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Aloe vera Polysaccharides as Biological Response Modifiers in Chickens

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Abstract

In the present study, polysaccharides have been isolated and evaluated as biological response modifiers in chickens. *Aloe* (*A.*) *vera* polysaccharides were administered in chickens for three consecutive days and used for immunological evaluation. Results showed significantly higher (p<0.05) lymphoproliferative response to PHA-P and Con-A in chickens of polysaccharides administered groups as compared to control. Carbon particle clearance assay showed significantly higher clearance index (K) in control group, indicating presence of more carbon particles in blood. Phagocytic index (α) showed significantly higher response in all three polysaccharides administered groups. Significantly higher humoral immune responses [total immunoglobulins (Igs), IgG and IgM titers] were also detected in treatment groups. Biomolecule administered chickens showed better feed conversion ratios and significantly higher (p<0.05) weekly weight gains. From the current study, it was concluded that *A. vera* derived polysaccharides have potential to be used as immunotherapeutic agent(s) in chickens.

Keywords: *Aloe vera*; Polysaccharides; Biological response modifiers; Chickens

Introduction

Biological response modifiers induced modification(s) in the functioning of immune system and have unique effects on the physiology of human beings and animals. These substances also reduced the immunosuppressive effects of different remedies and simultaneously increased their effectiveness. In this regard, *Aloe (A.) vera* is considered as one of the promising candidate having immunomodulatory effects in different animal models (Krishnan, 2006).

The use of plants as therapeutic agents has a long traditional history; although isolation of their active compounds initiated in 19th century (Phillipson and Anderson, 2001). According to World Health Organization, 80% population of world used herbal medicines for different human diseases (Serrentino, 1991). In this regard, many plants and their constituents including *Angelica gigas*, *Cissampelos pareira*, *Astragalus membranaceus*, *Mangifera indica*, *Ganoderma lucidum*, *Ocimum sanctum*, *Zingiber officinale*, *Phyllanthus emblica*, *Allium sativum*, *Curcuma longa*, *Nyctanthus arbor-tritis*, *Saccharum officinarum* and *Aloe vera* (Minja, 1989; Waller *et al*., 2001; Fajimi and Taiwo, 2005; Githiori *et al*., 2006; Akhtar *et al*., 2012; Awais *et al*., 2013) have been reported for different medicinal properties.

Among these, *A. vera* (*Aloe barbadensis* Miller) is the most commonly used medicinal plant having historical importance. It is a succulent plant found in tropical and subtropical areas of many continents including Pakistan. Modern therapeutic use of *A. vera* started in 1920s and now it is found in many journals, topic of numerous researches and commercial literature on internet. It has been reported to cure variety of conditions including mild fever, burns, wounds, gastrointestinal disorders, sexual vitality, fertility problems, inflammation, arthritis, cancer, and acquired immunodeficiency syndrome (Bashir *et al*., 2011; Manvitha and Bidya, 2014). Now a day, *A. vera* is extensively used in many manufacturing industries of foods, pharmaceuticals and cosmetics (Yang *et al*., 2003).

A. vera is unique in nature having many biological active components including proteins, carbohydrates, vitamins, enzymes, minerals, sugar, lignin, saponins and salicylic acids (Surjushe *et al*., 2008). These compounds have numerous pharmacological activities including wounds healing, antinflammatory, antiarthritis, antioxidative, antidiabetic and anticarcinogen (Waihenya *et al*., 2002; Iji *et al*., 2010; Saritha *et al*., 2010).

A. vera polysaccharides, anthraquinones and lectins have immunomodulatory effects (Leung *et al*., 2004; Akev *et al*., 2007; Liu and Wang, 2007). Its immunomodulatory activities include stimulation of macrophages, which produced nitric oxide and thus showed effects on the antigen presenting cells (APC) to release cytokine(s) (Strickland, 2001). The most important component of *A.*

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vera gel is acemannan (a linear carbohydrate polymer containing acetylated mannose). It is a storage polysaccharide, present in parenchyma protoplast (Rodriguez *et al*., 2010) and has potential to act against viral infections, which reduced opportunistic infections and stimulate wound healing (Ni *et al*., 2004). Its immunomodulatory effects had been reported in different animal models (Krishnan, 2006). It stimulates the natural and adaptive defense mechanism(s) including cytokines and help the body to protect itself (Alamgir and Uddin, 2010). Due to its biological response modifying activities it shows beneficial prophylactic and therapeutic effects against pathogens. It can be successfully used in oncology, inflammatory diseases, transplantation medicine and autoimmune disorders.

As far as therapeutic efficacy of *A. vera* and its component(s) is concerned, it had been reported in different animal models and human beings with promising results (Strickland, 2001; Agarry *et al.,* 2005), although limited work had been conducted on poultry (Djeraba and Quere, 2000; Mwale *et al*., 2006; Akhtar *et al.,* 2012).

Keeping in view, the present project has been designed to investigate the biological response modifying effects of *A. vera* polysaccharides in chickens.

Materials and Methods

Procurement of *Aloe vera* Leaves

Fresh *A. vera* leaves were collected from Botanical Garden and authenticated by a botanist from the Department of Botany, University of Agriculture, Faisalabad (UAF), Pakistan. The *A. vera* specimens were kept in the Ethno Veterinary Research and Development Centre, Department of Parasitology, UAF (specimen voucher No. 0173).

Leaves were cleaned with water and chlorine (5–10 parts per million; ppm) solution then with a solution containing formalin (37 % formaldehyde; 0.001–0.005 ppm) and with distilled water in the last (Femenia *et al*., 1999).

A. vera Gel Preparation

Mucilaginous leaf gel was collected from *A. vera* leaves within three to four hours to reduce deterioration. For this purpose, one leaf was taken at a time, placed its flat side on a cutting wooden board and removed epidermis with a sharp knife. Afterwards, serrated edges of both sides of the leaf were cut off to make slices of the leaf lengthwise. With the help of a wooden spatula, gel was scrapped out, thoroughly homogenized followed by filtration through cheese cloth and stored in screw capped glass bottles at 4°C in a refrigerator for further use.

Extraction of *A. vera* Polysaccharides

Polysaccharides from the *A. vera* gel were extracted by the method adopted by Chang *et al*. (2010). Briefly, *A. vera* gel, was vigorously mixed with four volume of 95% (v/v) ethanol and set aside overnight at 4°C. The precipitate thus obtained was centrifuged at 6500 g for 10 min and supernatant was removed. The precipitate was mixed in double distilled water, kept overnight and again precipitated with four volume of 95% (v/v) ethanol. The precipitation and solubilization procedures conducted repeatedly to roughly remove all the colored materials. Finally the precipitate thus obtained was mixed in doubled distilled water and treated with Sevagreagent {1:4 (v/v) (1-butanol: chloroform)}. Free protein was removed by repeated oscillation and centrifugation (Staub, 1965). This solution was mixed with three volumes of ethanol (95%, v/v), which precipitated polysaccharides. The crude polysaccharides thus obtained were further purified by washing twice with absolute ethanol followed by acetone and ethyl ether, finally dried completely at 40°C for 48 h.

Characterization of Polysaccharides

Hydrolysis: The polysaccharides were hydrolysed to get the monosaccharides according to the procedure adopted by Osborn *et al*. (1999) with little modifications. The polysaccharides were refluxed in 2M Trifloroacetic acid (TFA) (Sigma-Aldrich®, USA) at 100°C for 2 h in a round-bottomed flask equipped with a reflux condenser. The TFA was then removed by evaporation at 75°C and the water was removed by freeze drying (-65°C) overnight to get purified hydrolyzed monosaccharides.

HPLC analysis: The monosaccharides were analysed on a Shimadzu-10A HPLC workstation (Japan) supplied with a quaternary gradient pump unit and a refractive index detector (RID). The analytical columns including Rezex RCM-Monosaccharide Ca^{+2} and Phenomenex were used to get absorption spectra at 80°C temperature and wavelength of 235 nm. Isocratic double distilled water was used as mobile phase; whereas, injection volume for all samples and standards was taken as 20µl (each).

Experimental chickens: One hundred and sixty industrial broiler chicks (day old) were procured from local hatchery and kept in hygienic environment at experimental station, Department of Parasitology, UAF. Chicks were given water and anti-coccidial free feed *ad libitum* throughout the study and routinely vaccinated (Awais, 2010).

Experimental design: After 5 days of acclimatization, chicks were at random separated into four groups namely A_1, A_2, A_3 and A_4 (control) each containing 40 chicks. They were administered with the following oral treatments for three consecutive days at day 5^{th}, 6^{th} and 7^{th} of age.

Group A_1: Polysaccharides from *A. vera*, 100 mg/Kg of body weight/day.

Group A_2: Polysaccharides from *A. vera*, 200 mg/Kg of body weight/day.

Group A_3: Polysaccharides from *A. vera*, 300 mg/Kg of body weight/day.

Group A_4: Control group: PBS 3 mL/day.

Chickens from all groups were used for immunological evaluation in separate cages on 14thday of *A. vera* polysaccharides administration.

Immunofunction Studies

Lymphoproliferative response to Phytohaemagglutinin-P (PHA-P): *In vivo* lymphoblastogenic response was quantified by using a classic toe-web assay according to procedure adopted by Corrier (1990). Briefly, PHA-P (Sigma-Aldrich, St. Louis, MO 63178), was injected (100µg/100µL/ chicken; intradermally) into the toe web of the left foot of 10 chickens from every group. The swelling response was calculated at 24, 48 and 72 h post injection, by following formula:

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

Lymphoproliferative response to concanavalin-A (Con-A): Peripheral blood leukocytes were incubated with Con-A (25 µg/2x 106 cells) in 96 wells plate. The extent of lymphoproliferative response was calculated by classical MTT assay (Qureshi *et al*., 2000).

Carbon Particle Clearance Assay

On 14th day post administration, carbon particle clearance assay was carried out (Zhang, 2007). The clearance index and phagocytic index were calculated to demonstrate the immune functions of polysaccharide preparations (Hou *et al*., 2010).

Antibody Response

Ten chickens from experimental and control groups were injected with one mL of 5% saline suspension of sheep red blood cells (SRBCs) via intravenous route. Blood samples were collected at 7th and 14th day after first SRBCs injection. At 14th day after first injection, booster injections of SRBCs were given. The blood samples were collected at 7 and 14 days post boosting injection. Anti-SRBCs antibody titers (total Igs, IgM and IgG) were measured by using microplate haemagglutination assay (Yamamoto and Glick, 1982).

Feed Conversion Ratios (FCR)

After*A. vera* extracts administration chickens and feed were weighed weekly. The obtained data was used to calculate the weekly FCR by using the formula as adopted by Singh and Panda (1992):

$$\text{Feed conversion ratio} = \frac{\text{Feed consumption (gm)}}{\text{Body weight gain (gm)}}$$

Statistical Analysis

The collected data was subjected to statistical analysis by using one way ANOVA and differences among different treatment groups were determined by Duncan's multiple range (DMR) test.

Results

Isolation and Characterization of *A. vera* Polysaccharides

High performance liquid chromatography (HPLC) analysis: *A. vera* monosaccharides quantitative HPLC analysis resulted in three distinct peaks on chromatogram at different retention time. These peaks were compared with standards, which showed three different monosaccharides including maltose, glucose and mannose in hydrolyzed solution of *A. vera* at retention times 9.423, 11.080 and 12.55, respectively. Molar concentrations (%)of these detected monosaccharides in hydrolyzed solution of *A. vera* polysaccharides are presented (Table 1).

***In vivo* Lymphoproliferative Response to Phytohaemagglutinin-P (PHA-P)**

The amplitude of toe-web swelling was measured at 24, 48 and 72 h after PHA-P injection in all experimental and control groups. *A. vera* polysaccharides administered groups showed significantly higher (p<0.05) lymphoproliferative response in all experimental groups as compared to control. Maximum swelling was calculated at 24 h after PHA-P injection in all groups followed by 48 and 72 h (Fig. 1).

***In vitro* Lymphoproliferative Response to Concanavalin-A (Con-A)**

In vitro response to concanavalin-A was detected at 7th and 14th day post administration of *A. vera* polysaccharides at graded doses in all groups by lymphoblastogenic response of chickens peripheral blood lymphocytes (PBLs) to Con-A. All the experimental groups showed significantly higher (p<0.05) response in the PBLs of chickens as compared to those of control group, both on 7th and 14th day post administration of *A. vera* polysaccharides. However, among the treatment groups, chickens administered with *A. vera* polysaccharides at the dose rate of 200 and 300 mg/kg of body weight showed significantly higher (p<0.05) response as compared to those administered with *A. vera* polysaccharides at dose rate of 100 mg/kg of body weight (Fig. 2).

Carbon Particle Clearance Assay

The results of clearance index showed significantly higher (p<0.05) clearance index in control group A_4

Table 1: Quantitative analysis of monosaccharides detected in the hydrolyzed sample of *Aloe vera* polysaccharides

Monosaccharide	Retention Time (minutes)	Area (mV.s)	Height (mV)	Quantity (molar %)
Maltose	9.423	11.837	0.540	0.04
Glucose	11.080	35.252	0.605	0.11
Mannose	12.550	36.885	0.612	0.02

Table 2: Carbon particle clearance assay

Groups	Carbon particle clearance assay post administration of *A. vera* polysaccharides	
	Clearance index (K) (Mean OD±SE)	Phagocytic index (α) (Mean OD±SE)
A_1	0.0106±0.0039^{b}	69.3054±13.6106^{b}
A_2	0.0073±0.0024^{c}	4.8283±9.3211^{a}
A_3	0.0124±0.0019^{ab}	51.2677±10.2013^{bc}
A_4	0.0185±0.0040^{a}	48.5554±9.8143^{c}

Means sharing similar letters in a column are statistically non-significant (p>0.05)

$A_{1=}$ *A. vera* polysaccharides @ 100 mg/kg of BW; A_2=*A. vera* polysaccharides @ 200 mg/kg of BW; A_3=*A. vera* polysaccharides @ 300 mg/kg of BW; A_4=Control

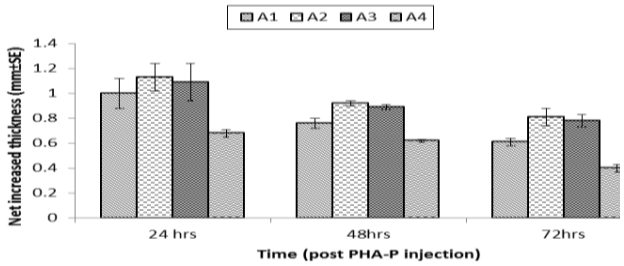

Fig. 1: *In vivo* lymphoproliferative response to PHA-P in experimental and control chickens

$A_{1=}$ *A. vera* polysaccharides @ 100mg/kg of BW; A_2=*A. vera* polysaccharides @ 200 mg/kg of BW; A_3=*A. vera* polysaccharides @ 300 mg/kg of BW; A_4=Control

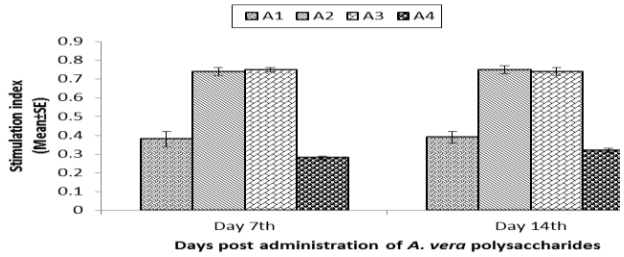

Fig. 2: *In vitro* lymphoproliferative response to Concanavalin-A in experimental and control chickens

$A_{1=}$ *A. vera* polysaccharides @ 100mg/kg of BW; A_2=*A. vera* polysaccharides @ 200 mg/kg of BW; A_3=*A. vera* polysaccharides @ 300 mg/kg of BW; A_4=Control

(0.0185±0.0040) and other experimental groups showed low clearance index values indicating less carbon in blood. Among the experimental groups, group A_2 showed lowest K value (0.0073±0.0024) followed by group A_1 (0.0106±0.0039) and A_3 (0.0124±0.0019). A_3 response was statistically similar to group A_1 and A_4 (Table 2).

The results of phagocytic index (α) showed significantly higher (p<0.05) response in all three experimental groups as compared to control. Maximum response was observed in group A_2, which was administered with *A. vera* polysaccharides at the dose rate of 200mg/kg body weight, followed by A_1 and A_3. Statistically, group A_2 showed significantly higher (p<0.05) phagocytic index followed by group A_1; whereas, A_3 showed response, which was similar to group A_1 and A_4 (Table 2).

Humoral Immune Responses

Antibody response to sheep red blood cells (SRBCs): All the experimental groups showed higher immunoglobulins (Igs, IgM and IgG) titers as compared to control. However, at 7thday post primary injection (PPI), polysaccharides administered group A_2 (at the dose rate of 200 mg/kg body weight) showed maximum Igs, IgM and IgG titers (73.52, 52.4, 21.11) followed by group A_3 (64, 48, 16) group A_1 (42.22, 28.29, 13.92) and A_4 (32, 19.87, 12.12). On day 14th PPI, geomean titer for total anti-SRBC antibodies was highest in group A_2 (55.72, 18.95, 36.75) followed by A_3 (42.22, 14.36, 27.85), A_1 (42.22, 17.97, 24.25) and A_4 group (24.25, 8.25, 16). Same trend was observed on day 7th and 14th post-secondary injections (PSI) (Table 3).

Effect on the Development of Lymphoid Organs of Chickens

Experimental groups showed apparently higher per cent organ-body weight ratios as compared to control group; whereas, the statistical difference was non-significant (p>0.05) in immune organs of all groups except thymus of experimental group A_2 showed statistically significant response as compared to control (Table 4).

Effects of Polysaccharides on Weight Gains and Feed Conversion ratio (FCR)

Experimental groups (A_2 and A_3) showed significantly higher weight gains as compared to group A_1 and A_4, control. Maximum weight gains (gm±SE) were observed at 6th week in group of chickens administered with *A. vera* polysaccharides. Among the experimental groups, maximum weight gains were observed in group A_3 followed by A_2, $A_1$and control group A_4 (Fig.3). Similarly, all experimental groups administered *A. vera* polysaccharides showed better FCR as compared to control. However, FCR was better in group A_3 (1.95) followed by group A_2 (1.96), A_1 (2.01) and control group A_4 (2.16).

Discussion

Biological response modifiers (BRM) are compounds, which induce modifications in the functioning of the

Table 3: Antibody response to sheep red blood cells in experimental and control chickens

Group	Total anti-SRBCs antibody titer			
	Day 7 PPI	Day 14 PPI	Day 7 PSI	Day 14 PSI
A_1	42.22	42.22	64	48.50
A_2	73.52	55.72	84.45	64
A_3	64	42.22	73.51	55.71
A_4	32	24.25	27.85	13.92
	----------Immunoglobulin-M--------			
A_1	28.29	17.97	36.14	11.74
A_2	52.40	18.95	47.69	15.49
A_3	48	14.36	45.65	13.49
A_4	19.87	8.25	15.73	4.73
	-------Immunoglobulin-G-----			
A_1	13.92	24.25	27.85	36.75
A_2	21.11	36.75	36.75	48.50
A_3	16	27.85	27.85	42.22
A_4	12.12	16	12.12	9.18

$A_{1=}$ *A. vera* polysaccharides @ 100 mg/kg of BW; A_2=*A. vera* polysaccharides @ 200 mg/kg of BW
A_3=*A. vera* polysaccharides @ 300 mg/kg of BW; A_4=Control

Table4: Organ-body weight ratio on day 14th post administration of *Aloe vera* polysaccharides in experimental and control chickens

Group	Thymus (Mean±SE)	Spleen (Mean±SE)	Bursa (Mean±SE)	Caecal tonsils (Mean±SE)
A_1	0.36±0.011[b]	0.23±0.011	0.26±0.008	0.09±0.008
A_2	0.38±0.008[a]	0.23±0.008	0.26±0.006	0.09±0.010
A_3	0.37±0.0158[ab]	0.23±0.007	0.26±0.0100	0.09±0.007
A_4	0.35±0.008[b]	0.22±0.006	0.25±0.016	0.08±0.007

Means sharing similar letters in a column are statistically non-significant (p>0.05)
$A_{1=}$ *A. vera* polysaccharides @ 100 mg/kg of BW; A_2=*A. vera* polysaccharides @ 200 mg/kg of BW; A_3=*A. vera* polysaccharides @ 300 mg/kg of BW; A_4=Control

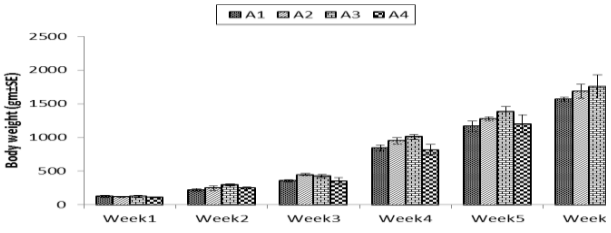

Fig. 3: Weekly (1st-6th) weight gains in experimental and control groups
$A_{1=}$ *A. vera* polysaccharides @ 100mg/kg of BW; A_2=*A. vera* polysaccharides @ 200 mg/kg of BW; A_3=*A. vera* polysaccharides @ 300 mg/kg of BW; A_4=Control

immune system and produce effects on the physiology of man and animals (Murthy *et al*., 2010). In this regard, *Aloe (A.) vera* is considered as one of the promising candidate having biological response modifying effects in different animal models (Akhtar *et al*., 2012; Farahnejad *et al*., 2013; Manvitha and Bidya, 2014).

A. vera reported to cure variety of conditions including fever, burns and wound healing, gastrointestinal disorders, sexual vitality and fertility problems, inflammation, ulcer, arthritis, cancer, immunosupression, AIDS, and coccidiosis (Mwale *et al*., 2005; Bashir *et al*., 2011; Akhtar *et al*., 2012). It is also considered an effective tool to enhance the immunity in broiler chicks and increasing microvilli density (Jinag *et al*., 2005; Akhtar *et al*., 2012; Yim *et al*., 2011).

Therapeutic efficacy of *A. vera* components had been reported in different animal models and human beings with promising results (Strickland, 2001; Agarry *et al*., 2005; Mwale *et al*., 2006), although limited work has been conducted on poultry (Mwale *et al*., 2005; Akhtar *et al*., 2012; Yim *et al*., 2011). Polysaccharides are carbohydrates, containing monosaccharide units joined to each other by glycosidic linkages (Varki *et al*., 2008). Polysaccharides have shown biological response modifying activities (Ramberg *et al*., 2010), by affecting both the humoral and cellular immune responses (Xue and Meng, 1996; Tzianabos, 2000). *A. vera* is a rich source of many carbohydrate units including polysaccharide, mannose-6-phosphate and acemannan (Hamman, 2008). Acemannan (β-(1, 4)-linked acetylated mannose) has shown immunomodulatory activities by macrophages activation; enhanced cytokine release, stimulated macrophages interactions and increased production of cytotoxic T-lymphocytes (Roberts and Travis, 1995).

In the current study, purified polysaccharides from *A. vera* were analyzed by using high performance liquid chromatography (HPLC). Three different monosaccharides including maltose, glucose and mannose in hydrolyzed solution of *A. vera* at retention times 9.423, 11.080 and 12.55, respectively were observed.

Major polysaccharides containing Mannose named Acemannan has been isolated by various researchers (Hart *et al*., 1989; Luta *et al*., 2009). Acemannan is a linear polysaccharide made of (1, 4)-linked mannosyl residues (Femenia *et al*., 1999). Moghaddasi and Verma (2011) described that *A. vera* contained two polysaccharides acemannan and glucomannan; whereas, saccharides were mannose, aldopentose, L-rhamnose and glucose. Chang *et al*. (2010) isolated mannose glucose and galactose from gel juice. Present study indicated the presence of glucose, maltose and mannose, which were also reported by Luta *et al*. (2009).

Results of *in vivo* lymphoproliferative response to PHA-P showed significantly higher (p<0.05) response in chickens administered with *A. vera* polysaccharides as compared to control. In polysaccharides given groups (100, 200 and 300 mg/kg of body wt.) chickens of group A_2 revealed a significantly higher (p<0.05) response as compared to control, which was best lymphoproliferative response calculated as compared to all other groups. During body defenses phagocytosis immediately effect invading foreign materials; whereas T cells take time to be stimulated and proliferated before responding invasion (Lamont, 1986). PHA-P (T-cell mitogen) induces proliferation of T-lymphocytes. Its particular site injection induces localized *in*

vivo T-cell lymphoproliferation (Cheema *et al*., 2003). These higher responses in group A_2 may be due to *A. vera* polysaccharides (Acemannan). From these results, it can be speculated that *A. vera* polysaccharides induced cellular immune response that may play role in controlling and clearing the organism (Kougt *et al*., 1994) and thus enhance resistance against diseases in chickens (Parmentier *et al*., 2001).

Concanavalin-A was used to evaluate *in vitro* cell mediated immune responses (lymphoblastogenic response) of chicken peripheral blood leukocytes (PBLs) to Con-A at 7th and 14th day post administration of *A. vera* polysaccharides. Results showed higher lymphoproliferation response in administered chickens than control. This increased stimulation index in treated groups was due to direct contact of T-lymphocytes mitogen receptors, which came direct contact with Con- A (T-cell mitogen). It caused lymphocytes cell division, which result in increased response to Con-A (Qureshi *et al*., 2000).

In the current study, carbon particle clearance test was used as an indicator of phagocytic activity of reticuloendothelial system measured by clearance of carbon particles from blood stream (Neha and Mishra, 2011). These cells have an important role in particle clearance from blood (Ismail and Asad, 2009). Phagocytosis is a defensive mechanism, which showed its effects immediately after invasion of foreign particles (Lamont, 1986). Macrophages phagocytic activity is an important marker of immune system of an organism, which can be increased by herbal polysaccharides (Shin *et al*., 2002).

In current study, when India ink injected intravenously into systemic circulation of birds, carbon particles were engulfed by macrophages. The clearance rate of carbon particles and phagocytic index, was calculated. The results of present study showed significantly higher clearance index in control group than other groups. The results of phagocytic index (α) showed significantly higher response in all three medicated groups as compared to control. Maximum response was observed in group A_2, which were administered with *A. vera* polysaccharides. Results of present study contrast with study of Neha and Mishra study on immunomodulator activity of Trikatu mega Ext. Results indicated higher phagocytic index of treated groups (at dose rate of 100 and 200 mg/kg body weight), which may be due to reticuloendothelial system stimulation (Neha and Mishra, 2011; Hajra *et al*., 2012).

Results of humoral immune response showed significantly higher total Igs, IgG and IgM titers in groups administered with *A. vera* polysaccharide. Effect of polysaccharides to stimulate the humoral immune responses is reported in mice in some previous studies (Madan *et al*., 2008; Yu *et al*., 2009). Present study showed contrast results to findings of other scientists, who reported an increased antibody titer after feeding *A. vera* against new castle virus (Jinag *et al*., 2005). Present study results were similar to Yu *et al*., 2009, who concluded that *A. vera* polysaccharides administered groups of rats showed significant increase in Ig A, Ig M and Ig G levels. In the current study, immune organ index was calculated in terms of organ-body weight ratios. Experimental groups administered with *A. vera* polysaccharides showed apparently higher per cent organ-body weight ratios as compared to control group; although, the difference was statistically non-significant ($p>0.05$) except thymus of experimental group A_2 which showed significant response as compared to control.

Maximum weight gains (gms±sd) at 6th week were observed in groups administered with *A. vera* polysaccharides. These results were in accordance to observations of (Jinag *et al*., 2005; Mehmet *et al.* 2005; Durrani *et al*. 2007). Increased body weight gain and FCR had also been reported in other studies (Durrani *et al*., 2008); however, *A. vera* combination with *Curcuma longa* showed non-significant difference in weight gain but numerically higher body weight gains (Mehala and Moorthy, 2008). Further, several carbohydrate polymers (glucomannans) present in *A. vera* played role in healing process (Shelton, 1991) and inhibited cyclooxygenase pathway resulting in decreased prostaglandin production from arachidonic acids (Grindlay and Reynolds, 1986).

Results of current study indicated that *A. vera* polysaccharides has the potential to stimulate the humoral and cellular arms of the immune responses and released cytokines to induce localized inflammatory reactions, which increased vascular permeability; caused vasodilation, activation of macrophages; enhanced phagocytic activity and more lytic enzyme production for destruction of pathogen(s) (Dashputre and Naikwade, 2010; Neha and Mishra, 2011).

From the current study, it was concluded that *A. vera* derived polysaccharides have potential to be used as immunotherapeutic agent(s) in chickens and can be commercialized.

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