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Full Length Article

Immunotherapeutic Activities of Mushroom Derived Polysaccharides in Chicken

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Abstract

Mushrooms including *Pleurotus ostreatus* (PO) and *Pleurotus sajor-caju* (PSC) were processed to isolate the polysaccharides through ion exchange (DEAE Cellulose) and size exclusion (Sephadex G-100) chromatography. Monosaccharides including maltose, glucose, mannose and fructose were identified through HPLC. Polysaccharides of PO and PSC were administered in chickens. Lymphoproliferative response to phytohaemagglutinin-P (PHA-P) and antibody response to sheep RBCs were used to demonstrate the cell mediated immunity and humoral immunity in chicken. Polysaccharides purified from PO and PSC showed significantly higher ($P<0.05$) cellular immune response at 48 and 72 h post administration of PHA-P relative to control. Significantly higher antibody titers (total Ig, IgM and IgG) were recorded in polysaccharide extract of PO and PSC at day 7th and 14th post-primary and post-secondary injections of sheep RBCs. Challenged infection of mixed species of genus *Eimeria* revealed significantly higher ($P<0.05$) oocyst count in control group relative to groups administered with polysaccharide extracts of PO and PSC. Moreover, significantly higher ($P<0.05$) per cent protection and protection against coccidial lesions were observed in chickens administered with polysaccharide extracts of PO and PSC. From the results, it was concluded that polysaccharides purified from PO and PSC had the immunotherapeutic potential and could be used in poultry to boost up the immune response in chickens.

Keywords: *Pleurotus ostreatus*; *Pleurotus sajor-caju*; Polysaccharide; Immunomodulatory; Coccidiosis; Chicken

Introduction

Mushrooms belonging to different genera have been recognized well for centuries due to their excellent therapeutic effects against different ailments (Chihara *et al*., 1969). However, modern world is heading towards structural elucidation to isolate the compounds, which are responsible for these therapeutic activities. Metabolites from various species of medicinal mushrooms are used for the treatment of various human diseases including allergic asthma (Li *et al*., 2000; Liu *et al*., 2003), food allergy (Li *et al*., 2001; Hsieh *et al*., 2003), atopic dermatitis (Kuo *et al*., 2002; Babar *et al*., 2012), inflammation (Jose *et al*., 2004; Kim *et al*., 2004), autoimmune joint inflammation such as rheumatoid arthritis (Kim *et al*., 2003a; Kim *et al.*, 2012), atherosclerosis (Bobek and Galbavy, 1999; Yamada *et al*., 2002), hyperglycemia (Gray and Flatt, 1998), thrombosis (Yoon *et al*., 2003), human immunodeficiency virus (HIV) infection (Nanba *et al*., 2000; Ngai and Ng, 2003), listeriosis (Kodama *et al*., 2001), tuberculosis (Markova *et al*., 2003), septic shock (Kim *et al*., 2003b) and cancer (Kodama *et al*., 2003; Ho *et al*., 2004; Nakamura *et al*., 2004; Lee *et al*., 2006).

Among the mushroom species genus *Pleurotus* is worldwide in distribution. These are commonly known as 'OYSTER' mushroom. Most of the species belonging to genus *Pleurotus* are edible and many are produced commercially these days (Santos-Neves *et al*., 2008). They are famous due to increased nutritional properties including high contents of dietary fibers, superior quality of proteins with essential amino acids, minerals and vitamins (Gunde-Cimerman, 1999). *Pleurotus* spp. had been investigated in several studies with noticeable antioxidant, anti-inflammatory and analgesic properties (Bobek and Galbavy, 1999; Smiderle *et al*., 2008). Annually, 900,000 tons of different species of *Pleurotus* have been produced in different parts of the world (Synytsya *et al*., 2009) that showed diverse pharmacological activities (Ragunathan *et al*., 1996). Sulfated β-glucans isolalted from derivatives of *Pleurotus* species showed tremendous activities against herpes simplex virus (Zhang *et al*., 2004) and different tumors (Tao *et al*., 2006). Similarly, α- glucans isolated from *Pleurotus florida* and *Pleurotus ostreatus* showed remarkable macrophage activation and anti-proliferative activity against colonic cancer cells, respectively (Lavi *et al*., 2006).

Immunomodulatory and therapeutic efficacy of mushroom polysaccharides have been extensively studied in various experimental models including rats (Lu *et al*., 2003;

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Li *et al*., 2008), mice (Takimoto *et al*., 2004; Yuminamochi *et al*., 2007) and rabbits (Ikuzawa *et al*., 1988) with promising results; although a very limited studies in this regard has been conducted in avian birds (Guo *et al*., 2004).

Keeping in view, the present study reports the immunomodulatory activity of *Pleurotus ostreatus* and *Pleurotus sajor-caju* derived polysaccharides and its subsequent therapeutic efficacy against *Eimeria* infection in chicken.

Materials and Methods

Mushroom species, *Pleurotus ostreatus* (PO) and *P. sajor-caju* (PSC), used in the present study were procured from local growers and authenticated from a botanist. Voucher specimens (174 and 175) were kept for future reference in Ethno Veterinary Research and Development Center, Department of Parasitology University of Agriculture Faisalabad, Pakistan. Dried mushrooms at 50^{O}C were cut into small pieces and subjected to electrical grinder to obtain powder which was then passed through sieve (2mm). The powder thus obtained was used for extraction of polysaccharides.

Polysaccharide Extraction

Polysaccharides were extracted from mushroom powder of PO and PSC following the method of Zhao *et al*. (2010) with minor modifications. Briefly, mushroom powder (200 gm) was immersed in 95% ethanol (500 mL; Riedel-de Haen, Germany) in a covered beaker for 24 h to remove impurities. After filtration, degreased powder obtained was suspended in double distilled water (500 mL). The suspension was subjected to ultra-sonication for (60 × 30) seconds followed by centrifugation at 1600×g for 20 minutes. The supernatant thus collected was precipitated with anhydrous ethanol (4-fold, v/v) and then incubated at 4ºC for 24 h. After centrifugation, the precipitate obtained was washed with anhydrous ethanol followed by acetone (MERCK, Germany) and ether (ACI LABSCAN, Poland). Sediment thus obtained was dried at room temperature (26°C) to yield crude polysaccharides.

The crude polysaccharides were further purified by dissolving in distilled water (20 mL) followed by filtration (0.45 μm and 0.22 μm). Filtrate (5 mL) obtained was passed through DEAE column (2.6 × 30 cm) eluted with distilled water and different concentrations of NaCl (0.01M, 0.03M, 0.05 M, 0.07 M, 0.09 M). Elutions (120×1 mL) were taken and subjected to phenol-sulphuric acid method to identify polysaccharide fractions (Dubois *et al*., 1956). Neutral and acidic polysaccharide fractions identified by ELISA reader (Bio Tek, USA) at 490 nm were pooled separately and subjected to size exclusion chromatography by Sephadex G-100 (MP Biomedicals, USA) column (2.6 × 60 cm) eluted with 0.05 M NaCl solution. Sixty elutions (1 mL each) were taken and subjected to phenol sulphuric acid method and absorbance was observed at 490 nm through ELISA reader to identify purified polysaccharide fractions. Fractions identified were pooled, concentrated and dialyzed against several changes of PBS. The dialysate thus obtained was lyophilized and used in further study.

HPLC Analysis

Lyophilized purified polysaccharides were hydrolyzed following the method of Chen *et al*. (2005) with minor modifications. Briefly, purified polysaccharides (1 mg) of each species were added to 1 mL of 4.95 N Trifluoroacetic acid (TFA) at 80^{O}C for 4 h. The mixture was cooled, evaporated and suspended in water for 30 min and then analyzed through HPLC (Shimadzu, Japan Model: LC-10AT) for qualitative and quantitative analysis to demonstrate the monosaccharide in the purified polysaccharides. Mobile phase was distilled deionized water and the flow rate was set to 0.6 mL/min. Refractive index detector and Rezex RCM-Monosaccharide Ca+2, Phenomenex column having 25 cm length, 7.8 mm diameter were attached with HPLC.

Infective Material Preparation

Chicken guts suspected to be naturally infected with coccidiosis were collected from local poultry sale points and outbreak cases in and around Faisalabad, Pakistan. Briefly, contents from the positive guts were subjected to sporulation at 37^{O}C and 60-70% relative humidity (Reid and Long, 1979). Sporulation was confirmed with direct microscopic examination. Sporulated oocysts were washed thrice with phosphate buffered saline and were subjected to morphometric analysis to identify the species of genus *Eimeria* (Ryley *et al*., 1976). Morphometric analysis of sporulated oocysts revealed the presence of *Eimeria tenella*, *E. acervulina*, *E. maxima* and *E. necatrix*, which were used in the challenge experiment by adjusting the dose at 6.5×10^{4}- 7.0×10^{4} with PBS.

Experimental Design

A total of 150 days old industrial broiler chicks (Hubbard) procured from the local hatchery were reared under standard management conditions at Experiment Station, *Institute of Microbiology* University of Agriculture Faisalabad, Pakistan. All the chicks were provided with withdrawal feed and water *ad-libitum*. Chicks were divided into 3 groups viz A, B and C (n=50) at 5th day of age, and were administered the polysaccharide extract according to the following schedule to the experimental and control groups for three consecutive days at days (7th, 8th and 9th of age).

Group A: Polysaccharides of PO @ 25 mg per Kg body weight.

Group B: Polysaccharides of PSC @ 25 mg per Kg body weight.

Group C: Kept as control.

At day 15th post administration of polysaccharide extracts, chickens in all the groups (A, B and C) were further subdivided into A_1, A_2, B_1, B_2, C_1, C_2, respectively. Subgroups A_1, B_1 and C_1 were used for immunological evaluation and A_2, B_2 and C_2 for therapeutic evaluation.

Immunological Evaluation

Lymphoproliferative response to phytohaemagglutinin-P (PHA-P) was used to evaluate *in vivo* cellular immune response (Corrier, 1990). Thickness of the inter-digital space was measured at 24, 48 and 72 h post administration of (PHA-P) with pressure sensitive screw gauge. Lymphoproliferative response was calculated by using the formula:

Lymphoproliferative response = (PHA-P response, right foot) − (PBS response, left foot)

Sheep RBCs were used as T-dependent antigen to demonstrate the antibody response (total Ig, IgM and IgG titers) in the experimental chicken in comparison to control (Qureshi and Havenstein, 1994). Washed RBCs (5%); (v/v) was administered in chickens followed by booster injection of sheep RBCs at an interval of 14 days. Sera samples were collected at day 7th and 14th post administration of first and second injection of sheep RBCs. Anti-sheep RBCs antibody titers (total Ig, IgM and IgG) were detected following the procedure of Yamamoto and Glick (1982) and results were expressed in terms of geomean titers (GMT; Burgh, 1978).

Therapeutic Evaluation

Therapeutic efficacy of polysaccharides extracts was detected in *Eimeria* induced infected chickens. At day 14th post administration of polysaccharide extracts, half of the chickens were challenged with mixed specie of *Eimeria* at dose of 7.0×10^4 per bird and per cent protection, oocyst count and lesion scorings were assessed from day 4th to day 12th post infection to demonstrate the therapeutic efficacy (Ryley *et al*., 1976; Johnson and Reid, 1970).

Statistical Analysis

One way analysis of variance (ANOVA) and tuckeys range test were used for the determination of statistical significance using statistical software (SPSS®, Ver.16). Data in antibody response to sheep RBCs was analyzed by geometric mean titer (GMT). Data in per cent protection and protection against lesions were analyzed by using the Chi square test. Value of ($p<0.05$) was considered to be statistically significant.

Results

In the present study, size exclusion chromatography was used to purify the polysaccharides from mushroom species. Two fractions (27-50, 76-87 and 27-41, 86-95) came off after running the samples of PO and PSC through DEAE cellulose column, respectively (Fig. 1a and 2a). These two fractions pooled separately were run through Sephadex G-100 column and fractions came off from PO were (17-27; 10-17) and from PSC were (15-27; 09-17), respectively (Fig. 1b, 1c; 2b, 2c).

These fractions were then subjected to HPLC analysis. Maltose, glucose, mannose and fructose were identified in the extracted polysaccharides of PO and PSC. The relative molar percentage of maltose, glucose, mannose and fructose

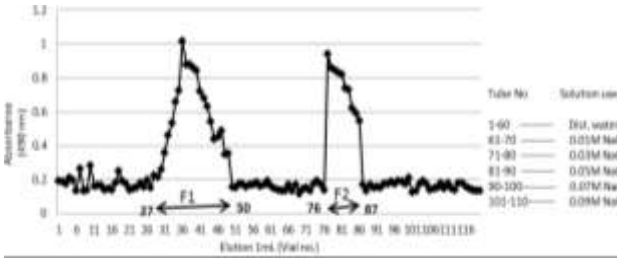

Fig. 1a: Elution profile of *Pleurotus ostreatus* through DEAE cellulose column

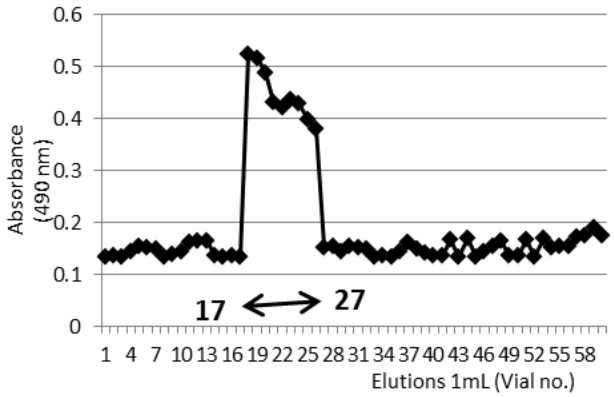

Fig. 1b: Elution profile of F1 obtained from DEAE cellulose column of *Pleurotus ostreatus* through Sephadex G100 column

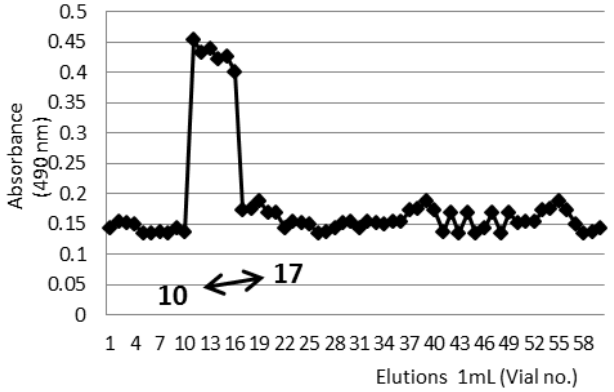

Fig. 1c: Elution profile of F2 obtained from DEAE cellulose column of *Pleurotus ostreatus* through Sephadex G100 column

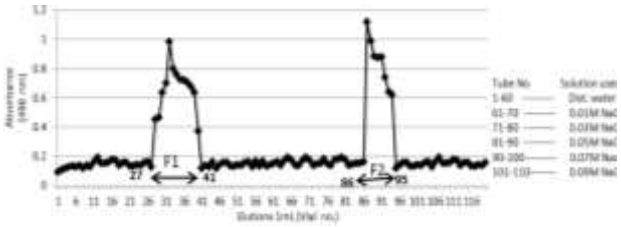

Fig. 2a: Elution profile of *Pleurotus sajor-caju* through DEAE cellulose column

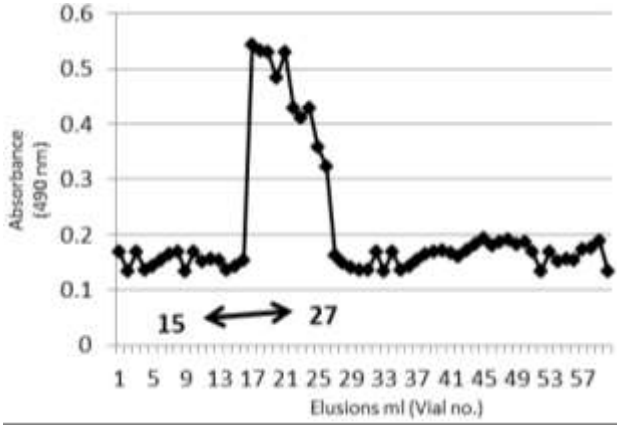

Fig. 2b: Elution profile of F1 obtained from DEAE cellulose column of *Pleurotus sajor-caju* through Sephadex G100 column

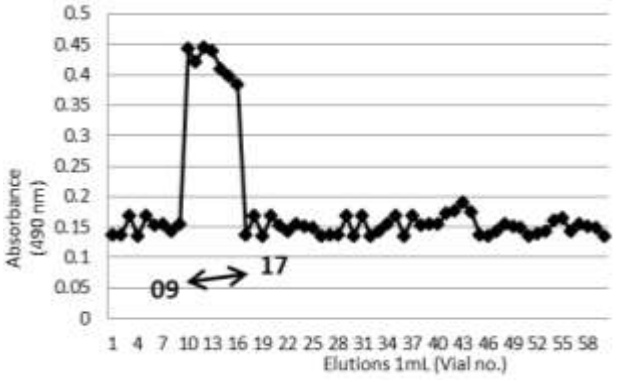

Fig. 2c: Elution profile of F2 obtained from DEAE cellulose column of *Pleurotus sajor-caju* through Sephadex G100 column

identified from PO were (0.342%, 0.378%, 0.379%, 0.045%) and those of PSC were (0.556%, 0.562%, 0.947%, 0.279%), respectively (Fig. 3a, 3b; Table 1).

Lymphoproliferative response recorded in polysaccharide extracts of PO and PSC in comparison to control were non-significant (P>0.05) at 24 h post PHA-P injection; however at 48 and 72 h post PHA-P injection significant (P<0.05) enhancement in lymphoproliferative response was observed in chickens administered with

Table 1: Relative ratios of different analytes calculated after HPLC analysis of mushroom samples

Mushroom	Analytes	RT(min)	Area (mV.s)	Height (mV)	Amount (%)
	Maltose	9.003	85.48	2.258	0.342
Pleurotus	Glucose	11.06	119.087	2.924	0.378
ostreatus	Mannose	12.423	57.117	1.419	0.379
	Fructose	13.547	10.160	.577	0.045
	Maltose	8.990	138.840	0.642	0.556
Pleurotus	Glucose	11.013	176.910	3.336	0.562
sajor-caju	Mannose	12.443	142.788	2.180	0.947
	Fructose	13.940	62.753	1.626	0.279

RT: retention times, m.V.s(mili volts per second), mV (mili volt)

Table 2: Antibody (total Ig, IgM, IgG titers) response to sheep RBCs in experimental and control chickens at day 7th and 14th post-primary and post-secondary injections of sheep RBCs

	Total Immunoglobulins Ig (GMT)			
Groups	DAY 7PPI	DAY 14 PPI	DAY 7 PSI	DAY 14 PSI
A_{1b}	35.91^{b}	28.50^{a}	40.30^{b}	35.91^{b}
B_{1b}	40.93^{a}	28.50^{a}	42.91^{a}	39.91^{a}
C_{1b}	25.39^{c}	20.15^{b}	28.91^{c}	28.50^{c}
IgM (GMT)				
Groups	DAY 7 PPI	DAY 14 PPI	DAY 7 PSI	DAY 14 PSI
A_{1b}	21.66^{b}	15.80^{a}	22.35^{a}	10.50^{a}
B_{1b}	23.21^{a}	17.19^{a}	15.75^{b}	7.40^{b}
C_{1b}	16.41^{c}	12.15^{b}	13.15^{c}	5.84^{c}
IgG (GMT)				
Groups	DAY 7 PPI	DAY 14 PPI	DAY 7 PSI	DAY 14 PSI
A_{1b}	14.25^{b}	12.7^{a}	18^{b}	25.4^{b}
B_{1b}	17.72^{a}	11.31^{a}	27.2^{a}	32.5^{a}
C_{1b}	8.98^{c}	8^{b}	15.8^{c}	22.7^{c}

Means sharing similar letters in a row are statistically non-significant (P>0.05). A_{1b}- polysaccharide extract of *Pleurotus ostreatus*, B_{1b}- polysaccharide extract of *Pleurotus sajor-caju*, C_{1b}- control, PPI- post primary injection, PSI-post secondary injection, GMT (geometric mean titer)

polysaccharide extracts of PSC and PO, respectively in comparison to control. Results indicated that polysaccharide extracts of PO and PSC showed higher cell mediated immune response at 48 and 72 h in comparison to control (Fig. 4).

In the current study, *in vivo* antibody titers (total Ig, IgG and IgM) at days 7th and 14th post-primary and post-secondary injection of sheep RBCs in groups administered with polysaccharide extract of PO and PSC were significantly higher (p<0.05) as compared to control. However, at day 7th post-primary injection of sheep RBCs, polysaccharide extract of PSC showed significantly higher (P<0.05) antibody titers (total Ig, IgM, IgG) as compared to polysaccharide extract of PO. At day 14th post- primary injection of sheep RBCs differences in antibody titers (total Ig, IgM, IgG titers) of PO and PSC were non-significant (P>0.05). At day 7th post-secondary injection of sheep RBCs significantly higher (P<0.05) IgM titers were noticed in polysaccharide extract of PO and significantly higher (P<0.05) total Ig and IgG titers were noticed in PSC extract. At day 14th post-secondary injections of sheep RBCs

Table 3: Per cent protection, lesion scoring, mean lesion scoring and protection against lesions post *Eimeria* infection

Groups (n)	Protection (%)	Lesion scoring of the birds					Mean Lesion score	Protection against lesions (%)
		0	1	2	3	4		
A_2(25)	60[a]	4	5	4	5	7	2.24	44.00[a]
B_2(25)	45[a]	3	6	4	4	8	2.32	42.00[a]
C_2(25)	20[b]	-	2	4	5	14	3.24	19.00 [b]

Values sharing similar letter in a column are statistically non-significant (P>0.05). A2- Polysaccharide extract of *Pleurotus ostreatus*, B2-Polysaccharide extract of *Pleurotus sajor-caju*, C- control, n-no of birds per group

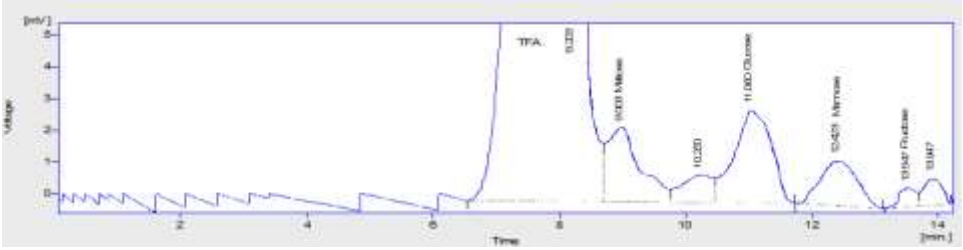

Fig. 3a: HPLC analysis of mushroom *Pleurotus ostreatus*

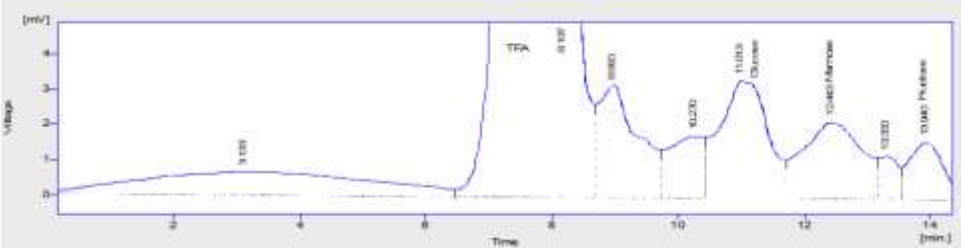

Fig. 3b: HPLC analysis of mushroom *Pleurotus sajor-caju*

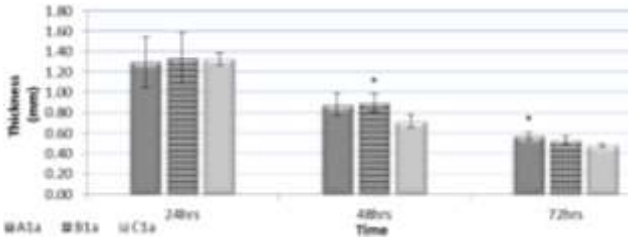

Fig. 4: Lymphoproliferative response to Phytohaemagglutinin-P (PHAP) in experimental and control groups. A1a: polysaccharides of *Pleurotus ostreatus*. B1a: polysaccharide of *Pleurotus sajor-caju*, C2a: control. *statistical significance (P<0.05)

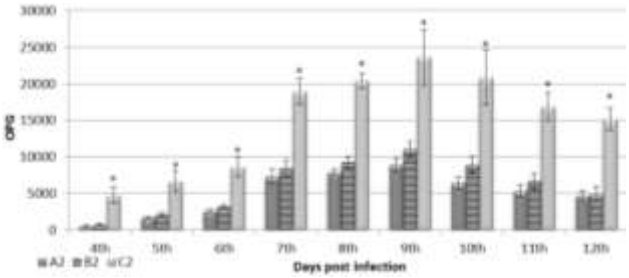

Fig. 5: Post infection oocyst per gram (OPG) of droppings in experiment and control groups

A2- Polysaccharide extract of *Pleurotus ostreatus*, B2-Polysaccharide extract of *Pleurotus sajor-caju*, C- control, * statistical significance (P<0.05)

polysaccharide extract of PSC showed significantly higher (P<0.05) antibody titers (total Ig, IgG) as compared to PO extract (Table 2). These results indicated that polysaccharide extract of PO and PSC showed profound humoral immune response in comparison to control.

Oocyst count, lesion scorings and percent protection after mixed species infection of the *Eimeria* were the parameters to evaluate therapeutic efficacy of polysaccharides. Significantly higher (P<0.05) oocyst count was recorded in the control group as compared to polysaccharide extracts of PO and PSC (Fig. 5). Caeca and intestines of the control groups showed extensive hemorrhagic lesions of dead and survived birds. Protection against lesions was statistically significant (P<0.05) in polysaccharide extract of PO (44%) and PSC (42%) in comparison to control (19%). However, differences observed between PO and PSC were statistically non-significant (P>0.05). In the current study, highest protection (60%) was recorded in polysaccharide extract of PO followed by PSC (45%) and control (20%) (Table 3).

Discussion

Mushrooms have been used since centuries for the treatment of different ailments and efforts were made to isolate different compounds from the mushrooms responsible for medicinal activities (Wasser, 2002). In this regard, several compounds had been isolated from different mushroom species with immunorestorative activities and their subsequent effects against wide variety of bacterial and parasitic diseases in different animal models and human beings (Lee *et al.*, 2006; Antonyuk *et al.*, 2010; Liu *et al.*, 2012). There are several bioactive compounds present in mushrooms including homo- or hetero- saccharides (Mono, Di and Poly), glycosides (anthraquinones, cardiac glycosides, coumarin, cyanogenic glycosides, flavonoid glycosides, phenolic glycosides and saponins), volatile oils, lactones, alkaloids, resin, tannin, proteins, phytochrome, lactones and aminoacid (Yang and Feng, 1998). Among these, polysaccharides are of paramount importance and their biological activities had been documented in human and animal models against viral, bacterial and parasitic diseases (Yuan *et al.*, 1993; Hu *et al.*, 1998; Pang *et al.*, 2000; Yu and Zhu, 2000).

Though, relationship of bioactive functions with respect to molecular structure of the polysaccharides is not well understood, however, polysaccharide bioactivity can be characterized by monosaccharide composition, three-dimensional complex structure and molecular weight ranging from 10^4 to 10^7 KDa (Xue and Meng, 1996). Therefore, in the current study, purified polysaccharides from PO and PSC were analyzed with high performance liquid chromatography (HPLC) to detect the monosaccharaides. In this regard, different *Pleurotus* spp. had been investigated and reported to contain mannose (2%), galactose (5%), 3-*O* methayl-galactose (3%) and glucose (90%) (Carbonero *et al.*, 2006). Guo *et al.* (2003) isolated fructose, galactose, glucose, mannose, xylose and glucuronic acid from *Lentinus edodes* with molar ratios 0.1, 0.2, 9.1, 0.1, 0.5, 0.1; and fructose, galactose, glucose, mannose, xylose and glucuronic acid from *Tremella fuciformis* with molar ratios 0.9, 0.1, 3.5, 1.5, 4.1 and 0.5, respectively. In another study, xylose, maltose, fructose, glucose and sucrose had been identified from polysaccharide extract of *Ganoderma lucidum* with relative molar percentage 0.4, 50.9, 14.4, 12.8 and 0.7, respectively (Yang *et al*., 2010). Similarly, in another study, HPLC analysis of polysaccharide extracts revealed the presence of L-arabinose, D-mannose, D-glucose and D-galactose, with the molar ratio of 0.7, 2.8, 24.8 and 1.0 per cent, respectively (Cui *et al*., 2011). The variation in the molar percentage among various studies including the current study may be due to difference in physicochemical properties of mushroom species, purity of polysaccharides and the methods used (Guo *et al*., 2003; 2005).

The higher lymphoproliferative response post PHAP administration may be due to the activation of natural killer (NK) cells, higher production of interferons and cytokines (IL-1 and Il-8), enhanced complement activation and by potentiating the activities of phagocytes or by prevention of leukocyte reduction (Wargovich *et al.*, 2001; Shamtsyan *et al*., 2004). Similar results with polysaccharides extracted from *Lentinus edodes* had been reported by Chen *et al*. (2003) in which enhanced cell-mediated immune response through delayed type of hypersensitivity reaction was shown through enhancement in proliferation of splenocytes and increased production of TNF-α and IFN-γ.

Higher humoral immune response in *Fomitella fraxinea* administered chickens had also been reported in the literature (Dalloul and Lillehoj, 2005). Immune potentiating effects of mushrooms may be due to regulated through cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) involved in immune cells proliferation, differentiation and secretions (Wu and Wang, 1999; Lee *et al.*, 2002).

Various mushrooms had been reported to cause significant reduction in mortality during viral (Liu *et al*., 1999; Yu and Zhu, 2000), bacterial (Yuan *et al*., 1993) and parasitic (Hu *et al*., 1998; Pang *et al*., 2000) diseases in chicken. Protection in control group (20%) observed in the study may be due to self-limiting mechanisms occurred during avian coccidiosis (Sharma, 1991).

From the present study, it was concluded that polysaccharides extracted from *P. ostreatus* and *P. sajor-caju* had immunotherapeutic potential and could be used in poultry to boost up the immune response in diseased conditions like coccidiosis in poultry.

Acknowledgements

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References

Antonyuk, V.O., Y. Klyuchivska and R.S. Stoika, 2010. Cytotoxic proteins of *Amanita virosa* Secr. mushroom: Purification, characteristics and action towards mammalian cells. *Toxicon*, 55: 1297–1305

Babar, W., Z. Iqbal, M.N. Khan and G. Muhammad, 2012. An inventory of the plants used for parasitic ailments of animals. *Pak. Vet. J.,* 32: 183-187

Bobek, P., and S. Galbavy, 1999. Hypocholesterolemic and antiatherogenic effect of oyster mushroom (*Pleurotus ostreatus)* in rabbits. *Nahrung*, 43: 339–342

Burgh, M.A., 1978. Simple method for recording and analyzing serological data. *Av. Dis.*, 22: 362–365

Carbonero, E.R., A.H.P. Gracher, F.R. Smiderle, F.R. Rosado, G.L. Sassaki, P.A.J. Gorin and M. Iacomini, 2006. A β-glucan from the fruit bodies of edible mushrooms *Pleurotus eryngii* and *Pleurotus ostreatoroseus*. *Carb. Polym.*, 66: 252–257

Chen, H.L., D.F. Li, B.Y. Chang, L.Y. Gong, X.S. Piao, G.F. Yi and JX Zhang, 2003. Effects of Lentinan on broiler splenocyte proliferation, interleukin-2 production and signal transduction. *Poult. Sci.*, 80: 760–766

Chen, S.C., M.K. Lu, J.J. Cheng and D.L. Wang, 2005. Antiangiogenic activities of polysaccharides isolated from medicinal fungi. *FEMS Microbiol. Lett.*, 249: 247–254

Chihara, G., Y. Maeda, T. Sasaki and F. Fukuoka, 1969. Inhibition of mouse sarcoma 180 by polysaccharides from *Lentinus edodes* (Berk.). *Nature*, 222: 687–688

Corrier, D.E., 1990. Comparison of phytohemagglutinin induced cutaneous hypersensitivity reactions in the interdigital skin of broiler and layer chicks. *Av. Dis.*, 34: 369–373

Cui, H.L., Y. Chen, S.S. Wang, G.Q. Kai and Y.M. Fang, 2011. Isolation, partial characterization and immunomodulatory activities of polysaccharide from *Morchella esculenta*. *J. Sci. Food Agric*., 91: 2180–2185

Dalloul, R.A. and H.S. Lillehoj, 2005. Recent advances in immunomodulation and vaccination strategies against coccidiosis. *Av. Dis.*, 49: 1–8

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.*, 28: 350–356

Gray, A.M. and R.R. Flatt, 1998. Insulin-releasing and insulin like activity of *Agaricus campestris* (mushroom). *J. Endocrinol*., 157: 259–266

Gunde-Cimerman, N., 1999. Medicinal value of the genus Pleurotus (Fr.) P. Karst. (Agaricales S.R., Basidiomycetes). *Int. J. Med. Mush.*, 1: 69–80

Guo, F.C., R.P. Kwakkel, B.A. Williams, H.K. Parmentier, W.K. Li, Z.Q. Yang and M.W. Verstegen, 2004. Effects of mushroom and herb polysaccharides on cellular and humoral immune responses of *Eimeria tenella* infected chickens. *Poult. Sci.*, 83: 1124–1132

Guo, F.C., R.P. Kwakkel, C.B. Williams, W.K. Li and M.W. Verstqen, 2005. Coccidiosis immunization: effects of mushroom and herb polysaccharides on immune responses of chickens infected with *Eimeria tenella*. *Av. Dis.*, 49: 70–73

Guo, F.C., B. A. Williams, R.P. Kwakkel and M.W.A. Verstegen, 2003. *In vitro* fermentation characteristics of two mushrooms, an herb and their polysaccharide fractions, using chicken cecal contents as inoculum. *Poult. Sci.*, 82: 608–615

Ho, C.Y., C.B.S. Lau, C.F. Kim, K.N. Leung, K.P. Fung, T.F. Tse, H.H.L. Chan and M.S.S. Chow, 2004. Differential effect of *Coriolus versicolor* (Yunzhi) extract on cytokine production by murine lymphocytes *in vitro*. *Int. Immunopharmacol.*, 4: 1549–1557

Hsieh, K.Y., C.I. Hsu, J.Y. Lin, C.C. Tsai and R.H. Lin, 2003. Oral administration of an edible-mushroom-derived protein inhibits the development of food-allergic reactions in mice. *Clin. Exp. Allergy*., 33: 1595–1602

Hu, T.J., J.L. Liang and F.S. Cheng., 1998. The use of 8301 polysaccharide as an adjuvant in coccidial infected chickens. *J. Grass Livestock (In Chinese)*, 6: 5–7

Ikuzawa, M., K. Matsunaga, S. Nishiyama, S. Nakajima, Y. Kobayashi and T. Andoh, 1988. Fate and distribution of an antitumor protein-bound polysaccharide PSK (Krestin). *Int. J. Immunopharmacol.*, 10: 415–423

Johnson, J. and W.M. Reid, 1970. Anticoccidial drugs: Lesion scoring techniques in battery and floor pen experiments with chickens. *Exp. Parasitol.*, 28: 30–36

Jose, N., T.A. Ajith and K.K. Janardhanan, 2004. Methanol extract of the oyster mushroom, *Pleurotus florida*, inhibits inflammation and platelet aggregation. *Phytotherap. Res.*, 18: 43–46

Kim, G.Y., S.H. Kim, S.Y. Hwang, H.Y. Kim, Y.M. Park, S.K. Park, M.K. Lee, S.H. Lee, T.H. Lee and J.D. Lee, 2003a. Oral administration of proteoglycan isolated from *Phellinus linteus* in the prevention and treatment of collagen induced arthritis in mice. *Biol. Pharmacol. Bull.*, 26: 823–831

Kim, S.H., N.S. Kim, K.C. Lee, H.B. Lee and M.S. Kim, 2012. Treatment of multiple thoracolumbar intervertebral disc disease using electro-acupuncture and oriental herbal medicine in a dog. *Pak. Vet. J*., 32: 631–634

Kim, G.Y., S.I. Roh, S.K. Park, S.C. Ahn, Y.H. Oh, J.D. Lee and Y.M. Park, 2003b. Alleviation of experimental septic shock in mice by acidic polysaccharide isolated from the medicinal mushroom *Phellinus linteus*. *Biol. Pharmacol. Bull.*, 26: 1418–1423

Kim, S.H., Y.S. Song, S.K. Kim, B.C. Kim, C.J. Lim and E.H. Park, 2004. Anti-inflammatory and related pharmacological activities of the n-BuOH subfraction of mushroom *Phellinus linteus*. *J. Ethnopharmacol.*, 93: 141–146

Kodama, N., K. Komuta and H. Nanba, 2003. Effect of Maitake (*Grifola frondosa*) D-fraction on the activation of NK cells in cancer patients. *J. Med. Food*., 6: 371–377

Kodama, N., M. Yamada and H. Nanba, 2001. Addition of Maitake D-fraction reduces the effective dosage of vancomycin for the treatment of *Listeria* infected mice. *Jpn. J. Pharmacol*., 87: 327–332

Kuo, Y.C., Y.L. Huang, C.C. Chen, Y.S. Lin, K.A. Chuang and W.J. Tsai, 2002. Cell cycle progression and cytokine gene expression of human peripheral blood mononuclear cells modulated by *Agaricus blazei*. *J. Lab. Clin. Med*., 140: 176–187

Lavi, I., D. Friesem, S. Geresh, Y. Hadar and B. Schwartz, 2006. An aqueous polysaccharide extract from the edible mushroom Pleurotus ostreatus induces anti-proliferative and proapoptotic effects on HT-29 colon cancer cells. *Cancer Lett.*, 244: 61–70

Lee, H., Y.J. Kim, H.W. Kim, D.H. Lee, M.K. Sung and T. Park, 2006. Induction of apoptosis by *Cordyceps militaris* through activation of caspase-3 in leukemia HL-60 cells. *Biol. Pharmaceut. Bull.*, 29: 670−674

Lee, I.H., R.L. Huang, C.T. Chen, H.C. Chen, W.C. Hsu and M.K. Lu, 2002. *Antrodia camphorate* polysaccharides exhibit anti-hepatitis B virus effects. *FEMS Microbiol. Lett.*, 209: 63–67

Li, X., L.L. Jiao, X. Zhang, W.M. Tian, S. Chen and L.P. Zhang, 2008. Anti-tumor and immunomodulating activities of proteoglycans from mycelium of *Phellinus nigricans* and culture medium. *Int. Immunopharmacol.*, 8: 909–915

Li, X.M., C.K. Huang, T.F. Zhang, A.A. Terper, K. Srivastava, B.H. Schofield and H.A. Sampson, 2000. The Chinese herbal medicine formula MSSM-002 suppresses allergic airway hyper reactivity and modulates TH1/TH2 responses in a murine model of allergic asthma. *J. Allerg. Clin. Immunol*., 106: 660–668

Li, X.M., T.F. Zhang and C.K. Huang, 2001. Food allergy herbal formula-1 (FAHF-1) blocks peanut-induced anaphylaxis in a murine model. *J. Allerg. Clin. Immunol.*, 108: 639–646

Liu, F., V.E.C. Ooi and M.C. Fung, 1999. Analysis of immunomodulating cytokine mRNAs in the mouse induced by mushroom polysaccharides. *Life Sci.*, 64: 1005–1011

Liu, Y.H., C.F. Tsai, M.C. Kao, Y.L. Lai and J.J. Tsai, 2003. Effectiveness of Dp2 nasal therapy for Dp2-induced airway inflammation in mice: using oral *Ganoderma lucidum* as an immunomodulator. *J. Microbiol. Immunol. Inf.*, 36: 236–242

Liu, Y.T., J. Sun, Z.Y. Luo, S.Q. Rao, Y.J. Su, R.R. Xu and Y.J. Yang, 2012. Chemical composition of five wild edible mushrooms collected from Southwest China and their antihyperglycemic and antioxidant activity. *Food Chem. Toxicol*., 50: 1238–1244

Lu, H., E. Kyo, T. Uesaka, O. Katoh and H. Watanabe, 2003. A water-soluble extract from cultured medium of *Ganoderma lucidum* (Reishi) mycelia suppresses azoxymethane-induction of colon cancers in male F344 rats. *Onc. Rep*., 10: 375–379

Markova, N., V. Kussovski, I. Drandarska, S. Nikolaeva, N. Georgieva and T. Radoucheva, 2003. Protective activity of lentinan in experimental tuberculosis. *Int. Immunopharmacol.*, 3: 1557–1562

Nakamura, T., S. Matsugo, Y. Uzuka, S. Matsuo and H. Kawagishi, 2004. Fractionation and anti-tumor activity of the mycelia of liquid-cultured *Phellinus linteus*. *Biosci. Biotech. Biochem.*, 68: 868–872

Nanba, H., N. Kodama, D. Schar and D. Turner, 2000. Effects of Maitake (*Grifola frondosa)* glucan in HIV- infected patients. *Mycoscience*, 41: 293–295

Ngai, P.H. and T.B. Ng, 2003. Lentin, a novel and potent antifungal protein from Shiitake mushroom with inhibitory effects on activity of human immunodeficiency virus-1 reverse transcriptase and proliferation of leukemia cells. *Life Sci*., 73: 3363–3374

Pang, F.H., M.Q. Xie and H.H. Ling, 2000. The investigation of Immunodulators tested for the results on the control of a coccidial infection. *Chin. J. Vet. Parasitol*., 8: 1–3

Qureshi, M.A. and G.B. Havenstein, 1994. A comparison of the immune performance of a 1991 commercial broiler with a 1957 random bred strain when fed "typical" 1957 and 1991 broiler diets. *Poult. Sci.*, 73: 1805–1812

Ragunathan, R., R. Gurusamy, M. Palaniswamy and K. Swaminathan, 1996. Cultivation of Pleurotus spp. on various agro-residues. *Food Chem.*, 55: 139–144

Reid, W.M., and P.L. Long, 1979. *A Diagnostic Chart for Nine Species of Fowl Coccidian*, p: 335. University of Georgia Research Report

Ryley, J.F., R. Meade, J. Ifazulburst and T.E. Robinson, 1976. Methods in Coccidiosis Research, separation of oocyst from faeces. *Parasitology*, 73: 311–326

Santos-Neves, J.C., M.I. Pereira, E.R. Carbonero, A.H.P Gracher, P.A.J Gorin, G.L. Sassaki and M. Iacomini, 2008. A gel-forming β-glucan isolated fromthe fruit bodies of the edible mushroom *Pleurotus florida*. *Carbohydr. Res*., 343: 1456–1462

Shamtsyan, M., V. Konusova, Y. Maksimova, A. Goloshchev, A. Panchenco, A. Simbertsev, N. Petrischev and N. Denisova, 2004. Immunomodulating and anti-tumor action of extracts of several mushrooms. *J. Biotechnol.*, 113: 77–83

Sharma, J.M., 1991. *Avian Cellular Immunology*. CRC press, Inc., 2000. Corporate Blvd., N.W., Boca Raton, Florida, USA

Smiderle, F.R., L.M. Olsen, E.R. Carbonero, C.H. Baggio, C.S. Freitas, R. Marcon, A.R.S. Santos, P.A.J. Gorin and M. Iacomini, 2008. Anti-inflammatory and analgesic properties in a rodentmodel of a (1→3), (1→6)-linked β-glucan isolated from *Pleurotus pulmonarius*. *Euro. J. Pharmacol.*, 597: 86–91

Synytsya, A., K. Mickova, A. Synytsya, I. Jablonsky, J. Spevacek, V. Erban, E. Kovarikova and J. Copikova, 2009. Glucans from fruit bodies of cultivated mushrooms *Pleurotus ostreatus* and *Pleurotus eryngii*: Structure and potential prebiotic activity. *Carbohydr. Polym.*, 76: 548–556

Takimoto, H., D. Wakita, K. Kawaguchi and Y. Kumazawa, 2004. Potentiation of cytotoxic activity in naive and tumor-bearing mice by oral administration of hot-water extracts from *Agaricus blazei* fruiting bodies. *Biol. Pharmaceut. Bull*., 27: 404–406

Tao, Y., L. Zhang and P.C.K. Cheung, 2006. Physicochemical properties and antitumor activities of water-soluble native and sulfated hyperbranched mushroom polysaccharides. *Carbohydr. Res.*, 341: 2261–2269

Wargovich, M.J., C. Woods, D.M. Hollisand and M.E. Zander, 2001. Herbals, cancer prevention and health. *J. Nut.*, 131: 3034–3036

Wasser, S.P., 2002. Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. *Appl. Microbiol. Biotechnol*., 60: 258–274

Wu, Y.H., and F. Wang, 1999. Research status on herbs' two way immune regulating functions. *J. Trad. Chin. Vet. Med*., 4: 19–22

Xue, M. and X.S. Meng, 1996. Review on research progress and prosperous of immune activities of bio-active polysaccharides. *J. Trad. Chin. Vet. Med*., 3: 15–18

Yamada, T., T. Oinuma and M. Niihashi, 2002. Effects of *Lentinus edodes* mycelia on dietary-induced atherosclerotic involvement in rabbit aorta. *J. Atheroscl. Thromb.*, 9: 149–156

Yamamoto, Y. and B. Glick, 1982. A comparison of the immune response between two lines of chickens selected for differences in the weight of the bursa of fabricius. *Poult. Sci*., 61: 2129-2132

Yang, Q., S.W. Wang, Y.H. Xie, J.Y. Sun and J.B. Wang, 2010. HPLC analysis of *Ganoderma lucidum* polysaccharides and its effect on antioxidant enzymes activity and Bax, Bcl-2 expression. *Int. J. Biol. Macromol*., 46: 167–172

Yang, Y. and W.S. Feng, 1998. *Brochures of Isolation and Extraction of Chemical Components from Herbs (in Chinese) China*. Chinese Herb Medicine Press, Beijing

Yoon, S.J., M.A. Yu and Y.R. Pyun, 2003. The nontoxic mushroom *Auricularia auricula* contains a polysaccharide with anticoagulant activity mediated by antithrombin. *Thromb. Res*., 112: 151–158

Yu, J.G. and L.Y. Zhu, 2000. The use of *Astragalus Radix* polysaccharides against Infectious Bursa Disease in chickens. *J. Trad. Chin. Vet. Med.*, 6: 3–4

Yuan, Y.L., B.T. Fan and Y.X. Zhang, 1993. Efficacy of 8301 polysaccharides as an adjuvant against avian *Pasteurellosis* in chickens. *J. Trad. Chin. Vet. Med.*, 3: 6–10

Yuminamochi, E., T. Koike, K. Takeda, I. Horiuchi and K. Okumura, 2007. Interleukin-12- and interferon-gamma-mediated natural killer cell activation by *Agaricus blazei* Murill. *Immunology*, 121: 197–206

Zhang, W., Y. Wang and Y. Hou, 2004. Effects of Chinese medicinal fungus water extract on tumor metastasis and some parameters of immune function. *Int. Immunopharmacol.*, 4: 461–468

Zhao, L., Y. Dong, G. Chen and Q. Hu, 2010. Extraction, purification, characterization and antitumor activity of polysaccharides from *Ganoderma lucidum*. *Carbohyd. Polym.*, 80: 783–789

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