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Original Article

Therapeutic potential and structural elucidation of a water-soluble polysaccharide of a wild edible mushroom *Agaricus bisporus* against neurodegenerative disease, Alzheimer

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ABSTRACT

Water-soluble polysaccharide **(AB)** is soluble polysaccharides extracted by hot water from *Agaricus bisporus*. Its composition was analyzed by HPLC, IR spectroscopy, and gel permeation chromatography (GPC). The results indicated that **AB** was composed of glucose and galactose in molar ratio of 6:1 with molecular weight of 19.4 KDa. AB showed potent antioxidant effect in an *in-vitro* assay. It was powerful as radical scavenger, reducing agent, metal chelator and inhibitor for lipid peroxidation. AB has been tested on Alzheimer's disease (AD) in particular on its antioxidant potential and on AD brain in $AlCl_3$ modal. Rats were divided into four groups (n=15), control, $AlCl_3$ intoxicant treated with **AB**. Brain tissue was separated for enzymes assays including superoxide dismutase (SOD), catalase (CAT), TBARS level as an index of malondialdehyde (MDA), cholinergic markers and apoptotic. **AB** treated AD $ALCl_3$ intoxication of CAT and SOD activity, total antioxidant capacity and anti-apoptotic factor (Bcl-2 and BDNF) with enhancing cholenergic biomarker acetylcholine concentration and inhibited acetylcholine esterase activity however it reduced oxidative stress biomarkers (malondialdehyde, hydrogen peroxide and nitric oxide).

Key words: *Agaricus bisporus*, soluble polysaccharides, structural elucidation, antioxidant, Alzheimer

INTRODUCTION

Alzheimer's disease is a neurodegenerative disease that is found in many people over the age of 65 and predicted to become an epidemic for Baby Boomers. Cognitive deterioration and declining activities in daily living are two of the major signs of Alzheimer's disease. Many polysaccharides though have regenerative qualities and function as memory and learning enhancers. No significant evidence proving that they specifically help Alzheimer's disease has been proven [1]. In the last years, the search for natural antioxidant compounds has gained considerable attention and the number of publications on antioxidants and oxidative stress has nearly quadrupled. Antioxidants compounds play a role in a wide range of common diseases and age-related degenerative conditions. These include cardiovascular disease, inflammatory conditions and neurodegenerative disease such as Alzheimer's disease, mutations and cancer [1, 2]. The main claims in favor of adding polysaccharide supplements to the body seem to be that they aid in reducing inflammation, resisting infection by boosting the immune system, and (of most interest in regards to Alzheimer's) in enhancing memory. In recent years, polysaccharides from the fruit bodies of mushroom have drawn a great deal of attention in the area of biochemistry and pharmaceutical science due to their broad spectrum of therapeutic properties, including immune-stimulatory, anti-tumor, anti-inflammatory, antifungal, antidiabetic, antioxidant, and free radical scavenging as well [3-6]. There are many varieties of mushrooms species; of which *Pleurotus* are characterized by a white spore print, attached to the gills, often with an essentric stip, or no stip at all, and they are commonly known as Oyster mushrooms [7]. Some of these natural polymers have been successfully used in clinical oncology to increase the effectiveness of chemotherapeutic preparations and reduce their side effects, such as Lentinan from *Lentinus edodes*, Schizophyllan from *Schizophyllum commune* and krestin from *Coriolus versicolor*. Large amount of experimental and clinical trials demonstrated that these mushroom derived polysaccharides could prolong the survival and improve the quality of life for cancer patients [8]. Therefore, discovering novel structurally and biologically polysaccharides from mushroom,

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especially those unexploited species has become a hot spot of great interest. However, to our best knowledge, there is no publication documented about the structure characterization of the polysaccharide up to now. In the course of our seeking bioactive polysaccharides from the natural resources [9, 10], the fruit bodies of *S. asparatus* was investigated chemically and biologically. Herein, we describe the isolation, structural elucidation and immunological activity of a water-soluble polysaccharide from the fruit bodies of S. *aspratus*. The effects of extracts from Pleurotus species against some pathogenic organisms and inflammation have been widely reported by researchers [11-14]. However, no report is available on the antimicrobial and anti-inflammatory effects of polysaccharides from *P. pulmonarius.* The present study was carried out in an attempt to investigate the potential of polysaccharides from *Agaricus bioporus* act as anti-inflammatory agents do aid in the prevention Alzheimer's disease.

MATERIALS AND METHODS

Extraction and purification of the polysaccharide: Fresh fruit bodies (1 kg) of *Agricus bisporus* collected from Bloshia mushroom Company in Dokki, Egypt. The sample was washed with distilled water and boiled on a water bath for 6 h. The mixture was kept overnight at 4 °C and filtered through fresh linen cloth. The filtrate was centrifuged at 5000 rpm (Sigma-Laborzentrifugen, 2K 215, Sigma Co., and D37520 Osterode-am-Harz, Germany) for 45 min at 4°C. The supernatant solution was collected and the polysaccharide (**CAB**) was precipitated with EtOH (1:5, v/v). After keeping the precipitated material in the mixture overnight at 4 °C, it was centrifuged at 4 °C for 1h, and then the residue was freeze-dried (1.5 g). The dried material was dissolved in 4% NaOH solution and re-precipitated with ethanol. The re-precipitated material was collected through centrifugation and dissolved in minimum volume of water. The solution was then dialyzed against distilled water for 30 h (3×1000 mL) to remove alkali and low molecular weight materials. During this dialysis one portion got precipitated from this solution. The whole dialyzed solution was centrifuged at 8000 rpm at 4°C. The water soluble (**AB**) and insoluble (**ABI**) parts were lyophilized separately [15].

Analysis of monosaccharide composition: The polysaccharide (0.1 g) was hydrolyzed with 90% formic acid at 100°C in a sealed tube for 5 h. Excess acid was removed by flash evaporation on a water bath at a temperature of 40°C and co-distilled with water (3×1 mL) [16]. The monosaccharides contents were quantified by HPLC on a Shimadzu Shim-Pack SCR-101N column (7.9 mm × 30 cm), using deionized water as the mobile phase (flow rate 0.5 mL/min), as described by El-Sayed *et al.* [17].

Molecular weight determination: The molecular weight of the polysaccharide was determined by gel permeation chromatography (GPC) on Agilent 1100 series, Germany, Detector: Refractive Index FPlgel particle size (5μm), 3 columns of pore type (100, 104, 105 A°) on series, length 7.5 × 300 mm (1000, 5000000) For DMF solvent Styrogel HR-DMF, 3 μm (7.8 × 300 mm), Water Company Ireland. One column (5000 - 600000) for water solvent (polyethylene oxide/glycol standard) PL aquagel-OH 7.5 mm and 30um pore type 8um particle size. PL aquagel-OH 7.5 mm, 50 um pore type, 8um particle size, in series Mw from 100-1250000 g/mol. The sample 0.01 gm was dissolved in 2 ml of solvent, and then it filtrated by siring filter 0.45 then the sample but in GPC device. The polydispersity index calculated from the Mw/Mn ratio [18].

Infrared Spectroscopy: The Fourier-transform infrared (FT-IR) spectrum of the polysaccharide was measured using a Bucker scientific 500-IR Spectrophotometer. The exopolysaccharide was mixed with KBr powder, ground and pressed into a 1 mm pellets for FTIR measurements in the range of 400-4000 Cm^{-1} [19].

***In-vitro* antioxidant studies**

Free radical scavenging effect*:* The free radical scavenging activity of **AB** and standard compounds at different concentrations (75, 150, 300 and 600 μg/ml) was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH•) using the method of Yamaguchi *et al*. [20]. Briefly, 0.1 mM solution of DPPH• in ethanol was prepared. Then, 1 ml of this solution was added to 3 ml of samples and standards solution Vitamin C (VC) and Butylated hydroxy-toluene (BHT) at the concentrations polysac-charide. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm in a spectrophotometer (Schimadzu UV/VIS-240IPC). Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The DPPH• radical concentration in the reaction medium was calculated from the following equation: DPPH• scavenging effect (%) = $100 - [(A_0\text{-}A_1)/A_0) \times 100]$, Where A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the sample of polysaccharide [21].

Reduction capability: The reduction capability of **AB** and standards was determined according to the method of Oyaizu [22]. The different concent-

rations (75, 150, 300 and 600 µg/ml) in 1ml of methanol were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of TCA (10%) was added to the mixture, which was then centrifuged for 10 min at 1000 *xg* (MSE Mistral 2000, UK, and Serial No.: S693/02/444). The upper layer of solution (2.5 ml) was mixed with methanol (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm in a spectrophotometer. VC and BHT were used as controls. Higher absorbance of the reaction mixture was indicated greater reducing power.

Metal chelating effect: The chelating of ferrous ions by the **AB** and standards was estimated by the method of Dinis *et al.* [23]. Briefly, **AB** and standards (75, 150, 300 and 600 µg/ml) were added to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml) and the mixture was shaken vigorously and left standing at room temperature for ten minutes. After the mixture had reached equilibrium, the absorbance of the solution was then measured spectrophotometrically at 562 nm in a spectrophotometer. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was given by the formula: Inhibition (%) = $[(A_0-A_1)/A_0] \times 100$, Where A_0 was the absorbance of the control, and A_1 was the absorbance in the presence of the sample and standards. The control contains $FeCl_2$ and ferrozine [24].

Total antioxidant capacity: Total antioxidant capacity was measured according to the method described by Miller and Rice-Evans [25] and Arnao *et al.* [26]. Exactly 0.2 ml of peroxidase (4.4U/ ml), 0.2 ml of H_2O_2 (50 µM), 0.2 ml of ABTS (2, 2-azino-bis (3-ethylbenz-thiazoline-6-sulfonic cid, diammonium salt, 100 µM) and 1 ml methanol were mixed, and were kept in the dark for 1 h to form a bluish green complex after adding of 1 ml **AB** of different concentrations or VC and BHT, used as a control. All were tested in triplicates. The absorbance at 734 nm was measured to represent the total antioxidant capacity and then was calculated as follows: Total antioxidant activity (%) = $[1- (A_{sample}/A_{control})] \times 100$.

Lipid peroxidation by lionleic assay: Inhibition of lipid peroxidation by **AB** and standards was determined according to the method of Gulcin *et al.* [27], with some modifications. A pre emulsion was prepared by mixing 175 µg Tween 20, 155 µl linoleic acid, and 0.04 M potassium phosphate buffer (pH 7.0). A 1 ml of sample in 99.5% ethanol was mixed with 4.1 ml linoleic emulsion, 0.02 M phosphate buffer (pH 7.8) and distilled water. The mixed solutions of all samples (21 ml) were incubated in screw cap-tubes under dark conditions at 40°C at certain time intervals. To 0.1 ml of this mixture was pipetted and added with 9.7 ml of 75% and 0.1 ml of 30% ammonium thiocyanate sequentially. After 3 min, 0.1 ml of 0.02 M ferrous chloride in 3.5% HCl was added to the reaction mixture. The peroxide level was determined by reading daily of the absorbance at 500 nm in a spectrophotometer. The same assay for VC and BHT was also determined for comparison. All test data was the average of three replicate analyses. The inhibition of lipid peroxidation percentage was calculated by the following equation: Inhibition (%) = $[(A_0 - A_1) /A_0] \times 100$, Where A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of extracts or standard compounds.

***In-vivo* study**

Anti-Alzheimer experiment: Adult male *sapargue dawely* rats from animal house National Research Centre weighing between 150-180g were maintained under normal laboratory conditions and kept in standard polypropylene cages at room temperature of 25-30°C, 60 to 65% relative humidity and provided with standard diet and water *ad libitum.* Four groups each of fifteen rats were used and treated as follows;

Group I: received the vehicle (saline solution) orally and it was served as negative control group for 135 days.

Group II: received $ALCl_3$ orally (4.2 mg/kg body weight /45 days), serves as positive control group.

Group III: received mushroom polysaccharide (**AB**) (100 mg/kg body weight/90 days) orally and served as positive control group.

Group IV: received $ALCl_3$ (4.2 mg/kg body weight/45 days) then treated with **AB** (100 mg/kg body weight /90 days).

At the end of the experiment, rats fasted overnight, were subjected to anesthesia by diethyl ether then, sacrificed. The whole brain of each rat was rapidly dissected and washed with isotonic saline and dried on filter paper. Brain was weighed and homogenized to give 10% (w/v) homogenate in ice cold medium containing 50 mM Tris-HCl and 300 mM sucrose at pH, 7.4 [27]. The homogenate was centrifuged at 4ºC. The supernatant (10%) was stored at -80ºC and were used in biochemical analyses including oxidative stress biomarker (nitric oxide concentration, hydrogen peroxide concentration, glutathione concentration and malondialdehyde concentration), antioxidant status (total antioxidant capacity, superoxide dismutase activity and catalase activity). The reduced glutathione level in the brain tissue was determined according to the method of Griffith [28]. Brain superoxide dismutase (SOD) activity was estimated

by the method of Kakkar *et al.* [29]. Catalase (CAT) activity was measured by following decomposition of H_2O_2 according to the method of Berr's and Sizer [30]. The TBARS level in brain tissue, an index of malondialdehyde (MDA) production was determined by the method of Ohkawa *et al.* [31]. Cholinergic markers (choline esterase and acetylcholine esterase activities) (kits were purchased from Quimica Clinica Aplicada S.A.) as well as anti-apoptotic marker β cell lymphoma 2 (Bcl-2) and brain derived neurotrophic factor (BDNF) (kits were purchased from Glory science Co., Ltd, USA) were determined in brain tissue. Brain total protein concentration was measured for calculation of enzyme specific activity [32]. The assessment was done by ELISA reader (Dynatech laboratories MRW microplate reader, 2CXB2445) the sensitivity of assay was 20 pg/ml. Oxidative stress biomarker and antioxidant status were determined by colorimetric method using kits purchased from Biodiagnostic Co., Cairo, Egypt.

Statistical Analysis: In all cases analyses each sample were performed in triplicate and data were averaged over the three measurements. The standard deviation (SD) was also calculated. Data were treated for multiple comparisons by analysis of variance (ANOVA) using SPSS program, version 9.05, followed by the least significance difference at level $P<0.05$. IC_{50} values were calculated for the *in-vitro* tests.

RESULTS

Chemical analysis of fresh mushroom: Moisture contents of the fruit bodies was 7.2% and the crude protein levels of fresh was remarkable 27.3% the crude fat contents of sample was 3.8% The ash content of the fruit bodies was significantly 9.3% while total sugars content were 43% **(Table 1).**

Table (1). Chemical composition of mushroom

Components	(%)
Moisture	7.2 ± 0.15
Ash	9.3 ± 0.13
Lipids	3.8 ± 0.02
Proteins	27.3 ± 0.21
Total sugars	43.0 ± 0.23
Reducing sugars	3.15± 0.23

Data are presented as mean of triplicates ±SD

Extraction and purification: One kilogram of fresh mushroom fruit body's *A. bisporus* was washed thoroughly with distilled water and then boiled with water for 6 h followed by centrifugation, precipitation in EtOH and freeze drying to yield 1.5 g crude polysaccharide (**CAB**). On treatment with 4% NaOH followed by centrifugation and dialysis the crude polysaccharide yielded two fractions, water soluble (**AB**) 98.7% and insoluble (**ABI**) 1.3%. **AB** was further purified by using gel permeation chromatography. **AB** was further dissolved in 4% NaOH solution and insoluble material was removed by centrifugation. The filtrate was dialyzed several times against distilled water followed by precipitation, centrifugation and freeze drying to yield pure **AB**. Now, the structure of the **AB** was analyzed in the following way.

Analysis of monosaccharide composition of AB: The semi-purified polysaccharide (**AB**) was analyzed for monosaccharide composition. The monosaccharide composition was analyzed by HPLC. The main monosaccharides of the **AB** are glucose and galactose in a molar ratio of 6:1, respectively.

Structural Characterization of AB: The bands in the region of 3463.53 cm^{-1} were due to the hydroxyl stretching vibration of the polysaccharides. The bands in the region of 2927.41 cm^{-1} were due to C–H stretching vibration, and the bands in the region of 1664.27 cm^{-1} were due to associated water. Moreover, the characteristic absorptions at 833.09 cm^{-1} in the IR spectra indicated that α-configurations were simultaneously present in **AB**. The actual molecular weight and distribution of the AB were determined by gel permeation chromatography (GPC). The **AB** polysaccharide in the GPC chromatogram **(Figure 1)** was widely dispersed molecules polydispersity index of 1.79 and had an overall weight average molecular weight (Mw) of 2.08 $\times 10^6$ g/mol and number average molecular weight (Mn) of 1.61 $\times$ 10^6 g/mol.

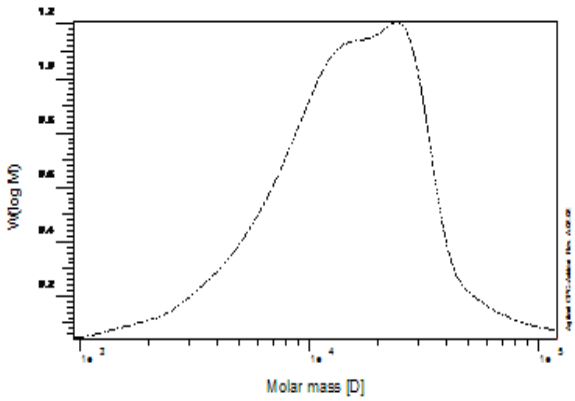

Figure (1). Molecular weight distributions of *A. bisporus* polysaccharide (AB)

In-vitro antioxidant activity: To evaluate the antioxidant properties of both fractions, five different assays were carried out: scavenging activity on DPPH• radicals, reducing power, metal chelation, total antioxidant activity and inhibition of lipid peroxidation. Mushroom polysaccharide

showed potent radical scavenging effect when tested with DPPH• radicals (IC_{50}= 79.34 µg/ml) but VC and BHT remained the best effectors. **AB** and standards was arranged in the following order; VC>BHT> AB **Table (2).** The effect of **AB** as reducing agent was determined by transformation of potassium ferricyanide into potassium ferrocyanide in the presence of **AB**. **AB** produced a valuable reduction capability (IC_{50}= 200 µg/ ml) which is near to BHT effect (150 µg/ ml) while the potent reducible effect was observed with VC (130 µg/ ml). **AB** showed weak metal chelating effect (300 µg/ ml) as compared to VC (80.76 µg/ ml) and BHT (77.91 µg/ ml). **AB** produced high antioxidant capacity (IC_{50}= 85 µg/ ml). The effect of **AB** as radical scavenger and reducing gent as well as metal chelator was accompanied with inhibition of lipid peroxidation more than level of standard drugs, VC and BHT. **AB** significantly inhibited lipid peroxidation (IC_{50}= 84.33 µg/ ml) while VC and BHT showed nearly the same effect IC_{50}= 82.79 µg/ml and IC_{50}= 82.56 µg/ ml, respectively)**.**

Table (2). Antioxidant activity of Mushroom polysaccharide and reference materials

Sample	IC_{50} (µg/ml)				
	DPPH scavenging	Reductive capability	Metal ion chelation	Total antioxidant capacity	Lipid peroxidation inhibition
AB	79.34±1.04^{ab}	200±2.03^{ab}	300 ± 1.67^{ab}	50 ± 2.17^{ab}	84.33 ±2.18^{ab}
VC	76.25 ±1.51^{b}	130 ± 2.00^{b}	80.76±1.93^{b}	77.48 ±2.08^{b}	82.79 ± 2.64^{b}
BHT	80.29 ±1.43^{a}	150 ± 1.94^{a}	77.91±2.06^{a}	75.35±1.76^{a}	82.56 ± 1.04^{a}

Data are presented as IC_{50} ± SD. a, significant as compared to VC while b, significant to BHT

Anti-Alzheimer effect of AB: Most of polysaccharides purified from medicinal herbs showed anti-tumor, antioxidant and immune-stimulating effects. However, little is known about their effects on Neuroprotection. Malondialdehyde, hydrogen peroxide and nitric oxide concentration were determined in brain tissue as oxidative stress biomarkers. Consumption of $AlCl_3$ for 90 days significantly magnified MDA by 173 % and H_2O_2 by more than 100% while nitric oxide was highly produced in brain tissue to be more than control group by 383% **(Table 3).** On the other hand, treating animals with mushroom polysaccharide only as positive control significantly ($P< 0.05$) decreased MDA, H_2O_2 and nitric oxide production by 11.92%, 19.34 and 5.76%, respectively as compared to negative control. Treating Al-intoxicant group with **AB** at 0.10% of LD_{50} significantly reduced the elevation in oxidative stress parameters produced by $AlCl_3$. **AB** reduced MDA concentration by 38.63% as compared to Al-intoxicated group also H_2O_2 was decreased by nearly the same percentage (39.2%) while the maximum reduction was recorded with nitric oxide concentration, reduction percentage= 73.67%. The reduction in determined oxidative stress parameter was accompanied with significant increments in brain antioxidant parameters. Oral administration of **AB** significantly induced catalase activity (6.73U/mg protein) with significant induction in SOD activity (5.14 U/mg protein) more than catalase. These increments in enzyme activation reflected on total antioxidant capacity which increased to be 15.44 mmol/mg protein **(Table 4).**

Table (3). Effect of AB on brain oxidative stress biomarkers in normal and AL-intoxicant groups

Groups	Malondialdehyde (nmol/mg protein)	Hydrogen peroxide (nmol/mg protein)	Nitric oxide (µmol/mg protein)
Negative control	4.11 ± 1.37^{b}	4.86 ± 2.16^{b}	1.91 ± 1.13^{b}
Positive control (AB group)	3.62 ± 2.17^{a} (11.92%) ↓	3.92 ± 2.70^{a} (19.34%) ↓	1.80 ± 1.66^{a} (5.76%) ↓
$AlCl_3$-intoxicant group	11.26 ± 2.63^{a} (173%) ↑	10.41 ± 1.97^{a} (114.19%) ↑	9.23 ± 1.52^{a} (383.24%) ↑
$AlCl_3$-intoxicant treated with AB	6.91 ± 1.94^{ab} (38.63%) ↓	6.33 ± 2.18^{ab} (39.19%) ↓	2.43 ± 1.47^{ab} (73.67%) ↓

Data are presented as mean of triplicates ±SD followed with inhibition percentage or increment percentage. a, significant change at $P<0.05$ for negative control group. b, significant with Al-intoxicant control group

Table (4). brain antioxidant status as affected with mushroom polysaccharide (AB)

Groups	Catalase activity (U/mg protein)	Superoxide dismutase activity (U/mg protein)	Total antioxidant capacity (mmol/mg protein)
Negative control	5.46 ± 0.65 [b]	3.11 ± 0.91 [b]	12.46 ± 1.22 [b]
Positive control (AB group)	6.78 ± 0.78[a] (24.18%) ↑	5.14 ± 0.65[a] (65.27%) ↑	15.44 ± 1.05[b] (23.91%) ↑
$AlCl_3$-intoxicant group	3.26 ± 2.11[a] (40.29%) ↓	2.81 ± 0.99[a] (9.64%) ↓	6.48 ± 1.94[a] (47.99%) ↓
$AlCl_3$-intoxicant treated with AB	4.51± 1.42[ab] (38.34%) ↑	3.17 ± 1.01[ab] (12.81%) ↑	10.58 ± 1.03[ab] (63.27%) ↑

Data are presented as mean of triplicates ± SD. a, significant change at *P<0.05* for negative control group. b, significant with Al-intoxicant control group. Percentages were calculated as compared to negative control for positive control and intoxicant while the treated was compared to intoxicant group.

Consumption of $AlCl_3$ showed adverse effect on CAT and SOD activities to be 3.26 and 2.81U/mg protein, respectively. However, treating AL-intoxicant for 90 days significantly increased CAT and SOD activities to be nearly negative control group (4.51 and 3.17 U/mg protein, respectively) with enhanced the total antioxidant capacity (10.58 mmol/mg protein). Brain anti-apoptotic factor, Brain derived neurotrophic factor and acetylcholine biomarkers were determined as a marker for brain activity. Induction of brain intoxication, AD disease, was carried out by $AlCl_3$ for 45 days which significantly (P<0.05) decreased Bcl-2 level (32.46 ng/mg protein) in brain tissue with significant reduction in BDNF concentration (1.08 $\times 10^{-2}$ pg/mg protein). These adverse effects were accompanied with significant increment in acetyl-choline level (9.77 $\times$ 10^{-2} nmol/mg protein) and reduction of acetylcholine esterase activity (316.21 U/mg protein) **(Table 5).** On the other hand, **AB** without brain intoxication significantly increased Bcl-2, BDNF and ACh levels (89.15 ng/mg protein, 2.44 $\times$ 10^{-1} pg/mg protein and 9.77 $\times 10^{-2}$ nmol/mg protein, respectively) with significant reduction on acetylcholine esterase activity (316.21 U/mg protein) **(Table 5).** Administration of **AB** after $AlCl_3$ intoxication significantly increased Bcl-2, BDNF, Ach concentrations in brain tissue as compared to intoxicant group (55.31 ng/mg protein, 1.41×10^{-1} pg/mg protein, 7.15×10^{-2} nmol/ mg protein and 631.55 U/mg protein, respectively).

Table (5). Effect of mushroom polysaccharide (AB) on brain anti-apoptotic factor, Brain derived neurotrophic factor and acetylcholine biomarkers

Groups	Bcl-2 (ng/mg protein)	BDNF (pg/mg protein)	ACh (nmol/mg protein)	AChE (U/mg protein)
Negative control	73.32 ± 1.27 [b]	1.54×10^{-1} ± 1.45[b]	7.89×10^{-2} ± 3.11[b]	498.56 ± 2.27[b]
Positive control (AB group)	89.15 ± 2.01[a] (21.59%) ↑	2.44×10^{-1} ± 1.33[a] (58.44%) ↑	9.77×10^{-2} ± 2.43[a] (23.3%) ↑	316.21 ± 2.38[a] (36.58%) ↓
$AlCl_3$-intoxicant group	32.46 ± 2.41[a] (55.72%) ↓	1.08×10^{-2} ± 2.41[a] (92.98%) ↓	5.76×10^{-2} ± 1.53[a] (26.99%) ↓	915.34 ± 3.41[a] (83.59%) ↑
$AlCl_3$-intoxicant treated with AB	55.31 ± 1.38[ab] (70.39%) ↑	1.41×10^{-1} ± 1.79[ab] (120%) ↑	7.15×10^{-2} ±1.76[ab] (24.13%) ↑	631.55 ± 2.15[ab] (31.01%) ↓

Data are presented as mean of triplicates ± SD. a, significant change at *P<0.05* for negative control group. b, significant with Al-intoxicant control group. Percentages were calculated as compared to negative control for positive control and intoxicant while the treated was compared to intoxicant group.

DISCUSSION

Basidiomycetes present different kinds of glucans and heteropolysaccharides. The common monosaccharide composition of these polysaccharides is glucose, galactose, xylose, mannose and fucose. Generally (1→3), (1→6)-β-glucans are extracted from these organisms, and also galactomannans, heteroglycans, and fucogalactans [33, 34]. Mushrooms included in the same genera show more similarities in their composition, including the structure of carbohydrates [35]. Mushroom polysaccharide has traditionally been used for the prevention and treatment of a multitude of disorders like infectious illnesses, cancers and various autoimmune diseases. Many polysaccharides though have regenerative qualities and function as memory and learning enhancers. No significant evidence proving that they specifically help Alzheimer's disease has been proven. However, research is still on-going so there is still a lot of promise in the area of polysaccharides being able to reverse some of the effects of Alzheimer's disease [33]. The downside to glucose though, is the fact that too much can raise insulin levels and result in obesity or diabetes. With that being said, studies have shown that glucose is vital to brain function and is often disturbed in those with depression, anorexia, and bulimia. Alzheimer's disease patients have also registered much lower glucose levels than those with other forms of brain malfunction. Galactose or mannose is another polysaccharide that is a particularly powerful anti-inflammatory agent when paired with glucose [34,35]. However, when paired with fucose, mannose becomes a super machine often helping to eliminate any inflammation completely and repair any tissue damage. The molecular weight of the polysaccharide in extract was 2.08×10^6 g/mol and the average molecular weight of mushroom suggested that the molecular weights of the polysaccharides may play an important role on their antioxidant activity. A relatively higher molecular weight of the cherry and cranberry F-4 fractions may have resulted in an increase in the antioxidant activity of these fractions. Polysaccharides was about 56.6 kDa, a value very similar to that of reported by Huiping et al. [36]. Free radicals and other reactive oxygen species are considered to be important causative factors in the development of diseases of aging such as neurodegenerative diseases, cancer and cardiovascular diseases. This relationship has led to considerable interest in assessing the antioxidant capacity of foods, botanicals and other nutritional antioxidant supplements [35, 37]. As plants produce significant amount of antioxidants to prevent the oxidative stress caused by photons and oxygen, they represent a potential source of new compounds with antioxidant activity. Thus, continued research is being undertaken all over the world on different plant species and their therapeutic principles [37]. Since a large amount of evidence demonstrates that oxidative stress is intimately involved in age-related neurodegenerative diseases, there have been a great number of studies which have examined the positive benefits of antioxidants to reduce or to block neuronal death occurring in the pathophysiology of these disorders [38]. In order to evaluate the ability of the polysaccharide to serve as antioxidants, three activities were measured its ability to scavenge DPPH$^\bullet$, reduction capability and metal ion chelation effect as well as total antioxidant capacity by ABTS scavenging ability. **AB** showed high scavenging effect against DPPH$^\bullet$ radicals which is near to BHT while it showed minimum effect as scavenger for ABTS radicals as compared to standards. Reduction capability of **AB** had the same trend with weak metal chelator effect. Although **AB** had lower effects than standards it produced nearly the lipid peroxidation inhibition effect which may attributed to other antioxidant effects. Oxidative stress accompanies with pathological changes in AD and is considered to be a crucial upstream factor in the pathogenesis of the disease [39]. Products of free radical damage, such as aldehydes or lipid hydroperoxides, may diffuse into the blood where they can be detected. Moreover, it has been found that blood-brain barrier (BBB) permeability significantly increases in both AD and vascular dementia as compared with ageing controls [40, 41]. Consequently, products of oxidative stress represent potential biomarkers in blood for diagnosis of AD. Oxidative stress is present in AD as a result of amyloid beta (Ab) misfolding, which is accompanied by the activation of microglia. The enzyme NADPH oxidase, localized in the microglia membranes, is activated in the brains of AD patients resulting in the production of free radicals [42, 43]. Furthermore, lipid peroxidation occurs before the formation of amyloid-β plaques in mouse models of AD [44]. These findings support the hypothesis that free radical damage is present in the brain in the preclinical stage of AD. Consumption of $AlCl_3$ significantly magnified MDA and H_2O_2 with magnification in nitric oxide level in brain tissue while administration of mushroom polysaccharide as positive control significantly decreased MDA, H_2O_2 and nitric oxide production. Treating Al-intoxicant group with MPS at 0.10% of LD_{50} significantly reduced the elevation in oxidative stress parameters produced by $AlCl_3$, MDA, H_2O_2 and nitric oxide concentration to be near control group. As a result of free radical overproduction, there is a reduced content of PUFA in the brain in AD [45] with non-enzymatically decomposition to a number of varying products, such as malondial-

dehyde and 4-hydroxynonenal (4-HNE), ketones, epoxides and hydrocarbons. Increased levels of MDA and 4-HNE in the brain in AD have been confirmed by several studies [46-48]. Aldehydes formed during lipid peroxidation of brain PUFA can diffuse from the primary sites and be used as markers of oxidative stress. MDA arises largely from the peroxidation of PUFA. It exists either in a free form or in bound to proteins. Free MDA in vivo is rapidly metabolized in tissues. A number of studies document elevated levels of MDA in AD [49- 52]. The reduction in determined oxidative stress parameter was accompanied with significant increments in antioxidant parameters. Oral administration of AB after AL-intoxication significantly induced catalase and SOD activities with reduction in MDA. Memory impairment in AD patients is a condition that makes them dependent upon their caregivers. Our data show that mushroom polysaccharide significantly repair the impairment produced in brain tissue by aluminum intoxication. Principal role of acetyl-choline esterase (AChE) is the termination of nerve impulse transmission at the cholinergic synapses by rapid hydrolysis of acetylcholine (ACh). Inhibition of AChE serves as a strategy for the treatment of Alzheimer's disease (AD), senile dementia, ataxia, myasthenia gravis and Parkinson's disease [53-55]. There are a few synthetic medicines, e.g. tacrine, donepezil, and the natural product-based rivastigmine for treatment of cognitive dysfunction and memory loss associated with AD [56]. These compounds have been reported to have their adverse effects including gastrointestinal distur-bances and problems associated with bioavaila-bility [57], which necessitates the interest in finding better AChE inhibitors from natural resources. AD is one of the most common forms of dementia affecting so many elderly people. Besides the neuropathologic hallmarks of this disease, namely neurofibrillary tangles and neuritic plaques, it is characterized neurochemically by a consistent deficit in cholinergic neurotransmission, particul-arly affecting cholinergic neurons in the basal forebrain [58]. The evidence stems from data of several authors that demonstrated the reduction in activity of enzymes involved in the synthesis of acetylcholine, *i.e.* choline acetyl transferase or excess degradation of Ach by AChE [59]. Current clinical strategy is to slow down the progression of deterioration in AD patients by using anti-cholinesterase inhibitors. Intoxication of animals with $AlCl_3$ significantly reduced the acetylcholine concentration in brain tissue with magnification in acetylcholine esterase activity this adverse effect was repaired by administration of MPS. It signify-cantly increased Ach concentration to reach control group with significant inhibition in AchE activity, as compared to intoxicant group. Biochemical Markers of Alzheimer's Disease stated that ideal biomarkers should be noninvasive, simple to perform, inexpensive, reliable, able to detect the neuropathological changes of AD, and validated through confirmed cases. One family of biomarkers that has received a great deal of attention in AD is the neurotrophic factors (NFTs). NFTs are small proteins that play key roles in neuronal survival, axonal guidance, cell morphology, as well as memory formation and cognition [57]. Many neurotrophic factors are synthesized in areas impacted by AD neuropathology early in the course of the disease (e.g. entorhinal cortex, hippocampal formation, amygdala). Additionally, axonal trans-port is essential for NFT signaling, as they are oftentimes synthesized away from their site of action; however, AD and other neurodegenerative dementias are frequently associated with axonal transport failure. Therefore, dysregulation of NFT is expected in neurodegenerative dementias such as AD [60]. Brain-derived neurotrophic factor (BDNF) is one NFT that has been linked with AD. Postmortem studies have documented decreased BDNF, pro-BDNF, and BDNF mRNA levels in brains of patients diagnosed with AD and Mild Cognitive Impairment (MCI) [60, 61]. BDNF is more highly expressed and widely distributed in the brain and its expression and growth promoting actions are critical for survival and plasticity of a variety of neurons throughout the brain, particularly in brain regions heavily affected in AD such as hippocampal, cortical, and cholinergic neurons. Moreover, in cell culture and animal models, functioning of the BDNF signaling pathway has been repeatedly demonstrated to be critical for neuronal differentiation, survival, plasticity, and cognition. Independent lines of evidence suggest that dysfunction in BDNF signaling may contribute to the neurodegeneration in AD. Brain regions associated with reduced BDNF expression are those displaying the highest levels of neurodegeneration (*e.g*. hippocampus) [57].] Paradis *et al.* [62] suggest the possibility that down regulation of Bcl-2 in the presence of amyloid β peptide (AB) renders the cell vulnerable to age-dependent stress. Although there is no overwhelming evidence for apoptotic neurons in AD brains, there are indications that such a mechanism can occur. Bcl-2 immunoreactivity generally is increased in AD neurons but decreased in degenerating neurons [62]. Other evidence of apoptosis in AD brains includes increased apopTag and c-jun immunoreactivity, evidence of DNA fragmentation, and Bcl-2-sensitive apoptosis induced by familial AD mutants of APP [63-66]. Induction of brain intoxication with $ALCl_3$ significantly decreased Bcl-2 level in brain tissue with significant reduction in BDNF concentration. These adverse effects were accompanied with

significant reduction in acetylcholine level and induction of acetylcholine esterase activity. However, Administration of **AB** after $AlCl_3$ intoxication significantly increased Bcl-2, BDNF, Ach concentrations in brain tissue as compared to intoxicant group. Interestingly, memory enhancing effect shared by **AB** in the present study might be due to its properties as antioxidant agent, acetyl-choline esterase inhibitor as well as its effect as BDNF and Bcl-2 inducible agent [67].

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