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White mushroom *Agaricus bisporus* extract inhibits oxidative DNA damage in human lymphocytes as assessed by comet assay

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Abstract

White mushroom *Agaricus bisporus*, one of the many known medicinal mushrooms, *Agaricus bisporus* contains antioxidant activities. The antioxidant effects of the mushroom may be partly explained by protection of cell components against free radicals. We evaluated the effect of aqueous *Agaricus bisporus* extracts for their potential for protecting against oxidative damage to DNA in human lymphocytes. Cells were pretreated with various concentrations (10, 50, 100 and 500 μg/mL) of the extract (Sclerotium and Mycelium) for 1 h at 37 ^{o}C. Cells were then treated with 100 μM of H_2O_2 for 5 min as an oxidative stress. Evaluation of oxidative damage was performed using single-cell gel electrophoresis for DNA fragmentation (Comet assay). Using image analysis, the degree of DNA damage was evaluated as the DNA tail moment. Cells pretreated with *Agaricus bisporus* extract showed over 30% reduction in DNA fragmentation compared with the positive control (100 μmol H_2O_2 treatment). Thus, *Agaricus bisporus* treatment affords cellular protection against endogenous DNA damage produced by H_2O_2.

Key words: White mushroom, *Agaricus bisporus*, comet assay, human lymphocyte, DNA damage.

Introduction

White mushroom *Agaricus bisporus* is one of the many known medicinal mushrooms, *Agaricus bisporus* is high nutritive value (Chen *et al*., 2006), also contains sodium, potassium, and phosphorus (Table 1), conjugated linoleic acid Protocatechuic acid and pyrocatechol (Delsignore *et al*., 1997), and antioxidants (Shi *et al*., 2002) *Agaricus bisporus* extracts have immunomodulatory activities *in vivo*, (Ren *et al*., 2008 and Dayong *et al*., 2007) and activity against several cancer cell lines (Lugang *et al*. 1997). A 2009 case control study of 2.018 women correlated a large decrease of breast cancer incidence in women who consumed mushrooms. Women in the study who consumed fresh mushrooms daily were 64% less likely to develop breast cancer, while those that combined a mushroom diet with regular green tea consumption reduced their risk of breast cancer by nearly 90% (Zhang *et al*., 2009) earlier researches indicating that the common mushroom can inhibit aromatase and therefore may be able to lower estrogen levels in the human body (Chen *et al*., 2006), which might reduce breast cancer susceptibility (Grube *et al.* 2001). A report suggested that a hot water extract of chaga (*Inonotus obliquus*) mushrooms might suppress cellular proliferation in a time-depended manner in human stomach cancer cell lines (Hwang *et al.,* 2003) using the aems test, also showed strong anti-mutagenic and anti-cytotoxic effects of ethanol extract , ethyl acetate fraction, water fraction and other sub-fractions from (*Inonotus obliquus*) (Ham *et al.* 2003; Ham *et al.* 2003). It is widely believed that oxidative nuclear DNA damage over the human lifespan contributes significantly to the age-related development of major cancers, such as those of the colon, prostate and breast. The presence of DNA damage has thus become one of the most sensitive biological markers for evaluating the oxidative stresses resulting from the imbalance between free radical generation and control through antioxidant system. The single cell gel electrophoresis assay (also known as comet assay) is an uncomplicated and sensitive technique for the detection of DNA damage at the level of the individual eukaryotic

Table (1): Nutritional value of White mushroom *Agaricus bisporus*.

Nutritional value per 100 g (3.5 oz)			
Energy 93 kJ (22 kcal)			
Carbohydrates	3.26 g	Folate (vit. B_9)	17 µg (4%)
Sugars	1.98 g	Vitamin B_{12}	0.04 µg (2%)
Dietary fibers	1 g	Vitamin C	2.1 mg (3%)
Fat	0.34 g	Vitamin D	0.2 µg (1%)
Protein	3.09 g	Iron	0.5 mg (4%)
Water	92.45 g	Magnesium	9 mg (3%)
Thiamine (vit. B_1)	0.08mg(7%)	Phosphorus	86 mg (12%)
Riboflavin (vit. B_2)	0.402 mg (34%)	Potassium	318 mg (7%)
Niacin (vit. B_3)	3.607 mg (24%)	Sodium	3 mg (0%)
Pantothenic acid (B_5)	1.497 mg (30%)	Zinc	0.52 mg (5%)
Vitamin B_6	0.104 mg (8%)	Vitamin B_6	0.104 mg (8%)

cell It has since increased in popularity as a standard technique for evaluation of DNA damage/repair, biomonitoring and genotoxicity testing (Tice, 2000). The aim of this work was to evaluate the effect of *Agaricus bisporus* extracts on the potential for protection against oxidative damage to DNA produced by H_2O_2 treatment in human lymphocytes using comet assay technique.

Materials and Methods

Sample preparation: White mushrooms *Agaricus bisporus* were directly obtained from different local market from Baghdad.

Cell preparation and treatment: Lymphocyte isolation for the comet assay was performed using Histopaque 1077-1 (Sigma Chemical), followed by a brief wash with phosphate buffered saline (PBS). Cells were pretreated with various concentrations of sclerotium or mycelium extracts (10, 50, 100, 500 μg/mL). Cells were harvested and then treated with 100 μM of H_2O_2 for 5 min to induce oxidative stress.

Alkaline single cell gel electrophorsis (comet assay): The comet assay was performed under alkaline conditions. essentially according to the procedure described by (Singh *et al.,* 1988) with a slight modification (Avishai *et al.,* 2003). Briefly, a fully frosted microscope slide glass was coated with 1% (w/v) normal melting - point agarose, and the coverslip and stored at 4C for 10 min to solidify. After removing the coverslip, a mixture of 100 μ L whole blood and 100 μ L of 0.5 (w/v) low melting point agarose was rapidly overlaid to solidify at 4°C for 10 min. After removing the coverslip, each slid was immersed in a lysing solution containing 2.5 M NaCl, 0.1 M EDTA, 10mM Tris-HCl buffer (pH 10.0), 1% (w/v) Triton X-100, and 10% (w/v) dimethylsulfoxide at 4°C for 2h. The glass slides were then kept at 4°C for 40 min in an electrophoresis buffer (0.3 M NaCl, 1 mM EDTA) to allow DNA unwinding and to express alkali-lable site. electrophoresis were performed at 18 V for 30 min in the same buffer. Glass slides were neutralized by washing three times with 0.4 M Tris-HCl buffer (pH 7.5) and stained with 50 μ L ethedium bromide. Then the slides were immersed in anhydrous alcohol for 1h, then dried.

Analysis: Endogenous lymphocyte DNA damage was analyzed using the alkaline Comet assay (single-cell gel electrophoresis) with little modification. Images of 100 randomly selected cells (50 cells from each of two replicate slides) were analyzed. Measurements of DNA density were performed using image analysis(comet score™) determining tail moment (TM, calculated as the percentage of total cellular DNA in the tail and as tail length) and relative DNA damage scores were calculated as TM values normalized against positive controls treated with 100 μM H_2O_2 or against negative untreated controls.

Results and Discussion

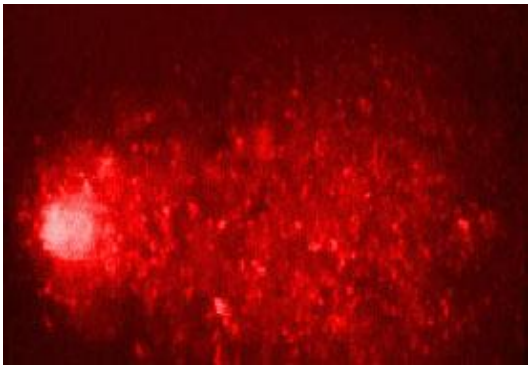
Table (2) showed the inhibitory effect of *Agaricus bisporus* extracts expressed as relative TM scores of DNA damage Figures (1, 2 and 3). Pretreatment with sclerotium extracts at 10, 50, 100 and 500 µg \mL reduced the degree of 60%, 73%, 64% and 38%, respectively compared with the positive controls. Similarly, pretreatment with mycelium extracts at 10, 50, 100 and 500 µg /mL reduced the degree of DNA damage by 71%, 68%, 58% and 30%, respectively (Table 3).

Table (2): Protection of *Agaricus bisporus* extracts of sclerotium on oxidative DNA damage in Human lymphocytes induced by H_2O_2 treatment.

Treatment	DNA damage (%)	Significant differences* at (p< 0.05)
Negative control (DW)	0	a
Positive control (100 µl /mL H_2O_2)	100	b
Sclerotium µg /mL		
10	60	c
50	73	d
100	64	cd
500	38	e

*Different letters represented Significant differences at (p< 0.05) by ANOVA.

Figure (1): Negative Control (Normal cell)



Figure(2): Positive Control (100 µ /mL H_2O_2)

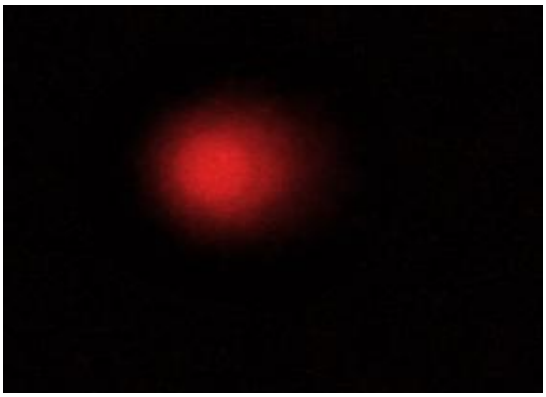


Figure (3): 500 µg /mL of Sclerotium.

Table (3): Protection of *Agaricus bisporus* extracts of Mycelium on oxidative DNA damage in human lymphocytes induced by H_2O_2 treatment.

Treatment	DNA damage (%)	Significant differences* at (p< 0.05)
Negative control (D.W)	0	a
Positive control (100 µl /mL H_2O_2)	100	b
Mycelium µg /ml		
10	70	c
50	68	c
100	58	d
500	30	e

*Different letters represented Significant differences at (p< 0.05) by ANOVA.

In of antimicrobial against tested organisms and a validation of its antioxidant activity. Some of the bioactive components as revealed in this research, like catechin had been labeled as anticancer, thus the possibility of *A. bisporus* as anticancer (Abah and Abha, 2010). The antioxidant activity of *A. bisporus* methanolic extract was also due to these bioactive compounds as most of them exhibited both antimicrobial and antioxidant activity. Flavonoid and phenolic compounds are potent water soluble and free radical scavenger which

prevent oxidative cell damage (Del- Rio and Ortuno, 1997; Okwu, 2004; Marja and Anu, 1999). Presence of ascorbic acid and phenolic compound in A. bisporus comfirms its antioxidant activity (Niki and Mino, 1994).

One of studies demonstrate that chaga *(Inonotus obliquus*) mushrooms extracts afford protection against cellular DNA damage produced by H_2O_2 in healthy human lymphocytes at levels similar to those provided by other known antioxidants (Yoo Kyoung *et al.,* 2004).

Our observations here suggest that (*Agaricus bisporus*) mushroom extracts might represent a valuable source of biologically active compounds with potential for protecting cellular DNA from oxidative damage *in vitro*. Understanding the chemical properties of the extracts as well as the mechanisms by which they might effect such protection are thus important issues and deserve further study.

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