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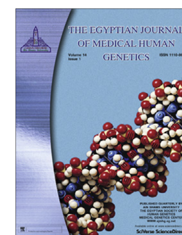

ORIGINAL ARTICLE

Antigenotoxic and anticlastogenic potential of *Agaricus bisporus* against MMS induced toxicity in human lymphocyte cultures and in bone marrow cells of mice

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KEYWORDS

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Sister chromatid exchanges,
Replication index

Abstract The aim of the present study is to evaluate, for the first time, antigenotoxic potential of *Agaricus bisporus* against methyl methanesulphonate induced toxicity in human lymphocyte culture *in vitro* and in bone marrow cells of albino mice *in vivo*. The parameters studied included total aberrant cells and the frequencies of aberrations in the bone marrow cells at three exposure durations viz., 16, 24 and 32 h, and for the *in vitro* method using chromosomal aberrations, sister chromatid exchanges and replication indices as markers. The alcoholic extract of *A. bisporus* was taken in five increasing concentrations of 200, 250, 300, 350 and 400 mg/kg body weight for three *in vivo* exposure durations viz., 16, 24 and 32 h. Similarly, four doses of extracts viz., 150, 200, 250 and 300 μg/ml of culture were taken for *in vitro* durations of 24, 48 and 72 h in the presence as well as the absence of S_9-mix. The treatment reduced the total number of aberrant cells ranging from 10.0% to 46.15% and it reduced the total frequencies of aberrations ranging from 198 to 96 against very high aberrations i.e., 227 caused due to methyl methanesulphonate *in vivo*. The same trends were observed in the *in vitro* experiments i.e., it reduced chromosomal aberrations from (42.00%, 71.25%, and 83.00% to 20.00%, 39.50%, and 43.00%) at 24, 48, and 72 h of exposure respectively.

Abbreviations: CA, chromosomal aberration, SCE, sister chromatid exchanges, RI, replication index, MMS, methyl methanesulphonate, SE, standard error

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However when experiments were carried out in the presence of liver S_9 fraction, these values were respectively 52.38, 44.56, and 48.34% significant at < 0.05 level, likewise it also reduced sister chromatid exchanges from 14.86 ± 1.44 down to 8.84 ± 0.75 per cell, whereas the replication index got enhanced from 1.45 to 1.64.

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1. Introduction

Dietary mushrooms have been used globally since millennia to promote health and prevent and treat various diseases due to their multitude of medicinal qualities [1]. The number of different mushroom species on earth is estimated at 140,000 of which only 10% is known [2]. In general, medicinal dietary mushrooms have been shown to improve cardiovascular health, stimulate immune function and contribute to glucose homeostasis and to modulate detoxification, as well as exert anti-allergic, antiviral, antibacterial, antifungal and anti-inflammatory activities [3].

The use of mushrooms with potential therapeutic properties has greatly raised global interest in the scientific as well as clinical community based on two main reasons. Firstly, mushrooms demonstrate their efficiency against numerous diseases including metabolic disturbances as serious as cancer and degenerative diseases. Secondly, these therapeutic effects seem to encompass multiple complex pharmacological actions on different cellular and molecular targets [4]. The most significant medicinal effects of mushrooms and their metabolites that have attracted the attention of the public are their antitumor properties [5,6]. The medicinal use of edible mushroom extracts seems to be more of a natural and less expensive approach and, in general, involves minimal unwanted side effects. Moreover, purified bioactive compounds derived from edible mushrooms might be potentially important new sources of anticancer agents. It has been known for many years that selected mushrooms of higher basidiomycetes origin are effective against certain types of cancer which have stirred a great interest in the use of such mushrooms across industry, media and the scientific community [2].

In recent years, multiple drug resistance in human pathogenic microorganisms has been reported to indiscriminate the use of commercially used antimicrobial drugs for the treatment of infectious diseases. Extracts from fruiting bodies and the mycelia of various mushrooms have been reported to cause antimicrobial activity against a wide range of infectious bacteria [7].

2. Materials and methods

The whole plant of *A. bisporus* was dried in the shade at room temperature. The shade dried plants were powdered and 60 g of coarse powder was defatted with petroleum ether and extracted exhaustively with 95% methanol at a temperature of 60 °C. The extract was dried by a vacuum evaporator. Methanolic extract of *A. bisporus* was dissolved in dimethyl sulphoxide (DMSO) to prepare different optimum concentrations for studies following earlier methodology [8].

2.1. In vivo method

Albino mice 8–10 weeks old (25–35 gm in weight) were exposed to different test chemicals by appropriate routes (intra peritoneal i.e., I.P injection) and were sacrificed at sequential intervals of 16, 24, and 32 h of stipulated treatment time. Animals were treated with each test substance as shown in the tables (Table A). Further processes of slide preparations, cells and chromosomal aberrations analyses are adopted from earlier published work [8].

The reduction factors due to test chemical treatments were calculated using the formula:

$$\%\text{Reduction} = \frac{(\text{Aberrant cells in MMS control} - \text{aberrant cells in MMS with } Agaricus \text{ extracts})}{(\text{Aberrant cells in MMS control} - \text{aberrant cells in negative control})}$$

2.2. In vitro lymphocytes culture method

Human peripheral blood lymphocytes are extremely sensitive indicators of the *in vitro* assay system. The chromosomal changes (numerical and structural) were utilized for investigation of the genotoxic as well as antigenotoxic potentiality of test chemicals. The parameters studied included chromosomal aberrations (CA), sister chromatid exchanges (SCEs) and cell growth kinetics (RI) both in the presence and in the absence of the exogenous metabolic activation system. The *in vitro* culture methods, preparation of S_9 (microsomal fraction), media preparation and analyses of chromosomal aberrations, sister chromatid exchanges, cell cycle kinetics and statistical analysis were followed as per earlier methodology.[8]

The replication index (RI) was calculated according to the formula of Tice et al. [9] as given below. The deviation from the controls was determined by using the Chi-square (χ^2) test.

$$\text{R.I} = \frac{(M_1X1) + (M_2X2) + (M_3X3)}{100}$$

3. Results

3.1. *In vivo* effects

The results following the treatment of *A. bisporus* extract with methyl methanesulphonate (MMS) were tabulated at three different durations of 16, 24 and 32 h. The results show that the value after treatment with MMS only at 16 h was 12.0%, while the same treatment with five different increasing concentrations of *A. bisporus* with MMS, reduced the values down to 11.9%, 10.8%, 9.1%, 8.9% and 8.5% respectively, the same values for normal and DMSO controls respectively were 2.1% and 2.0%. In terms of percent reduction in the number of aberrant cells, the observed values respectively were 08.3%, 10.0%, 24.16%, 25.83% and 29.16% for five different doses of *A. bisporus* extract. The results showed that *A. bisporus* extract at each concentration reduced the total aberrant cells significantly (Fig. 1).

The total numbers of frequency in the aberrant cells were 198 for MMS alone, whereas the same were 206, 169, 146, 138 and 124 in numbers for five different doses of *A. bisporus* extract respectively. The reduced values are statistically

Table A *In vivo* concentrations of phyto-chemicals.

Phyto-products	1st dose	2nd dose	3rd dose	4th dose	5th dose
Alcoholic extracts of *Agaricus bisporus in vivo* (mg/kg bw)	AB_1 200	AB_2 250	AB_3 300	AB_4 350	AB_5 400
Alcoholic extracts of *Agaricus bisporus in vitro*(μg/ml)	AB_1 150	AB_2 200	AB_3 250	AB_4 300	Nil
Positive and Negative Controls (μg/ml)	(+) MMS 5	(-)DMSO 5	Nil	Nil	Nil

Note: In the *in vivo* experiments five increasing doses were used whereas for *in vitro* experiments only four doses were tested, Methyl methanesulphonate and Dimethyl sulphoxide were used as positive and negative controls respectively.

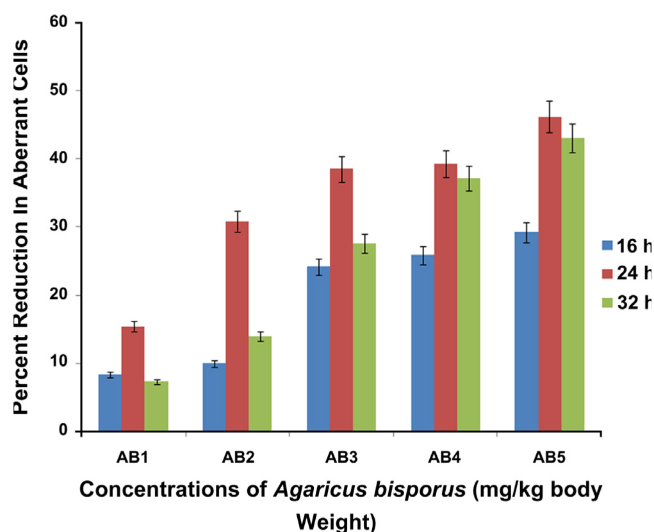

Figure 1 Shows *in vivo* anticarcinogenic effect of *Agaricus bisporus* extracts at 16, 24, and 31 h of treatment durations against MMS genotoxicity in albino mice bone marrow cell (significant at $P < 0.05$ level).

significant in comparison to MMS alone. As the doses of *A. bisporus* extract increases, the total frequency decreases, thus it shows a dose-dependent relationship. Cells with five or more aberrations were very few in numbers. A similar trend was obtained after 24 h of treatments. The obtained value of percent aberrant cells was 13.0% for MMS and 11.0%, 9.0%, 8.0%, 7.9% and 7.0% for five different concentrations of *A. bisporus* extract along with MMS. The percent reductions in the aberrant cells were 15.38%, 30.76%, 38.46%, 39.23% and 46.15% which shows very significant effect of different concentrations of the extract. It showed a dose dependent relationship between the doses of the extract and percentage reduction of the aberrant cells (Table 1 Fig. 1). The frequency of aberrant cells was recorded (Table 2). The values recorded were 222 aberrations for MMS and 145, 132, 107, 110 and 96 aberrations for the extracts plus MMS. The trends of reductions were dose dependent for the extracts (Table 2).

After 32 h of experiments the value obtained for the total number of aberrant cells was 151 for MMS alone while this number gets reduced to 140, 130, 110, 94 and 86 for their respective increasing concentrations of *A. bisporus* extract given with MMS. In terms of percent reductions in the number of aberrant cells, the extract showed a very much effective action in reducing the aberrant cells. The values for five concentrations of *A. bisporus* extract were 07.28%, 13.90%, 27.54%, 37.08% and 43.00% as shown in Fig. 1. The total frequency of aberrations followed a similar trend. The values recorded were 227 aberrations for MMS and 198, 171, 145, 119 and 119 aberrations for the extracts plus MMS.

3.2. In vitro effects

All the cultures were treated with Methyl methanesulphonate (MMS) at three different durations viz., 24, 48 and 72 h. The results of the treatment obtained in terms of clastogenic abnormalities in percent metaphase aberration, types of aberrations and aberrations per cell respectively are as follows viz., 40.00%, 58.50%, 69.75% and 0.40%, 059%, 0.70% aberrations calculated per cell respectively, while the control values for normal and DMSO plus *A. bisporus* extracts are 3.50, 4.75 percent at single standard doses at three different durations viz., 24, 48 and 72 h. The *A. bisporus* extract brings down aberrations from 40.00% to 32.00%, 27.75%, 23.25% and 21.00% with four consecutive doses at 24 h of duration, whereas at 48 h, it got lowered from 58.50% to 52.00%, 45.25%, 41.75% and 37.25% respectively by Ist to 4th concentrations of *A. bisporus* extracts. The same trend was noticed, when the treatment durations were increased to 72 h. These values show linear increasing trends with duration and doses of extracts. The maximum percentage reductions in the aberrations were 47.50% for 24 h and 36.32% and 42.65% for 48 and 72 h respectively, obtained due to the highest doses of *A. bisporus* extracts (Fig. 2).

Similarly when the experiment was setup along with the metabolic activation system ($+S_9$ mix), the effect of MMS got increased. The effect of the *A. bisporus* extract also showed similar increasing antigenotoxic trends as they lowered the clastogenic activity of MMS. The values showed linear increase with dosages (Table 3 and Fig. 3). The maximum effective percentage reductions were 52.38%, 44.56% and 48.35% for 24, 48 and 72 h respectively. The highest reduction for clastogeny of cells was noticed at 24 h durations; though the other values were also statistically significant at $P < 0.05$ level.

The data obtained from the experimental setup for sister chromatid exchanges are indicated in Table 4 and show a reduction in SCE in the absence as well as in the presence of the metabolic activation system; there was a lowering of the mean range and of the total SCEs and SCE per cell from 13.30 to 07.60 and from 14.86 to 8.84. For the analysis of SCE, only 48 h cultures were used and only 50 metaphases were scanned (Fig. 4).

The effects of *A. bisporus* extract on the replication index are shown in Table 5 and seem to elevate the level of the index when compared to the MMS treatment alone i.e. from 1.46 to 1.67; (though still lower than the normal level of 1.76). After being treated with the metabolic activation system, it was enhanced from 1.40 to 1.61. The *A. bisporus* extract, therefore,

Table 1 Effect of *Agaricus bisporus* on the frequency of cells with chromosome aberrations induced by methyl methanesulphonate (MMS x/kg.bw) at 24 h duration.

Treatment	*Agaricus bisporus* extract (Y/kg.bw)	Cells with pulverized chromosome	Types of chromatid aberrations				Aberrant cells		(%) Reduction
			Gaps	Breaks	Fragments	Exchanges	Number	Percentage	
DH_2O	0	00	03	04	16	00	20	2.0	
DMSO	0	00	02	02	17	00	19	1.9	
MMS + DMSO		13	98	40	73	02	130	13.0	
AB	AB_5	00	04	05	15	00	20	2.0	
MMS + AB	AB_1	09	35	36	65	00	110	11.0	15.38
	AB_2	05	55	32	51	01	90	9.0	30.76*
	AB_3	04	27	30	46	00	80	8.0	38.46*
	AB_4	04	31	28	47	00	79	7.9	39.23*
	AB_5	00	26	26	44	00	70	7.0	46.15*

Note: AB; concentrations of alcoholic extracts of *Agaricus bisporus,* MMS x/kg.bw; 5 μg/ml /kg body weight) at 24 h of treatment. Calculations were made excluding the gaps type of aberration and *significant at < 0.05 probability. Y/kg.bw is the concentration of alcoholic extracts of *Agaricus bisporus*.

is an effective anti-clastogenic agent as observed by these experiments.

4. Discussion

The role of phenolic compounds in acting as scavengers of free radicals is widely reported [10]; phenolic compounds play a major role in antioxidant activities shown by many vegetables [11]. It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when ingested daily from a diet rich in fruits and vegetables [12]. In a study, performed with *A. bisporus*, it was thought that the high free radical-scavenging activity and total antioxidant activity may result from the existence of the phenolics and the flavonoids.

The activity of ingredients against HL60 cells shows antiproliferative property, though there have been a number of reports which show that basidiomycetes have antitumor activity including antioxidant defense for the host immune system potential [13]. Many reports indicate that most of the polysaccharides or polysaccharide protein complexes from mushroom and natural sources cause direct cytotoxic effects on tumor

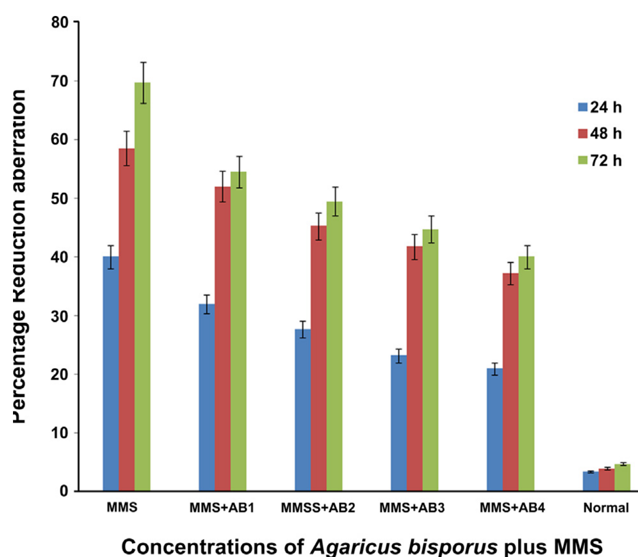

Figure 2 Comparative *in vitro* anticlastogenic effect of *Agaricus bisporus* extracts in the absence of S_9 mixture at 24, 48 and 72 h of treatment duration (significant at $P < 0.05$ level).

Table 2 Effect of *Agaricus bisporus* (AB) extract on the frequency of cells with chromosome aberrations induced by methyl methanesulphonate (MMS x/kg.bw) at 24 h duration.

Treatment	*Agaricus bisporus* extract (Y/kg.bw)	Cells with aberrations							Total number of aberrations
		0	1	2	3	4	5	6–9	
DH_2O	0	980	17	03	00	00	00	00	23
DH_2O + DMSO	0	981	17	02	00	00	00	00	21
MMS	0	870	73	20	16	06	04	01	222
AB	AB_5	980	16	04	00	00	00	00	24
MMS + AB	AB_1	890	81	15	07	02	01	00	145*
	AB_2	910	62	12	08	02	01	01	132*
	AB_3	920	60	10	02	04	01	00	107*
	AB_4	921	52	10	05	03	02	00	110*
	AB_5	930	57	06	02	04	01	00	96*

Note: AB; concentrations of alcoholic extracts of *Agaricus bisporus,* MMS x/kg.bw; 5 μg/ml /kg body weight) at 24 h of treatment. Calculations were made excluding the gaps type of aberration and *significant at < 0.05 probability. Y/kg.bw is the concentration of alcoholic extracts of *Agaricus bisporus*. The animals were sacrificed 24 h after MMS treatment 1000 cells from 10 animals were analyzed for each point. Y/kg.bw is the concentration of alcoholic extracts of *Agaricus bisporus*.

Table 3 Analysis of chromosomal aberration after treatment with Methyl methanesulphonate (MMS) along with *Agaricus bisporus* (AB) extract *in vitro,* in the presence of S_9 mix.

Treatments	Durations (h)	Metaphase scored	Percent aberration metaphase		Types of Aberration (%)			$\frac{Aberration}{Cell} \pm SE$
			Including gaps	Excluding gaps	Chromatid	Chromosome	Total	
MMS	24	200	40.50	38.25	31.50	10.50	42.00	0.42 ± 0.03
	48	200	42.75	41.50	47.00	24.25	71.25	0.71 ± 0.05
	72	200	52.00	46.50	54.25	29.00	83.25	0.83 ± 0.07
MMS + AB_1	24	200	25.75	21.00	23.00	11.00	34.00	0.34 ± 0.02
	48	200	34.00	29.75	40.50	20.50	61.00	0.61 ± 0.04
	72	200	29.00	28.75	42.75	24.00	65.75	0.66 ± 0.06
MMS + AB_2	24	200	26.75	21.00	18.25	10.00	28.25	0.28 ± 0.03
	48	200	34.75	29.25	38.25	17.25	55.50	0.56 ± 0.04
	72	200	32.25	28.75	36.75	20.75	57.50	0.58 ± 0.06
MMS + AB_3	24	200	22.75	19.50	12.00	09.75	21.75	0.22 ± 0.03
	48	200	32.75	28.50	31.25	12.50	43.75	0.44 ± 0.05
	72	200	25.00	22.25	32.00	16.75	48.75	0.49 ± 0.04
MMS_+AB_4	24	200	19.75	16.75	12.50	07.50	20.00	0.20 ± 0.03
	48	200	30.00	27.75	28.00	11.50	39.50	0.40 ± 0.04
	72	200	38.25	33.50	30.00	13.00	43.00	0.43 ± 0.06
CONTROL								
Normal	72	200	4.00	3.25	2.00	1.25	3.25	4.50 ± 0.01
DMSO + AB_2	72	200	5.75	4.25	2.75	1.50	4.75	6.25 ± 0.01

Note: AB; concentrations of alcoholic extracts of *Agaricus bisporus* MMS x/kg.bw; Methyl methanesulphonate 5 µg/ml/culture, gaps type of aberration is not included, SE; Standard error, Calculations were significant at <0.05 probability level.

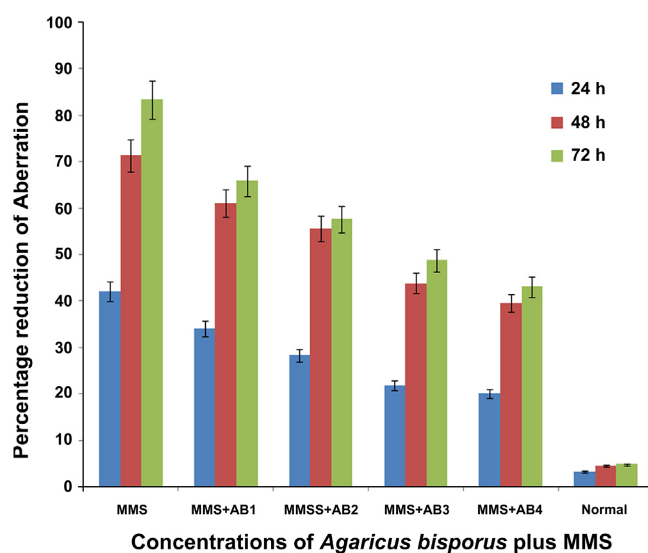

Figure 3 *In vitro* anticlastogenic effect of *Agaricus bisporus* extracts in the presence of S_9 mixture at 24, 48 and 72 h of treatment duration (significant at $P < 0.05$ level).

cells, mainly through host mediated immune response, this type of inhibitory action may be interwoven and the mechanism of action may vary from person to person as also the type of cancer [14]. Ergosterol, a phenolic compound extracted from the white button mushroom, shows the inhibitory effect on breast cancer cell line *in vitro* by aromatase inhibition without side effects [15].

A frequently reported protective mechanism exerted by mushrooms against cancer is the capacity to stimulate the immune system response where β-glucan, a water-soluble polysaccharide, is presumed to activate certain immune cells and the proteins that attack cancer, including macrophages, T-cells, natural killer cells, and interleukin-1 and interleukin-2 [2,16]. Dietary mushrooms such as *A. bisporus* have also been shown to alter aromatase activity, an enzyme that is involved in the conversion of androgens to proliferative estrogenic intermediates, which are closely linked to breast cancer development [17]. Mushroom extracts contain other components such as lentinan and lectins, which are directly cytotoxic and cytostatic to tumor cells such as MCF-7 breast cancer cells [18]) *A. bisporus* also contains bioactive antioxidants and anticarcinogenic substances including ergothioneine, selenium and polyphenols. Furthermore, studies have indicated that non-polysaccharide constituents in mushrooms have biological activity against murine skin cancer and human prostate carcinoma cells [19]. Collectively, there are many bioactive agents in mushrooms that can exert beneficial effects acting co-operatively in systems with different cell types, i.e. immunity, or exhibit cytotoxicity directly on the cancer cells.

Many diverse species of mushrooms and their respective bioactive agents have also demonstrated the capacity to inhibit cellular proliferation of human breast cancer cells *in vitro*. Isolated lectins from *Pholiata adipose* and *Inocybe umbrinella* exert strong antiproliferative activity toward MCF-7 and HepG2 human hepatoma cells with IC_{50} in the range of 1.9–7.5 µmol/L [20,21]). An isolated *A. bisporus*, or WB, lectin inhibits the incorporation of ^{3}H-thymidine into DNA by as much as 50% in MCF-7 cells and by 87% in HT29 colon cancer cells, about 16% in Caco-2 colon cancer cells, and by 55% in rat mammary fibroblasts (after 24 h incubation). This is in agreement with our observations since we have noted 33% reduction in BrdU incorporation after 24 h of incubation using *in vitro* human lymphocyte cultures. Numerous species of mushrooms when delivered as whole mushroom extracts or their isolated bioactive components, and their metabolites,

Table 4 Analysis of sister chromatid exchanges (SCE) after treatment with Methyl methanesulphonate along with *Agaricus bisporus* (AB) extract *in vitro,* in the presence of S_9 mix.

Treatment	Duration (Hrs.)	Metabolic activation	Metaphase scanned	Total SCE	Range	SCE/Cell ± SE
Aflatoxin B_1	48	$+S_9$	50	743	3–12	14.86 ± 1.44
$AFB_1 + AB_1$	48	$+S_9$	50	646	2–11	12.92 ± 1.14
$AFB_1 + AB_2$	48	$+S_9$	50	570	1–10	11.40 ± 1.00
$AFB_1 + AB_3$	48	$+S_9$	50	522	1–9	10.44 ± 0.90
$AFB_1 + AB_4$	48	$+S_9$	50	442	0–7	8.84 ± 0.75
Normal	48	$+S_9$	50	115	0–4	2.30 ± 0.40
DMSO	48	$+S_9$	50	108	0–5	2.16 ± 0.40
DMSO + AB_2	48	$+S_9$	50	103	0–5	2.06 ± 0.40

Note: AB; concentrations of alcoholic extracts of *Agaricus bisporus,* MMS x/kg.bw; methyl methanesulphonate 5 μg/ml/ culture, DMSO; dimethyl sulphoxide, SCE; Sister chromatid exchange, SE; Standard error, Calculations were made at < 0.05 probability level.

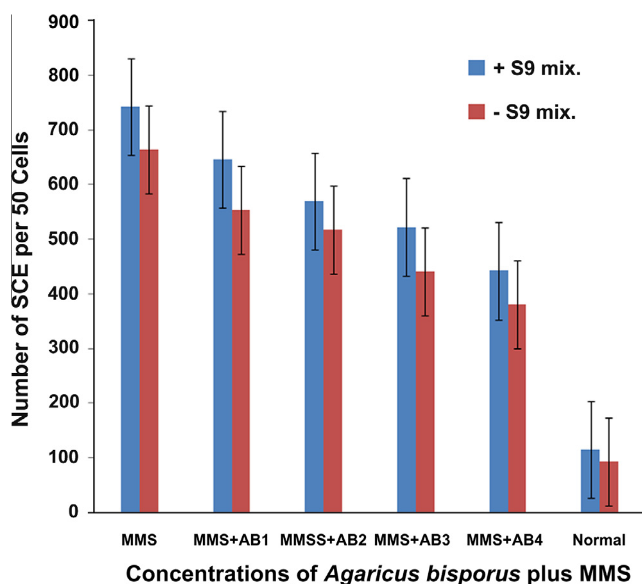

Figure 4 Antigenotoxic effect of *Agaricus bisporus* extracts on sister chromatid exchanges in the absence as well as in the presence of a metabolic activation system. Total 50 metaphase plates were scored at 48 h of treatment durations (significant at $P < 0.05$ level).

can inhibit cellular proliferation, suggesting that this is a key mechanism of action.

There have been many mechanisms proposed for the inhibitory action of mushrooms including that of the immune system and boosting the cell signaling inhibition. For example, studies show that shiitake has tumor-inhibitory effects on proliferation possibly through reduction in natural killer cell cytotoxicity in rodent studies [22]. In a study, it was confirmed that OYS mushroom extracts potently inhibit proliferation by inducing cell cycle arrest at the G_0/G_1 phase in MCF-7 cells [6]. Alternately, an extract of *A. blazei* inhibits cell growth in a dose-dependent manner through the arrest of cells in the G2/M phase and induction of apoptosis [23]. This is consistent with our results where proliferation was not altered as indicated by the replication index (RI) cell counts, though DNA replication clearly was decreased, suggesting an effect on the cell cycle. During the G_1 to S transition, cells are recruited for cellular proliferation through DNA replication, however if cells are arrested at this checkpoint, viz. G_0/S, then DNA replication will get reduced or even prevented. Our results thus indicate a ~30% reduction by the test of mushrooms on mutagenesis, as measured by BrdU incorporation, indicative of a block in DNA replication.

It was demonstrated that *Pleurotus ostreatus* induces G_0/G_1 cell cycle arrest of MCF-7 cells by the up-regulation of the expression of p53 and p21, whereas cell cycle arrest of HT-29 is induced at G_0/G_1 by the up-regulation of the expression of p21 [24]. Deregulated cell cycle progression is a common abnormality observed in human cancers. Progression through the cell-division cycle is regulated by the coordinated activities

Table 5 Analysis of cell cycle kinetics after treatment with methyl methanesulphonate along with *Agaricus bisporus* (AB) extract *in vitro,* in the presence of S_9 mix.

Treatment	Cell scored	Percent aberration metaphase			Replication Index	2 × 3 Chi square (χ^2) test
		M1	M2	M3		
MMS	200	55	40	05	1.46	
MMS + AB_1	200	53	42	05	1.52	**Significant
MMS + AB_2	200	49	44	07	1.58	*Significant
MMS + AB_3	200	45	45	10	1.65	*Significant
MMS + AB_4	200	45	43	12	1.67	*Significant
Normal	200	41	46	13	1.72	
DMSO	200	33	50	17	1.84	
DMSO + AB_2	200	33	49	18	1.85	

Note: 2 × 3 Chi square (χ^2) test was conducted, AB; concentrations of alcoholic extracts of *Agaricus bisporus,* MMS x/kg.bw; Methyl methanesulphonate 5 μg/ml/ culture. Calculations were made at < 0.05 probability level.

of cyclin/cyclin-dependent kinases (CDK) complexes. One level of regulation of these cyclin-CDK complexes is provided by their binding to CDK inhibitors (CKIs) [25]. In a study it was observed, using cDNA microarray technology and Western blot analysis, *P. ostreatus* up-regulated the expression of p21 and p53 in MCF-7 cells which had wild-type p53 [26]. Thus, treatment of MCF-7 cells by *P. ostreatus* may lead to the induction of p21 by p53 dependent mechanisms in MCF-7 cells. The increase of p53 protein levels in MCF-7 cells treated with *P. ostreatus* suggests that its inhibitory properties for growth may involve the induction of DNA damage response. The C terminus of p21, containing its nuclear localization signal, binds to or inhibits PCNA, thereby blocking DNA replication [27]. In the study, it was demonstrated that *P. ostreatus* slightly decreased the protein level of PCNA in MCF-7 treated cells, which may also contribute to the decreased DNA synthesis. However, expression of PCNA in HT-29 treated cells was not affected by *P. ostreatus* [26].

5. Conclusion

Alcoholic extracts of *A. bisporus* reduce the total aberrant cells ranging from 10.0% to 46.15% and it reduces the total frequencies of aberration from 198 to 96 against 227 aberrations caused due to MMS alone *in vivo*. The same trends were observed in the *in vitro* experiments i.e., it reduces chromosomal aberrations to 47.50% for 24 h and 36.32% and 42.65% for 48 and 72 h respectively; however when experiments were carried out in the presence of the liver S_9 fraction, these values are 52.38%, 44.56% and 48.35% respectively, being significant at <0.05 level, likewise it also reduces sister chromatid exchange from 14.86 to 8.84 per cell and enhances the replication index from 1.45 to 1.64.

Alcoholic extracts of *A. bisporus* significantly reduce the number of aberrant cells and the frequency of aberrations per cell at each concentration and duration of exposure *in vivo*; similarly it reduces chromosomal aberrations and sister chromatid exchange but enhances the replication index *in vitro*, both of which are statistically significant at <0.05 level.

Conflict of interest

The authors declare that there is no conflict of interest.

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