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*Full Length Research Paper*

# The effect of Aloe vera extract on humoral and cellular immune response in rabbit

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**Some plant polysaccharides are well known to possess immunostimulatory effects. *Aloe vera* possesses confirmed curative or healing actions. The aim of this study was to evaluate the effect of the administration of *A. vera* plant extract on cellular and humoral immune response in rabbits. 20 healthy male New Zealand white rabbits were randomly divided into five treatment groups: Groups consisted of: 1) control group (normal saline); 2) *A. vera* control; 3) vaccine control; 4) 50 mg *A. vera* extract + vaccine; 5) 150 mg *A. vera* extract + vaccine. The used vaccine was for myxomatosis. Blood samples were obtained at four time points: days 0, 7, 14 and 21 of the study. $CD^{4+}$ and $CD^{8+}$ lymphocytes frequency and serum immunoglobulin concentrations were evaluated. According to the results, oral administration of *A. vera* affected the composition of lymphocyte subsets and serum immunoglobulins positively. These findings demonstrated that *A. vera* may stimulate both cellular and humoral immune responses after immunization.**

**Key words:** *Aloe vera*, cellular and humoral immune, immunization, rabbits.

## INTRODUCTION

Immuno-stimulants are widely used for health management in animals (Pugh et al., 2001). Some plant polysaccharides are well known to possess immuno-stimulatory effects (Pugh et al., 2001; Im et al., 2005; Qiu et al., 2000). *Aloe vera* possesses confirmed curative or healing actions. A total of 360 *A. vera* species (commonly known as *A. vera*) are grown in the dry regions of North America, Europe and Asia. It is consumed both as a vegetable and as a traditional Chinese medicine in single and compounded prescriptions for treating fever, constipation and ringworm. Some specially prepared *A. vera* extracts possess many biological activities such as anti-inflammation, anti-cancer, antioxidant, anti-diabetes and macrophage activation (Grover et al., 2002; Krishnan, 2006; Xiao et al., 2007; Xu et al., 2008). Polysaccharide with biological response modifiers derived from *A. vera* are mainly mannose polymers (Leung et al., 2004). *A. vera*'s role as a biologically active immunomodulator has been well documented for years. Especially, these products have been extensively studied for their immunological effects (Yates et al., 1992; Im et al., 2005).

Administration of *A. vera* has been universally demonstrated to result in marked increase in phagocytic and proliferative activity of the reticuloendothelial system (Hanaue et al., 1989; Im et al., 2005). These products can modulate and stimulate both humoral and cellular immunity and also stimulate proliferation of murine pluripotent hematopoietic stem cells, granulocyte macrophage colony-forming cells, and cells forming myeloid and erythroid colonies (Egger et al., 1996; Im et al., 2005; Boudreau and Beland, 2006). It is demonstrated that immunomodulating and/or immunostimulating effects of these products are dependent on the activation of the innate immune cells (macrophages, neutrophils, lymphocytes and NK cells), synthesis and release of cytokines (TNF-α, IFN-α, IFN-γ, IL-1, IL-2, IL-6 and IL-8), generation of enhanced cell-mediated responses, and induction of nitric oxide production (Leung et al., 2004; Pugh et al., 2001; Im et al., 2005; Boudreau and Beland, 2006). Besides the role in cellular immunity, researchers also found that *A. vera* induces antibody responses. The

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effects on antibody response were proved by the significant increase in the number of B lymphocytes forming both IgM and IgG antibodies (Yates et al., 1992). The aim of this study was to evaluate the effect of *A. vera* administration on cellular and humoral immune response in rabbits.

## MATERIALS AND METHODS

### Preparation of *A. vera*

*A. vera* polysaccharides were prepared according to the method described by Wu et al. (2006). In brief, the leaves (300 g) of the plant, after blending in an electrical grinder, were extracted thrice with 2500 ml of distilled water at 70 to 80°C for 2 h. The filtrate of the obtained extract was condensed to syrup (ca. 500 ml), to which cold 95% ethanol (approximately 1500 ml) was added. The crude polysaccharide part was precipitated from the alcoholic liquor during its subsequent standing at 4°C overnight. The precipitate formed was collected by centrifugation at 12,000 x *g* and repeatedly washed sequentially with possibly less amounts of ethanol, acetone and ether, respectively. The refined crude polysaccharide (2.4 g) was re-dissolved in distilled water (240 ml) at a concentration of 1% (w/v) followed by filtration. The filtrate was deproteinized by treating it with trichloroacetic acid following the procedure detailed elsewhere (Yang et al., 1999). After centrifugation at 12,000 x *g* for 10 min, the supernatant was then precipitated with 3-fold volumes of 95% ethanol to obtain the crude polysaccharides.

### Animal preparation and study design

20 healthy male New Zealand white rabbits, weighing 2.0 to 2.5 kg, were used. The rabbits were kept for two weeks to adapt to the environmental conditions. They were housed in individual cages in a room with controlled temperature of 20 ± 22°C, and a 12 h light/dark cycle. The rabbits were fed standard diet and provided with fresh water *ad libitum*. Animals were randomly divided into five treatment groups (4 per group): Groups consisted of: 1) control group (normal saline, o.p.); 2) *A. vera* control o.p.; 3) vaccine control,;4) 50 mg *A. vera* extract o.p. + vaccine; 5) 150 mg *A. vera* extract o.p. + vaccine. The used vaccine was for myxomatosis (Mixohipra-FSA®, Laboratorios Hipra, S.A. one dose/rabbit). Blood samples were obtained from the central ear artery using 22 G catheter at four time points: days 0, 7, 14 and 21 of the study. Blood samples were transferred into heparinized and none heparinized tubes. Plasma was separated and stored frozen until assayed. For immunological analysis, the serum was separated by centrifugation at 3000 rpm/min for 10 min and stored at -20°C until analysis.

### Flow cytometric analysis

For evaluation of $CD^{4+}$ and $CD^{8+}$ lymphocytes frequency, single cell suspensions were prepared by mechanical tissue disaggregation in cold PBS and centrifuged at 800 x *g* for 4 min. Cells were washed twice with PBS. We used unlabeled CD16/CD32 to rule out any non-specific antibody binding and included IgG controls for these three colors. Cell suspensions (about $10^6$ cells) were incubated with a mixture of the anti-CD4-PE-cy5 + anti-CD8-R-PE antibodies at 4°C for 30 min in a dark environment; then cells were washed twice with PBS and suspended in PBS. The cells were analyzed using a flow cytometry (Beckman Coulter Epics XL-4). The data were analyzed using the System II version 3.0. About 20,000 lymphocytes were examined for each sample.

### Immunoglobulin content

Serum immunglobulin (IgG, IgM and IgA) concentrations were measured by ELISA technique (Microplate Reader® - DAS) using commercial test kit for IgA, IgG and IgM (MCA630®, Serotec), according to product description.

### Statistical analysis

Results were expressed as mean ± S.E.M. Multiple comparisons were performed by ANOVA followed by the Tukey honestly significant difference (HSD) test. In all the analyses, the level of significance was set at $P<0.05$.

## RESULTS

### Effect of *A. vera* polysaccharide on $CD^{4+}$ and $CD^{8+}$ lymphocytes

Table 1 shows the effect of *A. vera* on the $CD^{4+}$ and $CD^{8+}$ lymphocytes in the blood of rabbits in different groups. *A. vera* treatment produced a significant increase in the blood $CD^{4+}$ after 14 and 21 days, respectively ($P<0.05$) and $CD^{8+}$ lymphocytes after 7, 14 and 21 days ($P<0.05$), when compared with the control group. Therefore, *A. vera* treatment significantly enhanced blood $CD^{4+}$ and $CD^{8+}$ lymphocytes when compared with the control group dose dependently.

### Effect of *A. vera* polysaccharide on plasma IgM, IgG and IgA levels

According to Table 2, serum IgM concentrations in groups 3, 4 and 5 increased inconsiderately ($P<0.05$) on days 7, 14 and 21 when compared to the control. Serum IgG concentrations in group 3 increased significantly only on day 14 ($P<0.05$), whereas its levels in group 4 and 5 increased both on days 7, 14 and 21 ($P<0.05$). But serum IgG concentrations in groups 1 and 2 were not different significantly ($P>0.05$). There were no significant changes in serum IgA concentrations in all the groups, during the study.

## DISCUSSION

There are various indexes to evaluate the function of the immune system, for example, the composition of lymphocyte subsets and serum immunoglobulins are employed for the evaluation of the cellular and humoral immune responses, respectively. $CD^{4+}$ cells are a type of white blood cell. White blood cells are important in fighting infections. A $CD^{4+}$ count is a blood test to determine how well the immune system is working (Shirwan et al., 2003). $CD^{8+}$ is a transmembrane glycoprotein that serves as a co-receptor for the T cell receptor (TCR). Like the TCR, $CD^{8+}$ binds to a major histocompatibility complex (MHC)

**Table 1.** Effect of *A. vera* polysaccharide on $CD^{4+}$ and $CD^{8+}$ lymphocytes. The values are presented as mean ± SEM.

| CD | Day | G 1 | G 2 | G 3 | G 4 | G 5 |
|---|---|---|---|---|---|---|
| CD $^{4+}$ | 0 | 69.3 ± 3.3 | 72.4 ± 3.2 | 69.4 ± 3.4 | 71.6 ± 3.8 | 72.2 ± 4.4 |
| | 7 | 71.4 ± 3.8 | 80.3 ± 4.6 | 71.6 ± 3.5 | 74.6 ± 3.5 | 75.5 ± 3.9 |
| | 14 | 72.1 ± 4.2 | 81.5 ± 4.2$^{a}$ | 73.4 ± 4.3 | 80.8 ± 4.7$^{a}$ | 88.4 ± 4.3$^{b}$ |
| | 21 | 70.8 ± 3.7 | 89.2 ± 4.9$^{b}$ | 72.7 ± .3.6 | 86.2 ± 4.6$^{b}$ | 89.7 ± 4.6$^{b}$ |
| CD $^{8+}$ | 0 | 48.3 ± 1.8 | 50.4 ± 1.9 | 48.7 ± 1.7 | 51.2 ± 2.1 | 50.8 ± 1.9 |
| | 7 | 50.1 ± 2.1 | 51.8 ± 2.2$^{a}$ | 51.8 ± 2.5$^{a}$ | 58.5 ± 2.3$^{b}$ | 59.3 ± 2.7$^{b}$ |
| | 14 | 48.8 ± 1.7 | 57.4 ± 1.7$^{b}$ | 52.2 ± 2.3$^{a}$ | 64.4 ± 2.5$^{c}$ | 66.7 ± 2.5$^{c}$ |
| | 21 | 49.3 ± 2.0 | 52.7 ± 1.8$^{a}$ | 52.5 ± 2.4$^{a}$ | 60.5 ± 2.3$^{b}$ | 65.4 ± 2.8$^{c}$ |

a to c: Values with different superscripts differ significantly (P<0.05).

**Table 2.** Effect of *A. vera* polysaccharide on plasma IgM, IgG and IgA levels. The values are presented as mean ± SEM.

| Ig | Day | G 1 | G 2 | G 3 | G 4 | G 5 |
|---|---|---|---|---|---|---|
| Ig M | 0 | 154.4 ± 3.2 | 158.5 ± 2.8 | 160.6 ± 3.6 | 157.6 ± 3.1 | 159.1 ± 2.9 |
| | 7 | 161.5 ± 3.3 | 156.4 ± 3.0 | 188.4 ± 3.7$^{a}$ | 192.2 ± 3.5$^{a}$ | 189.4 ± 3.7$^{a}$ |
| | 14 | 158.7 ± 2.7 | 160.6 ± 3.1 | 205.7 ± 3.8$^{b}$ | 210.5 ± 4.1$^{c}$ | 212.4 ± 4.3$^{c}$ |
| | 21 | 156.2 ± 2.8 | 157.3 ± 2.2 | 187.3 ± 3.4$^{a}$ | 205.2 ± 3.8$^{b}$ | 208.7 ± 3.8$^{bc}$ |
| Ig G | 0 | 987.6 ± 9.8 | 992.7 ± 10.4 | 1067.5 ± 12.2 | 1024.8 ± 11.7 | 997.4 ± 10.6 |
| | 7 | 1032.8 ± 11.6 | 1052.4 ± 9.8 | 989.6 ± 8.6 | 1273.4 ± 11.8$^{a}$ | 1267.5 ± 12.5$^{a}$ |
| | 14 | 1067.4 ± 11.3 | 1073.9 ± 10.7 | 1248.3 ± 12.6$^{a}$ | 1481.5 ± 13.8$^{b}$ | 1563.3 ± 15.3$^{c}$ |
| | 21 | 996.3 ± 9.9 | 1098.5 ± 11.3 | 1182.7 ± 10.8 | 1287.8 ± 11.3$^{a}$ | 1446.9 ± 11.7$^{b}$ |
| Ig A | 0 | 48.23 ± 4.3 | 49.65 ± 3.8 | 48.66 ± 5.2 | 50.35 ± 4.9 | 52.87 ± 5.4 |
| | 7 | 51.32 ± 3.8 | 52.74 ± 4.6 | 49.54 ± 5.1 | 50.23 ± 4.8 | 51.82 ± 4.4 |
| | 14 | 50.71 ± 3.9 | 50.98 ± 3.8 | 49.88 ± 4.7 | 48.75 ± 5.3 | 49.69 ± 3.9 |
| | 21 | 50.42 ± 4.2 | 50.65 ± 4.4 | 48.74 ± 3.7 | 50.44 ± 5.1 | 50.75 ± 5.2 |

a to c: Values with different superscripts differ significantly (P<0.05).

molecule, but it is specific for the class I MHC protein. It is predominantly expressed on the surface of cytotoxic T cells, but can also be found on natural killer cells (Vecchione et al., 2002). In previous experimental studies, increase or stimulation in $CD^{4+}$, CD8 T lymphocytes and IgG concentrations were related to administration of *A. vera* (Hanaue et al., 1989; Yates et al., 1992; Leung et al., 2004; Sampedro et al., 2004; Boudreau and Beland, 2006). Similarly, in this study, $CD^{4+}$ T lymphocytes on days 14 and 21 and $CD^{8+}$ T lymphocytes on days 7, 14 and 21 increased in the study groups. Serum IgG and IgM concentrations on days 7, 14 and 21 AV increased in the study groups too. Immunostimulating action of *A. Vera* was reported to be induced by potentiation of synthesis and release of several cytokines such as TNF-α, IFN-γ, IL-1, IL-2 and IL-6 (Yates et al., 1992; Qiu et al., 2000; Leung et al., 2004; Im et al., 2005; Boudreau and Beland, 2006). Moreover, it has been shown that *A. vera* derived polyglucans significantly supported the formation of specific and nonspecific antibodies (Im et al., 2005; Boudreau and Beland, 2006). For this reason, increase in serum IgG after application of *A. vera* may be attributed to elevation in humoral immune response due to increase in both $CD^{4+}$ T lymphocyte and cytokines synthesis or release. All lymphocyte subsets ratios and serum immunoglobulin concentrations except for IgA, increased in the study groups. On the other hand, $CD^{4+}$ and $CD^{8+}$ T lymphocyte, serum IgG and IgM concentrations increased in the study groups. Therefore, increases in both $CD^{4+}$ and $CD^{8+}$ T lymphocytes, and serum IgG and IgM concentrations may be attributed to modulatory or stimulatory effects which suggest the effects of *A. vera* on both cellular and humoral immune response following vaccination. Similar observations on immune system by *A. vera* have also been reported (Hanaue et al., 1989; Sampedro et al., 2004; Im et al., 2005; Boudreau, Beland, 2006)

$CD^{8+}$ T lymphocyte is completely related to cellular immune response, while $CD^{4+}$ T lymphocyte takes part in both cellular and humoral immune responses. $CD^{4+}$ T

lymphocytes according to cytokines that they release are divided into Th1 and Th2 cells. Antigenic stimulation of Th1 cells induce T cell cytotoxicity and macrophage activation through release of cytokines like IL-2, IFN-γ and TNF-β and also in the stimulation of Th2 cells and production of B cells. Th2 cells plays role in the stimulation of IL-4, IL-5, IL-10 and IL-13 cytokines leading to production of B cells and antibodies 43-45. The findings relating to lymphocyte subsets confirm the findings of other studies where *A. vera* was used (Chinnah et al., 1992; Boudreau and Beland, 2006). Our results showed that serum IgM, serum IgG and IgM concentrations in post vaccination period in the study increased as reported by researchers (Boudreau and Beland, 2006; Chinnah et al., 1992; Im et al., 2005; Pugh et al., 2001). On the other hand, the authors' results on the evaluation of the effects of *A. vera* on antibody response in laboratory animals showed that their potential to increase antibody response was present only when they were used as adjuvant (Yates et al., 1992; Chinnah et al., 1992). These findings demonstrate that *A. vera* may stimulate humoral immune response as reported by some researchers (Leung et al., 2004; Sampedro et al., 2004; Im et al., 2005).

In conclusion, this study demonstrated that oral administration of *A. vera* affects various aspects of the immune system, including the effects on the composition of lymphocyte subsets and serum immunoglobulins. These findings demonstrate that *A. vera* extract may stimulate both cellular and humoral immune responses after immunization. The observed changes after administration of *A. Vera* extract following immunization might occur with similar mechanisms. However, since exact mechanisms of the stimulation of cell-mediated and humoral immunity related to *A. vera* application after vaccination still remain unknown, further investigations might be useful to understand the stimulatory mechanisms through investigation of Th1 and Th2 helper cells ratios, and cellular and humoral immune system specific cytokines.

**REFERENCES**


Boudreau MD, Beland FA (2006): An evaluation of the biological and toxicological properties of Aloe Barbadensis (Miller), *Aloe vera*. J. Environ. Sci. Health C. 24: 103-154.

Chinnah AD, Baig MA, Tizard IR, Kemp MC (1992). Antigen dependent adjuvant activity of a polydispersed beta-(1,4)-linked acetylated mannan (acemannan). Vaccine, 10: 551-557

Egger SF, Brown GS, Kelsey LS, Yates KM, Rosenberg LJ, Talmadge JE (1996). Studies on optimal dose and administration schedule of a hematopoietic stimulatory β-(1,4)-linked manan. Int. J. Immunopharmacol. 18: 113-126.

Grover JK, Yadav S, Vats V (2002). Medicinal plants of India with anti-diabetic potential. J. Ethnopharmacol. 81: 81–100.

Hanaue H, Tokuda Y, Machimura T, Kamijoh A, Kondo Y, Ogoshi K, Makuuchi H, Nakasaki H, Tajima T, Mitomi T, Kurosawa T (1989). Effects of oral lentinan on T-cell subsets in peripheral venous blood. Clin. Ther. 11: 614-622.

Im SA, Oh ST, Song S, Kim MR, Kim DS, Woo SS, Jo YI, Lee CK (2005). Identification of optimal molecular size of modified Aloe polysaccharides with maximum immunomodulatory activity. Int. Immunopharmacol. 5: 271-279.

Krishnan P (2006). The scientific study of herbal wound healing therapies: Current state of play. Curr. Anaesth. Crit. Care, 17: 21–27.

Leung MYK, Liu C, Zhu LF, Hui YZ, Yu B, Fung KP (2004). Chemical and biological characterization of a polysaccharide biological response modifier from *Aloe vera* L. var. chinensis (Haw.) Berg. Glycobiol. 14: 501-510.

Pugh N, Ross SA, ElSohly MA, Pasco DS (2001). Characterization of aloeride, a new high-molecular-weight polysaccharide from *Aloe vera* with potent immunostimulatory activity. J. Agric. Food. Chem. 49: 1030-1034.

Qiu Z, Jones K, Wylie M, Jia Q, Orndorf S (2000). Modified Aloe barbadensis polysaccharide with immunorequlatory activity. Planta. Med. 66: 152-156.

Sampedro MC, Artola RL, Murature M, Murature D, Ditamo Y, Roth GA, Kivatinitz S (2004). Mannan from Aloe saponaria inhibits tumoral cell activation and proliferation. Int. Immunopharmacol. 4: 411-418.

Shirwan H, Mhoyan A, Yolcu ES, Que XY, Ibrahim S (2003). Chronic cardiac allograft rejection in a rat model disparate for one single class I MHC molecule is associated with indirect recognition by CD4+ T cells. Transplant. Immunol. 11: 179–185.

Vecchione A, Catchpole B, D'Mello F, Kanellos T, Hamblin A (2002). Modulating immune responses with dendritic cells: An attainable goal in veterinary medicine? Vet. Immunol. Immunopathol. 87: 215–221.

Wu JH, Xu C, Shan CY, Tan RX (2006). Antioxidant properties and PC12 cell protective effects of APS-1, a polysaccharide from *Aloe vera* vera var. Chinensis. Life. Sci. 78: 622–630.

Xiao BX, Guo JM, Liu DH, Zhang S (2007). Aloe-emodin induces in vitro G2/M arrest and alkaline phosphatase activation in human oral cancer KB cells. Oral. Oncol. 43: 905–910.

Xu FG, Liu Y, Zhang ZJ, Song R, Dong HJ. Tian Y (2008). Rapid simultaneous quantification of five active constituents in rat plasma by high-performance liquid chromatography/tandem mass spectrometry after oral administration of Da-Cheng Qi decoction. J. Pharm. Biomed. Anal. 47: 586–595.

Yang F, Zhang T, Tian G, Cao H, Liu Q, Ito Y (1999). Preparative isolation and purification of hydroxyanthraquinones from Rheum officinale Baill by high-speed counter-current chromatography using pH-modulated stepwise elution. J. Chromatogr. A. 858: 103–107.

Yates KM, Rosenberg LJ, Harris CK, Bronstad DC, King GK, Biehle GA, Walker B, Ford CR, Hall JE, Tizard IR (1992). Pilot study of the effect of acemannan in cats infected with feline immunodeficiency virus. Vet. Immunol. Immunopathol. 35: 177-189.