs, of mice treated with solutions of mushroom (one single lineage) before treatment with CP. The results show that the mushroom solutions prepared at different temperatures did not significantly influence the mutagen-induced MN frequencies. The results also show that the frequencies of MN in PCEs or RETs of animals treated only with the solution of mushrooms are not different from those of untreated controls.

## 6. Discussion

*A. blazei* Murrill extracts have previously been shown to have anticarcinogenic properties [2,6]. The mechanism of the anticarcinogenic action is not completely understood; but it seems that the mushroom extract modulated the response of the immune system [6]. It was also found that extracts from *A. blazei* inhibited the mutagenicity of benzo(a)pyrene in the Ames *Salmonella* microsome assay [3]. These results suggest that antimutagenic activity might also be involved in the anticarcinogenic action of *A. blazei*. We therefore, started to investigate the antimutagenic potential of *A. blazei* in vivo. The results presented here actually indicate that aqueous solutions extracted from *A. blazei* exhibit antimutagenic activity against the in vivo DNA damaging effect of the indirectly acting alkylating agent CP. Aqueous solutions of a mixture from various lineages of the mushroom inhibited the induction of micronuclei by CP in mice bone marrow and peripheral blood cell. Because, in folk medicine, teas from *A. blazei* are prepared at different temperatures, we have also tested the possible influence of the kind of preparation on the antimutagenic effect. However, the results did not indicate any significant influence of the temperature at which the solution was prepared on the antimutagenic activity.

Solutions prepared at the three different temperatures (4, 21, and 60°C) led to reduced micronucleus frequencies without showing systematic differences. Interestingly, no antimutagenic activity was found for a single lineage of *A. blazei* in the MNT. In contrast to the mixture of lineages used in the first experiment, the single lineage did not lead to a reduction of CP-induced MN frequencies in either bone marrow or blood of mice. At present, there is no explanation for this conflicting result, but it might indicate that the antimutagenic component is not equally distributed between different lineages and/or that it is not equally present in the mushrooms at different periods of the year. Further studies are therefore, required to better characterize the antimutagenic activity of *A. blazei* extracts and to identify their active compounds and their mode of action. But our results strongly suggest that under certain circumstances these mushrooms exhibit antimutagenic activities that might contribute to an anticarcinogenic effect.

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