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Evaluation of *Agaricus blazei in vivo* for antigenotoxic, anticarcinogenic, phagocytic and immunomodulatory activities

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ABSTRACT

The development of various types of cancer results from the interaction among endogenous, environmental and hormonal factors, where the most notable of these factors is diet. The aim of the present study was to determine the antigenotoxic, anticarcinogenic, phagocytic and immunomodulatory activities of *Agaricus blazei*. The test antigenotoxicity (Comet Assay) and anticarcinogenic (Test of Aberrant Crypt Foci) assess changes in DNA and/or intestinal mucosa that correlate to cancer development. Tests of phagocytosis in the spleen and differential count in blood cells allow the inference of modulation of the immune system as well as to propose a way of eliminating cells with DNA damage. Supplementation with the mushroom was carried out under pre-treatment, simultaneous treatment, post-treatment and pre-treatment + continuous conditions. Statistical analysis demonstrated that the mushroom did not have genotoxic activity but showed antigenotoxic activity. Supplementation caused an increase in the number of monocytes and in phagocytic activity, suggesting that supplementation increases a proliferation of monocytes, consequently increasing phagocytic capacity especially in the groups pre-treatment, simultaneous and pre-treatment + continuous. The data suggest that *A. blazei* could act as a functional food capable of promoting immunomodulation which can account for the destruction of cells with DNA alterations that correlate with the development of cancer, since this mushroom was demonstrated to have a preventive effect against pre-neoplastic colorectal lesions evaluated by the aberrant crypt foci assay. According to these results and the literature, it is believed that supplementation with *A. blazei* can be an efficient method for the prevention of cancer as well as possibly being an important coadjuvant treatment in chemotherapy.

1. Introduction

In Brazil, cancer represents the second leading cause of death in the adult population. And the most common cancers in the Brazilian population is melanoma skin cancer, followed by tumors of the breast, prostate, lung, colon and rectum, stomach and cervix (INCA, 2008).

Diet represents the greatest interaction of humans with the environment. All foods that are ingested or nutrients that are absorbed can have a beneficial potential or present a risk to systemic pathologies and of gastrointestinal tract (object of study in this paper). The nutritional state together with the condition of the body can prevent or help fight disease, or inversely, can cause a greater susceptibility to disease (Hesketh et al., 2006).

The regular consumption of functional foods reduces the risk of various chronic diseases, mainly cardiovascular, cancer, diabetes, hypertension and osteoporosis (Hornstra et al., 1998; López-Varela et al., 2002).

Agaricus blazei is known in Brazil as the sun mushroom, in Japan as himematsutake, agarikusutake or kawarihiratake and in China as Ji Song Rong. It was brought from Japan due to its beneficial health effects, like prevention of diabetes, hyperlipidemia, arteriosclerosis and chronic hepatitis (Takaku et al., 2010). Nowadays, it is widely utilized in Oriental countries as an edible mushroom. Considered as a functional food (functional food is a part of an everyday diet and is demonstrated to offer health benefits and to reduce the risk of chronic disease beyond the widely accepted nutritional

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effects), it is vastly utilized in traditional medicine in the form of a medicinal extract for the prevention and treatment of cancer (Firenzuoli et al., 2007).

In general, the total composition of the mushrooms is water (90%), protein (2–40%), carbohydrates (1–55%), fiber (3–32%) and ash (8–10%). The ash content is made up mainly of salts, metals like calcium and magnesium (Firenzuoli et al., 2007). Among the carbohydrates are notably some biologically active polysaccharides, present in the basidiocarp and/or mycelium, such as the β-glucans which have attracted the attention of investigators (Mizuno, 1995). β-Glucans consist of units of D-glucopyranosyl and its structure depends on the source and methods of extraction utilized. β-Glucans extracted from the mushroom *A. blazei* have a structure principally formed by chains of D-glucose with β(1 → 3) linkages and branching with β(1 → 6) connections (Angeli et al., 2008).

Immunotherapy in utilized in many cancer patients, improving their quality of life, and is also an important form of adjuvant treatment (Ohno et al., 1999). Besides immunomodulatory capacity, antimutagenic, antigenotoxic, antitumor and antiteratogenic activities have also been described for *A. blazei* and different β-glucans (Di Luzio et al., 1979; Grüter et al., 1991; Saitô et al., 1991; Demleitner et al., 1992; Kishida et al., 1992; Chorvatovicová et al., 1996, 1999; Lohman et al., 2001; Slamenová et al., 2003; Tohamy et al., 2003; Lin et al., 2004; Oliveira et al., 2009).

Based on the above findings, the aim of the present study was to determine the antigenotoxic, anticarcinogenic, phagocytic and immunomodulatory activities of the mushroom *A. blazei* in Swiss mice.

2. Material and methods

2.1. Chemical agents

DNA damage was induced using 1,2-dimethylhydrazine (DMH; Sigma®, CAS No. 306-37-6) at a concentration of 20 mg/kg body weight (b.w.) diluted in EDTA solution (37 mg/mL) and administered to the animals intraperitoneal (i.p). The applications were according to the protocol proposed by Silva (1999), in which four doses of DMH were administered, where two doses per week were given for 2 weeks.

2.2. Preparation of diet and supplementation

The mushroom *A. blazei* was supplied by Prof. Dr. Mario Sergio Mantovani of the Genetic Toxicology Laboratory, Biological Sciences Center, Department of General Biology, State University of Londrina – UEL, Londrina, PR, Brazil. The commercial ration Nuvilab® was ground and the dried mushroom, also ground, was added at a proportion of 10%. The components were then mixed, moistened and pelletized, and the pellets were then dried in an oven at 40 °C. The concentration of *A. blazei* was defined according to Nosál'ová et al. (2001). The ration was offered to the animals every day, but amounts (100–150 g/day); otherwise, because of palatability, the mice tended to nibble at the ration without ingesting it.

2.3. Animals and experimental design

Male Swiss mice at reproductive age were used for the study; they were about 60 days old, with a mean weight of 30 g, and were from the Center for the Study of Nutrition and Genetic Toxicology – CENUGEN of the Centro Universitario Filadelfia – UniFil, Londrina, PR, Brazil. The experiments were conducted in the center's animal facility. Two experiments were conducted where the first determined antigenotoxicity, phagocytic activity and differential blood cell counts, and the second entailed the aberrant crypt foci assay. In the first experiment, the animals were divided into 7 experimental groups with 7 animals/group. However, in the second experiment the number of groups was the same but with 11 animals/group. The animals were kept in propylene cages lined with wood shavings which were changed daily. The animals spent a minimum acclimation period of seven days. Light and temperature were controlled using a 12-h photoperiod (12 h dark: 12 h light) with the temperature kept at 22 ± 2 °C and humidity at 55 ± 10%. Feeding was *ad libitum* and consisted of filtered water and commercial ration supplemented or not with 10% *A. blazei*, according to the experimental groups.

The experiment was performed according to the guidelines of the Brazilian College of Animal Experimentation (COBEA, 2004) and approved by the Committee of Ethics in Animal Experimentation of the State University of Londrina.

2.3.1. Experiment 1

The animals were treated for 4 weeks, the experimental groups are as follows: Control Group (EDTA-solution in which the DMH was diluted) – the animals were maintained for 2 weeks, receiving only commercial ration and filtered water. In the 3rd and 4th weeks of the experiment, they were given EDTA solution (0.1 ml/10 g b.w., i.p.), two times per week, together with commercial ration, everyday of this period; Group DMH – the animals were submitted to the same situations as in the control group; however, EDTA was substituted by DMH at a dose of 20 mg/kg b.w. (i.p.); Group *A. blazei* – the animals were offered ration supplemented with *A. blazei* at a proportion of 10% during the entire experimental period (4 weeks); the other procedures were performed as described for the control group; Group pre-treatment – the animals received for the first 2 weeks of the experiment ration supplemented with *A. blazei*. After the end of this period, the administration of 4 doses of DMH (20 mg/kg b.w., i.p.) was begun, where 2 doses were given in the 3rd and the other 2 doses in the 4th week; next, supplemented commercial ration was begun and this proceeded until the end of the experiment; Group Simultaneous – commercial ration was offered during the first 2 weeks of the experiment; in the following 2 weeks, the animals received 4 doses of DMH (20 mg/kg b.w., i.p.), where during the same time the animals were fed with commercial ration supplemented with *A. blazei*; Group Post-treatment – for 4 weeks the animals were given commercial ration, where in the 3rd and 4th weeks DMH was administered (20 mg/kg b.w., i.p.). After these 2 weeks, commercial ration supplemented with *A. blazei* was offered for the last 72 h of the experiment; Group pre-treatment + Continuous – commercial ration supplemented with *A. blazei* was offered during the whole experimental period, where in the 3rd and 4th weeks the animals were given DMH (20 mg/kg b.w., i.p.), as in the protocol described above, and supplementation proceeded up to the moment the animals were euthanized (Fig. 1).

2.3.2. Experiment 2

The experimental groups were the same as described in Section 2.3.1. However, this experiment lasted 10 weeks longer after the administration of the last dose of DMH. In this manner, the time of supplementation in post-treatment and pre-treatment + continuous were extended until the end of the experimental period (Fig. 2).

2.4. Biological assays

2.4.1. Experiment 1

2.4.1.1. Comet assay. The comet assay was performed according to Singh et al. (1988) and Tice (1995) with modifications. The slides were prepared by depositing on a slide, previously coated with

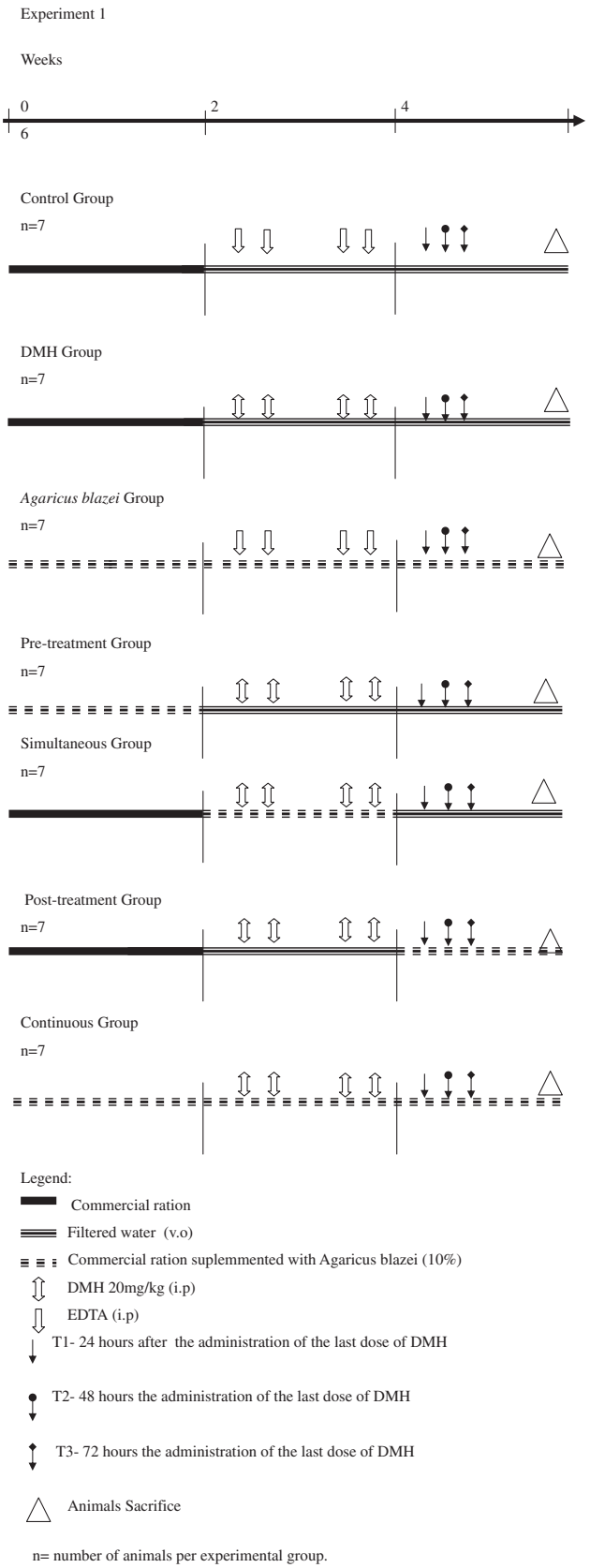

Fig. 1.

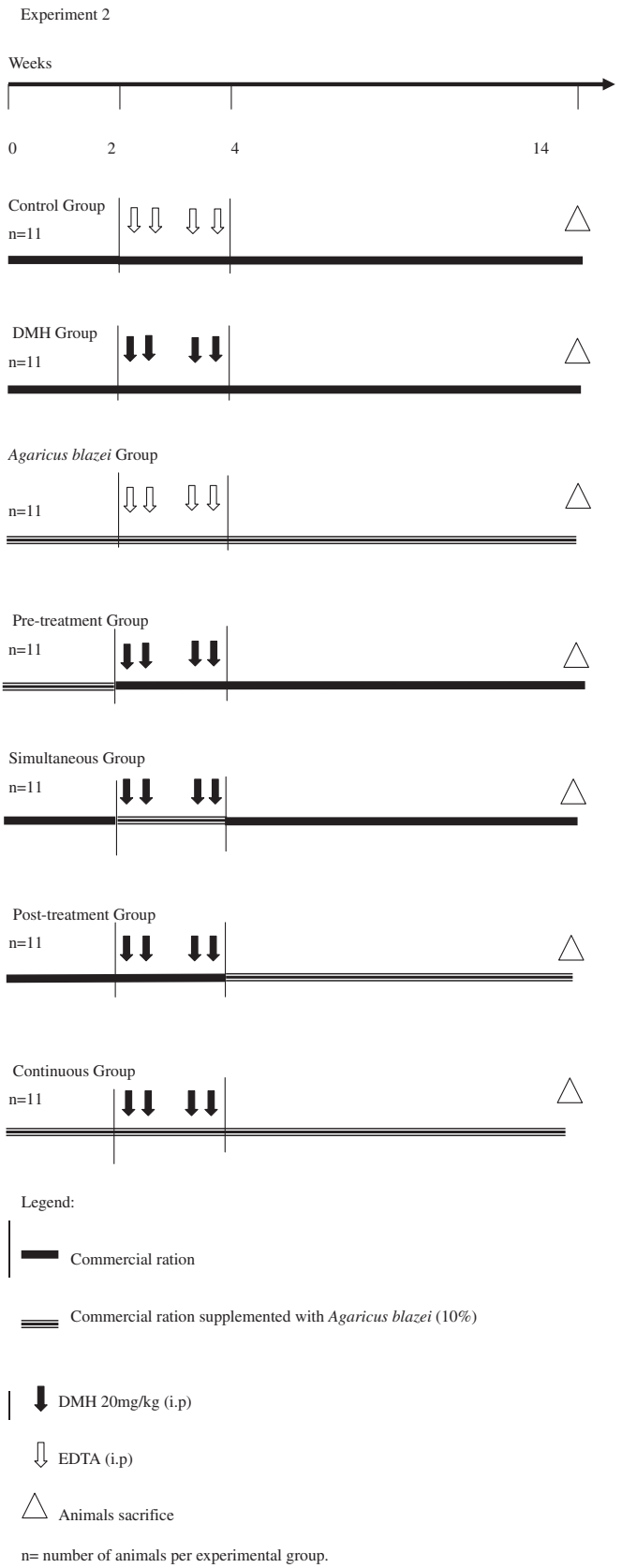

Fig. 2.

5% normal agar, 20.0 μL of peripheral blood, drawn 24 h after the last administration of DMH or its vehicle, with 120.0 μL LPM agarose (1.5%), at 37 °C. Next, the slides were covered with glass coverslip and chilled at 4 °C for 20 min. After removal of the coverslips, the slides were immersed in lysis solution, freshly prepared, composed of 89.0 mL lysis stock solution (2.5 M NaCl, 100.0 mM EDTA,

10.0 mM Tris, pH 10.0 adjusted with solid NaOH, 890.0 mL of distilled water and 1% sodium lauryl sarcosinate), 1.0 of mL Triton X-100 (Merck) and 10.0 mL of DMSO (dimethyl sulfoxide). Lysis was allowed to proceed for 1 h, at 4 °C, protected from light. Next, the slides were placed in a electrophoresis chamber with pH > 13.0 solution (300.0 mM NaOH and 1.0 mM EDTA, prepared from a stock solution of 10.0 N NaOH and 200.0 mM EDTA, pH 10.0) at 4 °C for 20 min to denature DNA. Electrophoresis was carried out at 25.0 V and 300.0 mA (1.25 V/cm). Afterward, the slides were neutralized with 0.4 M Tris–HCl buffer, pH 7.5, for 3 cycles of 5 min, air-dried, fixed in absolute ethanol for 10 min and saved for later analysis. For staining, the slides were covered with 100.0 μL of ethidium bromide (20.0 μg/mL) and a coverslip. The material was evaluated with a fluorescence microscope (Bioval, Model L 2000A) at 400 times magnification, with an excitation filter of 420–490 nm and emission filter of 520 nm.

A total of 100 cells per treatment were analyzed visually, classifying the comets as: class 0 – cells not damaged and that had no tail; class 1 – cells with tail smaller than the diameter of the nucleus; class 2 – cells with tail size between 1 and 2 times the diameter of the nucleus; class 3 – cells with tail larger than 2 times the diameter of the nucleus. Apoptotic cells, which showed a totally fragmented nucleus, were not counted (Kobayashi et al., 1995). The total score was calculated by summing the resulting values of the multiplication of total number of cells observed in each class of lesion by the value of the class (0–3). Statistical analysis was performed by ANOVA followed by Tukey's test ($p < 0.05$).

2.4.1.2. Differential blood cell counts. Peripheral blood was drawn for differential cell counts at 24, 48 and 72 h after the last administration of EDTA and DMH. A caudal vein puncture was performed and blood smears were made on slides. The slides were air-dried and submitted to rapid staining using the Kit Panotico developed by Laborclin Laboratory (Gurr, 1965, 1971).

Analysis of the slides was carried out with a light microscope (Microscope DBG) at 1000 times magnification. A total of 100 cells per animal were analyzed, in a blinded test, and differentiated as lymphocytes, neutrophils, monocytes, eosinophils and basophils.

Statistical analysis was performed by ANOVA followed by Tukey's test ($p < 0.05$).

2.4.1.3. Phagocytic activity of spleen cells. At 72 h after the last application of EDTA or DMH the animals were euthanized and the spleens collected. These organs were cut into pieces with scissors and then pressed through a stainless steel screen with 5 ml of sterile phosphate buffer, Ca^{2+} and Mg^{+2} free, pH 7.4. Repeated pipetting with a Pasteur pipet was used to obtain a homogeneous cell suspension. One hundred microliters of cell suspension were placed immediately in the center of a slide pre-coated with acridine orange (1 mg/mL) and slide was then coverslipped. The slides were stored in a freezer until time of analysis. The slides were viewed with a fluorescence microscope (Bioval, Model L 2000A) at 400 times magnification, with an excitation filter of 420–490 nm and emission filter of 520 nm. A total of 200 cells/animal were analyzed, in a blinded test, in two categories: absence or presence of phagocytosis based on the descriptions of Hayashi et al. (1990).

Statistical analysis was carried out using ANOVA and Tukey's test ($p < 0.05$).

2.5. Experiment 2

2.5.1. Assay for aberrant crypt foci – sampling, processing and analysis of colon

After the period of 14 weeks of experiment, the mice were euthanized by cervical dislocation, and an incision was then made on the ventral side, along the medial line from the pubic region up to the xiphoid process. The muscles of the abdomen were retracted laterally using incisions in the proximal and distal part. After localizing the proximal colon, a ligature was made with cotton thread. Afterwards, the colon was removed, from the proximal part up to the rectum, and washed with normal saline to remove the feces. After washing, the colon was opened longitudinally along the border of the mesenteric insertion, stretched out on Styrofoam plates and the ends fixed with pins. The plates were immersed in a trough with 10% buffered formalin, for at least 24 h. At the moment of analysis, each segment of colon was stained with 10% methylene blue for 10 min and then placed on a microscope slide, with the mucosa side up, for analysis using a light microscope (Microscope DBG) at 100 times magnification. All the mucosa was examined for the identification and quantification of aberrant crypt foci based on the criteria utilized by Bird (1987): (I) focus consisting of a single crypt – the aberrant crypt shows lining by a think epithelial layer, with elliptical luminal opening and larger in size (at least 2 times) than the surrounding normal crypts; (II) focus with two or more crypts – the aberrant crypts form distinct blocks and occupy an area greater than that occupied by an equivalent number of crypts of normal morphology, and there is no presence of normal crypts separating the aberrant crypts within these foci.

The total number of aberrant crypt foci, total aberrant crypts per foci and the ratio crypt/focus were compared among the different treatments utilizing ANOVA followed by Tukey's test, and the differences were considered statistically significant when $p < 0.05$.

3. Results

3.1. Experiment 1

Table 1 presents the means ± standard deviation of the frequency of lesioned cells, distribution among the classes of DNA damage and scores with regard to the comet assay in peripheral blood cells of mice. Statistical analysis demonstrated that there was no statistically significant difference between the control group and that supplemented with the mushroom, demonstrating the lack of genotoxic activity. The mean number of lesioned cells in the control group was 11.86 ± 3.80 and in the group supplemented with the mushroom 12.29 ± 5.71. When comparing the DMH groups and those where the drug was combined with the mushroom, antigenotoxic activity could be observed for all protocols. The percent reduction in damage was 24.24%, 38.76%, 45.52% and 50.09% for the treatment protocols simultaneous, pre-treatment + continuous, pre-treatment and post-treatment, respectively.

Differential blood cell counts were determined at 24, 48 and 72 h after the last administration of DMH, and the values are presented in Tables 2–4, respectively.

At 24 h, there were no statistically significant differences for any cell type between the different experimental groups. However, the number of monocytes was higher than reference values in all groups. The highest percentages were found in the groups that received DMH and/or *A. blazei,* and the percentages varied from 4.57% to 8.57% (reference values: 0.1–3.5%). Eosinophils were also higher in those groups that were given DMH, and the mean percentages varied from 0.43% to 1.00% (reference values: 0–0.4%). Basophil counts were increased only in the group that received treatment with DMH, and the mean value was 0.57% (reference values: 0–0.3%).

At 48 h, the lymphocyte, eosinophil and basophil counts did not show statistically significant differences. Lymphocytes were found to be within the range of reference values (55–95%) and the mean percentage varied from 63.86% to 70.28%. However, the number of eosinophils was found to be above the reference values (0–0.4%) in all experimental groups, and mean percentages varied from 0.57%

Table 1
Means ± standard deviation of the frequency of lesioned cells, distribution among the classes of DNA damage and scores with regard to the score in the tests for genotoxicity and antigenotoxicity of *A. blazei* in the comet assay in peripheral blood of male mice:

Experimental groups	Lesioned cells	Damage classes				Score
		0	1	2	3	
Genotoxicity						
Control	11.86 ± 3.80[a]	89.00 ± 3.65	9.57 ± 3.05	2.14 ± 2.19	0.14 ± 0.38	14.28 ± 5.06[a]
DMH	83.71 ± 6.10[d]	15.71 ± 6.63	60.71 ± 12.94	14.86 ± 10.59	8.71 ± 4.11	116.57 ± 20.98[d]
Agaricus	12.29 ± 5.71[a]	87.71 ± 5.71	11.43 ± 5.13	0.71 ± 1.11	0.14 ± 0.38	13.29 ± 6.77[a]
Antigenotoxicity						
Pre-treatment	51.00 ± 6.51[b,c]	48.86 ± 10.11	38.43 ± 8.42	8.86 ± 7.49	3.86 ± 4.26	67.71 ± 24.14[b]
Simultaneous	66.29 ± 11.78[c]	33.71 ± 11.77	39.00 ± 12.22	15.57 ± 6.50	11.71 ± 7.30	105.29 ± 25.57[c,d]
Post-treatment	47.14 ± 10.96[b,c]	52.43 ± 11.63	28.86 ± 3.24	16.00 ± 8.12	2.71 ± 1.50	69.00 ± 20.95[b]
Pre + Continuous	55.86 ± 14.04[c]	43.29 ± 14.02	34.29 ± 8.87	16.71 ± 6.16	4.86 ± 4.10	82.29 ± 25.81[b,c]

Control – negative control – EDTA 0.1 mL/10 g b.w. (body weight); DMH – DMH (dimethylhydrazine) 20 mg/kg b.w. (body weight) for 2 weeks (positive control); *A. blazei* – *A. blazei* 10%/kg c.r. for 4 weeks; pre-treatment – *A. blazei* 10%/kg c.r. during the first 2 weeks + DMH 20 mg/kg b.w. for 2 weeks; simultaneous – simultaneous treatment – *A. blazei* 10%/kg c.r. + DMH 20 mg/kg b.w. for 2 weeks; post-treatment – DMH 20 mg/kg b.w. during the first 2 weeks + *A. blazei* 10%/kg c.r. for 2 weeks; pre + continuous – pre-treatment + continuous – *A. blazei* 10%/kg c.r. during the first 2 weeks + *A. blazei* 10%/kg c.r.; DMH 20 mg/kg b.w. for 2 weeks. Different letters indicate statistically significant differences ($p < 0.05$; ANOVA/Tukey).

Table 2
Effects of supplementation of *A. blazei* on the blood cells 24 h.

Parameters	Ref. val.	Experimental groups						
		Control	DMH	*Agaricus*	Pre	Sim	Post	Pre + continuous
Lymphocyte	55–95%	65.71 ± 6.42[a]	70.28 ± 6.18[a]	65.86 ± 8.39[a]	66.28 ± 4.19[a]	63.86 ± 4.60[a]	67.43 ± 5.16[a]	63.86 ± 7.80[a]
Neutrophil	10–40%	29.86 ± 7.13[a]	21.71 ± 6.65[a]	27.86 ± 9.33[a]	26.28 ± 3.64[a]	26.68 ± 2.93[a]	27.14 ± 3.13[a]	27.57 ± 5.91[a]
Monocytes	0.1–3.5%	3.86 ± 1.57[a]	7.00 ± 2.31[a]	6.00 ± 4.55[a]	6.00 ± 2.45[a]	8.57 ± 2.99[a]	4.57 ± 2.99[a]	5.43 ± 2.30[a]
Eosinofil	0–0.4%	0.14 ± 0.38[a]	0.43 ± 0.79[a]	0.28 ± 0.49[a]	0.43 ± 0.53[a]	1.00 ± 1.15[a]	0.71 ± 0.75[a]	0.71 ± 0.95[a]
Basophil	0–0.3%	0.14 ± 0.38[a]	0.57 ± 0.97[a]	0.00 ± 0.00[a]	0.14 ± 0.38[a]	0.14 ± 0.38[a]	0.28 ± 0.49[a]	0.28 ± 0.49[a]

Ref. val – reference values; control – negative control – EDTA 0.1 mL/10 g b.w.; DMH – DMH (dimethylhydrazine) 20 mg/kg b.w. for 2 weeks (positive control); *A. blazei* – *A. blazei* 10%/kg c.r. for 4 weeks; Pre-treatment – *A. blazei* 10%/kg c.r. during the first 2 weeks + DMH 20 mg/kg b.w. for 2 weeks; simultaneous – simultaneous treatment – *A. blazei* 10%/kg c.r. + DMH 20 mg/kg b.w. for 2 weeks; post-treatment – DMH 20 mg/kg b.w. during the first 2 weeks + *A. blazei* 10%/kg c.r. for 2 weeks; pre + continuous – pre-treatment + continuous – *A. blazei* 10%/kg c.r. during the first 2 weeks + *A. blazei* 10%/kg c.r.; DMH 20 mg/kg b.w. for 2 weeks. Different letters indicate statistically significant differences ($p < 0.05$; ANOVA/Tukey).

Table 3
Effects of supplementation of *A. blazei* on the blood cells 48 h:

Parameters	Ref. val.	Experimental groups						
		Control	DMH	*Agaricus*	Pre	Sim	Post	Pre + continuous
Lymphocyte	55–95%	78.00 ± 2.00[a]	80.00 ± 2.00[a]	72.57 ± 7.63[a]	70.71 ± 4.07[a]	72.43 ± 9.02[a]	74.28 ± 8.54[a]	71.14 ± 3.34[a]
Neutrophil	10–40%	21.57 ± 6.60[a]	13.43 ± 3.55[b]	24.28 ± 7.41[a,b]	23.71 ± 4.78[a,b]	20.43 ± 6.95[a]	21.00 ± 7.02[a]	21.00 ± 3.87[a]
Monocytes	0.1–3.5%	6.71 ± 2.43[a]	6.00 ± 1.82[a]	2.42 ± 1.62[b]	4.86 ± 2.12[a,b]	6.14 ± 1.95[a]	2.14 ± 1.21[b]	6.86 ± 1.68[a]
Eosinofil	0–0.4%	1.00 ± 1.15[a]	2.28 ± 1.89[a]	0.57 ± 0.53[a]	0.57 ± 0.78[a]	0.85 ± 1.07[a]	0.86 ± 1.86[a]	0.71 ± 0.95[a]
Basophil	0–0.3%	0.14 ± 0.38[a]	0.43 ± 1.13[a]	0.14 ± 0.38[a]	0.00 ± 0.00[a]	0.14 ± 0.38[a]	0.14 ± 0.38[a]	0.28 ± 0.49[a]

Ref. val – reference values; control – negative control – EDTA 0.1 mL/10 g b.w.; DMH – DMH (dimethylhydrazine) 20 mg/kg b.w. for 2 weeks (positive control); *A. blazei* – *A. blazei* 10%/kg c.r. for 4 weeks; Pre-treatment – *A. blazei* 10%/kg c.r. during the first 2 weeks + DMH 20 mg/kg b.w. for 2 weeks; simultaneous – simultaneous treatment – *A. blazei* 10%/kg c.r. + DMH 20 mg/kg b.w. for 2 weeks; post-treatment – DMH 20 mg/kg b.w. during the first 2 weeks + *A. blazei* 10%/kg c.r. for 2 weeks; pre + continuous – pre-treatment + continuous – *A. blazei* 10%/kg c.r. during the first 2 weeks + *A. blazei* 10%/kg c.r.; DMH 20 mg/kg b.w. for 2 weeks. Different letters indicate statistically significant differences ($p < 0.05$; ANOVA/Tukey).

to 2.28%. The basophils were increased in the group treated only with DMH, where the mean percentage was 0.43% (reference values: 0–0.3%). The neutrophil counts were found to be within the range of reference values (10–40%) for all groups. However, the DMH group showed a statistically significant decline for this cell type in relation to the other groups. When evaluating the monocytes, it was possible to see that only the groups supplemented with *A. blazei* and the post-treatment group showed mean percentages of 2.42% and 2.14%, respectively, thus within the reference values (0.1–3.5%). However, the other groups showed means above the reference values, with statistically significant differences.

At 72-h evaluation, lymphocytes and neutrophils were found to be inside the range of reference values (lymphocytes: 55–95%; neutrophils: 10–40%), and the mean percentages varied from 63.43% to 79.43% and 12.86% to 28.57%, respectively. The lymphocytes did not show any significant variation. The neutrophils decreased significantly in number in the group that received DMH, in relation to other groups, while the groups that received DMH and *A. blazei*, except for the post-treatment protocol, showed a tendency for a reduction in this cell type. In relation to the monocytes, all groups showed numbers above the reference values with exception of groups *A. blazei* and post-treatment, where these two groups also showed statistically significant differences in relation to the others. The group that showed the highest mean percentage was the one given supplementation with *A. blazei* in the pre-treatment + continuous protocol, and the mean percentage of this group was 6.00% (reference values: 0.1–3.5%). Eosinophils and basophils did not show statistically significant differences. However, eosinophil count was increased in all experimental groups when compared to the reference values (0–0.4%), and the mean

Table 4
Effects of supplementation of *A. blazei* on the blood cells 72 h:

Parameters	Ref. val.	Experimental groups						
		Control	DMH	*Agaricus*	Pre	Sim	Post	Pre + continuous
Lymphocyte	55–95%	66.28 ± 9.94[a]	79.43 ± 5.32[a]	73.28 ± 8.79[a]	75.43 ± 8.60[a]	72.43 ± 6.24[a]	71.85 ± 10.68[a]	63.43 ± 13.02[a,b]
Neutrophil	10–40%	28.57 ± 9.20[a]	12.86 ± 4.41[b]	23.57 ± 8.52[a,b]	19.00 ± 9.90[a,b]	21.43 ± 3.91[a,b]	26.14 ± 11.00[a,b]	28.57 ± 13.43[a]
Monocytes	0.1–3.5%	3.71 ± 4.07[a,b]	3.57 ± 2.07[a,b]	1.71 ± 0.95[a]	4.43 ± 2.44[a,b]	4.57 ± 2.07[a,b]	1.57 ± 1.40[a]	6.00 ± 3.00[b]
Eosinofil	0–0.4%	1.14 ± 0.90[a]	2.14 ± 1.21[a]	1.00 ± 0.82[a]	1.29 ± 1.11[a]	1.00 ± 1.15[a]	0.43 ± 0.53[a]	1.57 ± 1.98[a]
Basophil	0–0.3%	0.29 ± 1.76[a]	1.00 ± 1.15[a]	0.43 ± 0.79[a]	0.29 ± 0.49[a]	0.71 ± 0.95[a]	0.43 ± 0.79[a]	0.43 ± 0.53[a]

Ref. val – reference values; control – negative control – EDTA 0.1 mL/10 g b.w.; DMH – DMH (dimethylhydrazine) 20 mg/kg b.w. for 2 weeks (positive control); *A. blazei* – *A. blazei* 10%/kg c.r. for 4 weeks; pre-treatment – *A. blazei* 10%/kg c.r. during the first 2 weeks + DMH 20 mg/kg b.w. for 2 weeks; simultaneous – simultaneous treatment – *A. blazei* 10%/kg c.r. + DMH 20 mg/kg b.w. for 2 weeks; post-treatment – DMH 20 mg/kg b.w. during the first 2 weeks + *A. blazei* 10%/kg c.r. for 2 weeks; pre + continuous – pre-treatment + Continuous – *A. blazei* 10%/kg c.r. during the first 2 weeks + *A. blazei* 10%/kg c.r.; DMH 20 mg/kg b.w. for 2 weeks. Different letters indicate statistically significant differences ($p < 0.05$; ANOVA/Tukey).

values varied from 0.43% to 2.14%. With respect to basophils, only the control and pre-treatment groups did not show values greater than the reference values (0–0.3%). In the other groups, the mean percentage varied from 0.43% to 1.00%.

Table 5 presents the values referring to phagocytic activity in the spleen. Statistical analysis indicated a difference between the groups. The greatest indication of phagocytic activity appeared in the groups with the pre-treatment + continuous, pre-treatment and simultaneous protocols. The control, *A. blazei* and post-treatment groups had similar activities but differed from the groups mentioned above. The group treated only with DMH showed the least phagocytic activity in spleen cells.

3.2. Experiment 2

Table 6 presents the mean values for initial weight, final weight and weight gain of the animals. There was a statistical difference among initial weights, varying between 36.91 ± 3.27 g and 49.09 ± 5.01 g, and among final weights, with a variation of 36.64 ± 2.66 g to 49.27 ± 5.00 g. The variation in weight gain was from 5.45 ± 3.24 g to 6.18 ± 2.75 g, which was statistically different. There was weight loss in animals of the groups *Agaricus*, post-treatment and pre-treatment + continuous, and weight gain in other groups, where the pre-treatment group showed the least weight gain.

Table 7 lists the mean absolute and relative weights of the animals. In relation to absolute weights, that of liver showed a statistically significant difference between the pre-treatment + continuous group, having the lowest mean, and the control and *Agaricus* groups. The relative weights of heart and liver differed statistically among the groups. The groups DMH and post-treatment had the lowest means for relative weight of heart, and the pre-treatment + continuous group the highest. In relation to relative weight of liver, the groups simultaneous and post-treatment showed the lowest values, and the *Agaricus* group the highest mean. With regard to the means for the other organs, the means of the relative and absolute weights did not show statistically significant differences.

Table 6
Mean values for initial weight, final weight and weight gain of the animals during the experimental period:

Experimental groups	Initial weight (g)	Final weight (g)	Weight gain(g)
Control	37.64 ± 3.98[a]	43.45 ± 4.66[a,b]	5.82 ± 3.63[b]
DMH	36.91 ± 3.27[a]	43.09 ± 4.32[a,b]	6.18 ± 2.75[b]
Agaricus	47.09 ± 4.93[b]	42.73 ± 3.50[a,b]	−4.36 ± 1.96[a]
Pre-treatment	46.55 ± 6.93[b]	49.27 ± 5.00[c]	2.73 ± 4.13[b]
Simultaneous	42.91 ± 5.39[a,b]	47.73 ± 4.82[b,c]	4.82 ± 2.40[b]
Post-treatment	49.09 ± 5.01[b]	45.82 ± 3.63[b,c]	−3.27 ± 3.00[a]
Pre + continuous	45.09 ± 4.32[b]	36.64 ± 2.66[a]	−5.45 ± 3.24[a]

g – gram; control – negative control – EDTA 0.1 mL/10 g b.w. for 2 weeks; DMH – DMH (dimethylhydrazine) 20 mg/kg b.w. for 2 weeks (positive control); *Agaricus* – *A. blazei* 10%/kg c.r. for 14 weeks; pre-treatment – *A. blazei* 10%/kg c.r. for 2 weeks + DMH 20 mg/kg b.w. for 2 weeks; simultaneous – simultaneous treatment – *A. blazei* 10%/kg c.r. for 2 weeks + DMH 20 mg/kg b.w. for 2 weeks; post-treatment – DMH 20 mg/kg b.w. for 2 weeks + *A. blazei* 10%/kg c.r. for 10 weeks; pre + conitnuous – pre-treatment + continuous – *A. blazei* 10%/kg c.r. for 14 weeks + DMH 20 mg/kg b.w. for 2 weeks. Different letters indicate statistically significant differences ($p < 0.05$; ANOVA/Tukey).

The number, distribution and reduction in damage with regard to aberrant crypt foci in the colon of the animals are shown in Table 8. The control group did not show aberrant crypt foci. The *Agaricus* group, despite showing a focus of two crypts resembled the control group statistically. Statistical analyses indicated the anticarcinogenic activity of the *A. blazei* group in relation to the pre-treatment and pre-treatment + continuous groups with a percent reduction in damage of 54.60% and 50.45%, respectively. The groups simultaneous and post-treatment showed numerous intermediates of aberrant crypt foci in comparison to the groups described above and the control groups. Thus, it was shown that

Table 5
Effects of supplementation of *A. blazei* on the frequency of splenic phagocytosis:

Experimental groups	No. cells analyzed	Total cells without evidence of phagocytosis			Total cells with evidence of phagocytosis		
		No. Abs.	Mean ± SD	Percentage	No. abs.	Mean ± SD	Percentage
Control	1400	425	63.28 ± 5.41[c]	31.64	975	139.28 ± 6.42[c]	69.65
DMH	1400	516	63.71 ± 4.89[d]	36.85	884	126.28 ± 4.89[d]	63.15
Agaricus	1400	416	54.43 ± 4.04[c]	29.71	984	140.57 ± 4.04[c]	70.29
Pre	1400	348	49.71 ± 5.99[a]	24.85	1052	150.28 ± 5.99[a]	75.15
Sim	1400	317	45.28 ± 6.52[a]	22.64	1083	154.71 ± 6.52[a]	77.36
Post	1400	443	60.71 ± 6.42[c]	30.35	957	136.71 ± 5.41[c]	68.35
Pre + continuous	1400	226	32.28 ± 4.75[b]	16.14	1174	167.71 ± 4.75[b]	83.86

Control – negative control – EDTA 0.1 mL/10 g b.w.; DMH – DMH (dimethylhydrazine) 20 mg/kg b.w. for 2 weeks (positive control); *A. blazei* – *A. blazei* 10%/kg c.r. for 4 weeks; pre-treatment – *A. blazei* 10%/kg c.r. during the first 2 weeks + DMH 20 mg/kg b.w. for 2 weeks; simultaneous – simultaneous treatment – *A. blazei* 10%/kg c.r. + DMH 20 mg/kg b.w. for 2 weeks; post-treatment – DMH 20 mg/kg b.w. during the first 2 weeks + *A. blazei* 10%/kg c.r. for 2 weeks; pre + continuous – pre-treatment + continuous – *A. blazei* 10%/kg c.r. during the first 2 weeks + *A. blazei* 10%/kg c.r.; DMH 20 mg/kg b.w. for 2 weeks. Different letters indicate statistically significant differences ($p < 0.05$; ANOVA/Tukey).

Table 7
Mean values of absolute and relative weights With regard to the means for the other organs of the animals after of experimentation period:

Experimental groups	Total weight (g)				Relative weight (g)			
	Heart	Lung	Liver	Kidneys	Heart	Lung	Liver	Kidneys
Control	0.2407 ± 0.0322[a]	0.2531 ± 0.0457[a]	2.2720 ± 0.3219[b]	0.6670 ± 0.1072[a]	0.0056 ± 0.0009[a,b]	0.0058 ± 0.0010[a]	0.0524 ± 0.0066[b,c]	0.0155 ± 0.0028[a]
DMH	0.2289 ± 0.0363[a]	0.2569 ± 0.0498[a]	2.0627 ± 0.2858[a,b]	0.6587 ± 0.0977[a]	0.0054 ± 0.0010[a]	0.0060 ± 0.0011[a]	0.0479 ± 0.0053[a,b,c]	0.0154 ± 0.0024[a]
Agaricus	0.2703 ± 0.0429[a]	0.2976 ± 0.1263[a]	2.2738 ± 0.3554[b]	0.6700 ± 0.1325[a]	0.0064 ± 0.0011[a,b]	0.0070 ± 0.0028[a]	0.0531 ± 0.0058[c]	0.0157 ± 0.0028[a]
Pre-treatment	0.2816 ± 0.0618[a]	0.2809 ± 0.0433[a]	2.2898 ± 0.2439[b]	0.6991 ± 0.1213[a]	0.0057 ± 0.0010[a,b]	0.0057 ± 0.0010[a]	0.0466 ± 0.0041[a,b]	0.0141 ± 0.0014[a]
Simultaneous	0.2736 ± 0.0343[a]	0.2711 ± 0.0573[a]	2.1710 ± 0.1761[a,b]	0.6595 ± 0.0729[a]	0.0057 ± 0.0008[a,b]	0.0057 ± 0.0012[a]	0.0457 ± 0.0038[a]	0.0138 ± 0.0008[a]
Post-treatment	0.2416 ± 0.0526[a]	0.2547 ± 0.0322[a]	2.0183 ± 0.2165[a,b]	0.6170 ± 0.0791[a]	0.0053 ± 0.0010[a]	0.0055 ± 0.0004[a]	0.0441 ± 0.0037[a]	0.0135 ± 0.0012[a]
Pre + continuous	0.2712 ± 0.0520[a]	0.2492 ± 0.0443[a]	1.9130 ± 0.1803[a]	0.5822 ± 0.0641[a]	0.0069 ± 0.0010[b]	0.0063 ± 0.0010[a]	0.0483 ± 0.0037[a,b]	0.0147 ± 0.0013[a]

g – gram; control – negative control – EDTA 0.1 mL/10 g b.w. for 2 weeks; DMH – DMH (dimethylhydrazine) 20 mg/kg b.w. for 2 weeks (positive control); *Agaricus* – *A. blazei* 10%/kg c.r. for 14 weeks; pre-treatment – *A. blazei* 10%/kg c.r. for 2 weeks + DMH 20 mg/kg b.w. for 2 weeks; simultaneous – simultaneous treatment – *A. blazei* 10%/kg c.r. for 2 weeks + DMH 20 mg/kg b.w. for 2 weeks; post-treatment – DMH 20 mg/kg b.w. for 2 weeks + *A. blazei* 10%/kg c.r. for 10 weeks; pre + conitnuous – pre-treatment + continuous – *A. blazei* 10%/kg c.r. for 14 weeks + DMH 20 mg/kg b.w. for 2 weeks. Different letters indicate statistically significant differences ($p < 0.05$; ANOVA/Tukey).

Table 8
Number, distribution and reduction in damage with regard to aberrant crypt foci (ACF) in the colon of male mice.

Treatment	Total of ACF		%RD	Total of AC	Absolute Values of AC/Foci				Relation AC/Foci
	Absolute Values	Mean ± EPM			1AC/Foci	2AC/Foci	3AC/Foci	4AC/Foci	
Carcinogenicity									
Control	0	0,00 ± 0,00[a]	-	0	0	0,00	0,00	0,00	0,00
DMH	337	30,64 ± 6,52[c]	-	639	137	117	64	19	1.90
Agaricus	1	0,09 ± 0,30[a]	-	2	0,00	1,00	0,00	0,00	2,00
Anticarcinogenicity									
Pre-treatment	153	13,91 ± 2,59[a,b]	54,60	307	55	54	32	12	2,01
Simultaneous	172	15,64 ± 2,73[b,c]	48,96	368	48	64	48	12	2,14
Post-treatment	234	21,27 ± 4,25[b,c]	30,56	458	90	82	44	18	1,96
Pre + Continuous	167	15,18 ± 2,36[b]	50,45	322	62	62	32	11	1,93

Legend: Control – Negative control – EDTA 0,1mL/10 g b.w. (body weight); DMH – DMH (dimethylhydrazine) 20 mg/Kg b.w. (body weight) for 2 weeks (positive control); Agaricus blazei – Agaricus blazei 10%/Kg c.r. (commercial ration) for 4 weeks; Pre-treatment – Agaricus blazei 10%/Kg c.r. (commercial ration) during the first 2 weeks + DMH 20 mg/Kg b.w. (body weight) for 2 weeks; Simultaneous - Simultaneous treatment – Agaricus blazei 10%/Kg c.r. (commercial ration) + DMH 20 mg/Kg b.w. (body weight) for 2 weeks; Post-treatment – DMH 20 mg/Kg b.w. (body weight) during the first 2 weeks + Agaricus blazei 10%/Kg c.r. (commercial ration) for two weeks; Pre + Continuous - Pre-treatment + Continuous – Agaricus blazei 10%/Kg c.r. (commercial ration) during the first 2 weeks + Agaricus blazei 10%/Kg c.r. (commercial ration); DMH 20 mg/Kg b.w. (body weight) for 2 weeks. Different letters indicate statistically significant differences (p < 0,05; ANOVA/Tukey).

despite that there were no statistically significant differences, the percent reduction in damage indicates a tendency toward prevention, where the percentages of prevention of foci were 48.96% for the simultaneous group and 30.56% for the post-treatment group.

4. Discussion

Various important substances with immunomodulatory (Kentaro et al., 2007; Kawamura et al., 2005; Akramienė et al., 2007) and antitumor activity (Firenzuoli et al., 2007; Talcott et al., 2007; Murakawa et al., 2007) were isolated from mushrooms, among which the polysaccharides stand out, particularly β-D-glucans, polysaccharopeptides, polysaccharide proteins and proteins. Also possessing these activities are triterpenes, lipids and phenols (Angeli et al., 2008).

In view of the known properties of mushrooms as functional foods, and due to their constituents and the use of *A. blazei* on a large scale in different population groups for treating cancer and enhancing the immune system, the present study evaluated the supplementation of this mushroom in the normal diet of mice, at a proportion of 10%, by means of the comet assay, aberrant crypt foci assay, differential cell counts in peripheral blood and splenic phagocytic activity.

In light of the results obtained, it appears that supplementation with *A. blazei* did not show genotoxic activity, and this finding allows us to infer that supplementation with this mushroom, based on this protocol, does not produce DNA damage that leads to the development of cancer. This finding is supported by the studies of Menoli et al. (2001), Delmanto et al. (2001), Oliveira et al. (2002), Bellini et al. (2003), Luiz et al. (2003), Guterrez et al. (2004), Machado et al. (2005) and Guterres et al. (2005), who reported that the administration of *A. blazei*, in different forms of extract and concentrations, did not produce genotoxic and/or mutagenic activity.

Other authors reported that *A. blazei* and β-glucan possess antigenotoxic activity (Chorvatovicová et al., 1999; Guterrez et al., 2004; Guterres et al., 2005; Angeli et al., 2008; Bellini et al., 2006). In the present study, this property was demonstrated for daily supplementation with 10% *A. blazei* in the normal diet. However, some authors such as Oliveira et al. (2007) report the lack of antigenotoxic activity with the administration of β-glucans isolated from *Saccharomyces cerevisiae* in Chinese hamster ovarian cells. Similarly, Oliveira et al. (2002) conducted a study with aqueous extract of *A. blazei* in mice, and demonstrated the lack of antigenotoxicity using the comet assay. Thus, the literature shows that there is the results of the various studies are not consistent with

respect to the antigenotoxic potential of this mushroom and that responses vary based on the protocols, doses and types of extracts or forms of treatments utilized.

When the comet data are correlated with the splenic phagocytosis findings, it can be seen that the frequency of phagocytosis is similar in the control, *A. blazei* and post-treatment groups. This fact suggests that the administration of mushroom probably does not increase the phagocytosis in the spleen in animals suffered no damage in the genetic material contained which could be visualized by means of the comet assay. Thus, supplementation with *A. blazei* could be correlated with an increase in monocytes and later macrophages. However, these become active and participate in phagocytosis only when need be. In case the body does not need this function, these cells remain present, but without exerting any phagocytic activity.

When evaluating the group that received DMH and those submitted to the protocols of pre-treatment, simultaneous, post-treatment and pre-treatment + continuous, it is seen that the frequency of lesioned cells observed by the comet test in these groups is better than that shown by the DMH group. An analysis of splenic phagocytosis for these same groups demonstrates that greater phagocytic capacity is found in the protocols of pre-treatment, simultaneous and pre-treatment + continuous and that the values found in the DMH group are lower in comparison with all the groups. This suggests that supplementation with *A. blazei* in a protocol of pre-treatment (2 weeks), simultaneous treatment (2 weeks) or pre-treatment + continuous (4 weeks) is capable of modulating phagocytic activity in spleen cells, and it appears that the longer the time of provision of supplemented ration, the better the efficacy of the spleen is to remove cells in the blood stream that have some type of genetic alteration and that could contribute indirectly to the development of cancer. However, this assay and the results presented here can help in understanding what could occur in cells of non-hematopoeitic tissues, since the metabolites of DMH reach all cell types and different tissues of the body. Thus, in analyzing the phagocytosis data, it can be seen that actually the increase in time of supplementation is related to an improvement in phagocytic capacity, in a statistically significant manner, and that this supplementation alone is not related to any damage observed in the animals, be it by the comet assay or by the demonstration of phagocytic activity.

These notions are reinforced by data from the analysis of differential blood cell counts, which demonstrated that at 24 h the number of monocytes in all the groups are above the reference value. This is especially notable in the groups DMH, *A. blazei*, pre-treatment, simultaneous and pre-treatment + continuous. As discussed earlier, the lack of elevation in monocyte counts in the post-treatment protocol is due to the short time of supplementation with mushroom. In analyzing the other groups, it can be inferred that the administration of DMH is capable of producing a picture of monocytosis, because of the inflammatory reaction that the drug causes, especially in the intestinal mucosa of mice (Wiebecke et al., 1973; Richards, 1977; Deschner, 1978; Wargovich et al., 1983; Krutovskikh and Turosov, 1994; Bain, 2007).

Another fact is that even without suffering injury the group that received only *A. blazei* also showed a picture of monocytosis. This could indicate that the supplementation of the diet with this mushroom modulates the immune system, making it more efficient with regard to phagocytic function, since monocytes in the blood represent a maturation phase of phagocytic mononuclear cells originating in the bone marrow. These cells enter the blood stream, where they remain only a few days, and by passing through the walls of the capillaries and venules, they penetrate some organs, including the spleen, transforming into macrophages which constitute a more advanced phase in the life of phagocytic mononuclear cells (Junqueira and Carneiro, 2008).

On the other hand, when the groups that received DMH combined with supplementation with *A. blazei* are evaluated, there was an even greater increase in the presence of circulating monocytes, except for the pre-treatment + continuous group. This finding suggests that in these groups there was a better splenic phagocytic capacity, since these monocytes can be found in the spleen in the form of macrophages at a later time. When evaluating phagocytosis in the spleen, this notion is confirmed, since this parameter was increased in these groups. Another fact that is called to our attention is that the pre-treatment + continuous group did not show such a marked increase in monocytes, when compared to the pre-treatment and simultaneous groups, because these monocytes could have already migrated to the spleen, which is corroborated by higher phagocytic activity found in this group.

In the evaluations at 48 and 72 h, monocytosis was found for all the groups except the *A. blazei* and post-treatment groups. The lack of monocytosis in the *A. blazei* group could be better understood when considering that these circulating cells migrate to other tissues that are not hematopoietic where the spleen is also considered among these. However, as these animals were not undergoing any process of injury, the technique of phagocytosis in the spleen did not demonstrate their effective presence, that is, resulting in phagocytosis. With respect to the lack of monocytosis in post-treatment at 48 and 72 h, where the mean values were always lower than those shown by the controls, albeit not statistically significant, it is possible that the short time of supplementation was not sufficient to modulate the immune system of the experimental animals.

The maintenance of monocytosis in the pre-treatment, simultaneous and pre-treatment + continuous groups could be explained by the fact that *A. blazei* is capable of modulating the immune system, so that the cells with genetic damage are sequestered by the spleen, thereby diminishing indirectly the maintenance of cells that could be initiated for the development of cancer in the body.

The results obtained are in accordance with those reported by Alves de Lima (2006) who demonstrated that supplementation with β-glucan was related to an increase in monocytes in peripheral blood and granulocyte progenitor cells both in spleen and bone marrow. These authors also considered that the macrophages are phagocytes and that their function is to remove foreign bodies, parasites and bacteria. Thus, it is assumed that reducing the frequency of micronuclei in reticulocytes of peripheral blood may be due to the improvement of phagocytic activity in spleen cells. It was from these considerations, the authors of this research were also the inferences made earlier.

However, despite these various considerations with respect to the biological activities favored by supplementation with *A. blazei,* which can be related indirectly to the prevention of cancer, the present research group evaluated another technique that can help even more in understanding the actual effectiveness of this supplementation in controlling pre-malignant lesions. These can be better in demonstrating the anticarcinogenic capacity of the mushroom in study. The assay in question was the aberrant crypt foci assay, which is a validated and common experimental model for determining the macro–microscopic and clinical behavior of pre-neoplastic lesions, where the results can be applied to humans, because these lesions are the first to appear in the carcinogenesis of the colon in both rodents and humans (Druckrey et al., 1978; Bird, 1995; Banerjee et al., 1998).

Thus, this study also demonstrated the efficacy of the mushroom *A. blazei* in preventing carcinogenesis induced by DMH. In rodents, the administration of DMH leads to a slight inflammatory reaction and an increase in cell proliferation in the mucosa of the colon (Krutovskikh and Turosov, 1994). Its successive application results in continuous proliferation, producing an increase in the number of cells in the DNA synthesis phase (Deschner, 1978;

Wargovich et al., 1983) and thereby a progressive hyperplasia of the crypts and hypertrophy of the mucosa (Wiebecke et al., 1973; Richards, 1977).

Different treatment protocols need to be studied to elucidate the mode of action of biological molecules or chemical compounds in antimutagenesis (Ferguson, 1994; Flagg et al., 1995; Antunes, 2000; De Flora and Ferguson, 2005; Oliveira et al., 2006, 2007). Therefore, in this study, male mice were exposed to DMH and β-glucan, utilizing the protocols of pre-treatment and post-treatment which can indicate a bioantimutagenic action, and the protocol of simultaneous treatment which can indicate desmutagenic and bioantimutagenic activities, in addition to the continuous treatment protocol (Morita et al., 1978; Kada, 1983; Ferguson, 1994; Flagg et al., 1995; Antunes, 2000; Oliveira et al., 2002; De Flora and Ferguson, 2005; Oliveira et al., 2006, 2007).

The pre-treatment group showed a percent reduction in damage of 54.60%. In the pre-treatment + continuous group there was a reduction of 50.45% in the incidence pre-neoplastic damage, demonstrating that the mushroom acts in a chemopreventive manner. The simultaneous and post-treatment groups showed a low rate of prevention of aberrant crypt foci. These data suggest that the mushroom act through bioantimutagenesis as well as desmutagenesis. However, it appears that the least effective action is through desmutagenesis, as suggested by the poor prevention of lesions in simultaneous treatment. This finding can be reinforced by the percent reduction in damage shown by at least the comet assay.

Bobek et al. (1998, 2001), Bobek and Galbavy (2001) and Nosál'ová et al. (2001) tested *Pleurotus ostreatus* in rats and observed a significant reduction in aberrant crypt foci, demonstrating the anticarcinogenic activity of this edible mushroom. In these studies, the authors used pre-treatment + continuous protocols, and their results also suggest effects involving desmutagenesis and bioantimutagenesis.

The decrease in the aberrant crypt foci can be explained by the soluble fibers acting as a selective substrate for bacteria that produce short-chain fatty acids such as butyrate which appears to be capable of promoting apoptosis in the human intestine, maintaining the tissue in a good state (Hague et al., 1993; Scheppach et al., 1995; Green et al., 1998). In addition, these fibers increase the number of bifidobacteria which produce little or no β-glucuronidase, an enzyme that decreases the metabolism of DMH and consequently diminishes carcinogenicity in the colorectal mucosa (Hauly and Moscatto, 2002) and other damage which can be visualized by other techniques in the peripheral blood and spleen, such as in the case of the comet assay and splenic phagocytosis assay, respectively, for example. These bacteria even produce lactic acid which decreases intestinal pH, and consequently generates a bactericidal medium for putrefactive bacteria, besides reducing the absorption of ammonia (Goldin and Gorbach, 1980; Koo and Rao, 1991; Gibson and Macfarlane, 1995).

Another possible mechanism of anticarcinogenic action is the reduction in carcinogenic substances, due to their adsorption to the cell wall of microbiota. The microbiota can even retain water causing increased intestinal transit, thereby diluting all the components present in the colon. In this manner, there are inhibitory effects in the stages of initiation and promotion of colon cancer (Reddy and Rivenson, 1993; Hague et al., 1993; Pool-Zobel et al., 1993; Scheppach et al., 1995; Pierre et al., 1997; Peridgon et al., 1998).

The literature even shows various other works that correlate the data here discussed, such as the recent study by Lin et al. (2004) These authors reported that a fraction of maitake, an extract isolated from the mushroom *Grifola frondosa*, in which the isolated active component is β-glucan, caused an improvement in colony formation of granulocytes/macrophages, improved response of progenitor cells in bone marrow and better recovery of granulocytes/macrophages in response to the administration of doxorubicin which causes bone marrow suppression and also increases the incidence of micronuclei in different tissues.

Rodrigues et al. (2003) demonstrated that the active principle of *A. blazei* produces immunomodulatory effects, namely the activation of macrophages and neutrophils, which cause tumor regression. Mizuno et al. (1998) demonstrated the stimulation of lymphocytes in mice treated with polysaccharides from *A. blazei*, suggesting that this mushroom could be an effective prophylactic, reducing the occurrence of cancer by increasing the production of lymphocytes. Chan et al. (2007) concluded that *A. blazei* causes a significant increase in phagocytic activity in rats, where seven parameters were tested and shown to improve in animals supplemented with *A. blazei*, demonstrating the appreciable capacity of this mushroom to modulate the function of the immune system in the animals evaluated and confirming its immunomodulating potential, as discussed here also.

In a clinical study carried out in human volunteers, the authors confirmed that *A. blazei* can help in alleviating symptoms related to diseases like obesity, hypertension, diabetes and cancer through its antiinflammatory and antitumor effects and its stimulation of the immune system (Liu et al., 2008).

Based on these considerations, *A. blazei* could act as an immunomodulatory food and be effective in improving phagocytic activity in the spleen. These facts suggest a mechanism for eliminating cells with DNA damage (genotoxic and mutagenic) related to the development of cancer. Finally, the findings suggest that mushroom has a true efficacy in preventing colorectal carcinogenesis, also through the capacity of modulating the intestinal microbiota responsible for the degradation of pro-carcinogens. However, further studies are needed to elucidate the mechanisms underlying the anticarcinogenicity of *A. blazei*, so that it can be better indicated as an immunomodulatory food and a chemopreventive agent effective in protecting against colorectal cancer.

Conflict of interest statement

The authors of this article have no conflict of interest.

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