



Effect of *Aloe vera* polysaccharides on immunity and antioxidant activities in oral ulcer animal models

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ABSTRACT

Aloe vera polysaccharides have traditionally been used in Asian cultures as medicinal plants to enhance immunity and reduce oxidative injury. The current investigation was conducted to examine the effects of *A. vera* polysaccharides on various in vivo parameters of innate immunity and antioxidant enzymes activities in oral ulcer animals. Forty wistar rats were randomly divided into the following 1 control group and 3 experimental groups (each group contained 10 rats). Rats in experimental groups were orally fed by *A. vera* polysaccharides. Rats in control group were orally fed by same volume of saline. The results showed that *A. vera* polysaccharides enhanced immunity activity and exerted antioxidant effects compared with vehicle controls. These results demonstrate, for the first time, that *A. vera* polysaccharides are effective in enhancing innate immunity and suppressing oxidative injury in oral ulcer animals.

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1. Introduction

Recurrent oral ulcer is one of the most common oral mucosal diseases. Oral ulcer (or mouth ulcers) can be very painful and the resulting lesions can be mild or severe. Although oral ulcer has been the subject of considerable studies, the etiology and pathogenesis of the disease have not yet been completely explained (Tran, Blair, Deeb, & Cardenas, 1996). Local and systemic conditions, genetic, immunological and infectious factors have been identified as potential etiological agents. Recurrent oral ulcer is a chronic systemic inflammatory disease. Inflammatory reactions trigger the oxidative stress and oxidants decrease the level of antioxidants. The free radical metabolism in the erythrocyte and serum of oral ulcer patients has been investigated in two recent studies (Katayama, Nishizawa, Hirano, Yamamura, & Momose, 2000; Orem et al., 2002). Also there are a few studies showing the active role of white blood cells in oral ulcer pathogenesis (Pande, Hiran, Pani, & Vishwanathan, 1995).

Referred to as a miracle plant, *Aloe vera* possesses confirmed curative or healing actions. A total of 360 *A. vera* species (commonly accepted as *A. vera vera*) are growing in the dry regions of North American, Europe and Asia. It has been consumed both as a vegetable and as a traditional Chinese medicine in single and compounding 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, Yadav, &

Vats, 2002; Krishnan, 2006; Xiao, Guo, Liu, & Zhang, 2007; Xu et al., 2008). Polysaccharides isolated from *A. vera* contribute to ethnomedicinal and bioactive properties of the plant. A few reports dealing with the characterization of chemical constituents of the polysaccharides are available (Ai, Wang, Yang, Zhu, & Lei, 2008; Chen, Bao, Huang, & Li, 2002; Yan, Cui, Zhang, & Chu, 2003; Anandan, Rekha, Saravanan, & Devaki, 1999; Vinson, Al Kharrat, & Andreoli, 2005).

The aim of our study was to evaluate the change in the immunity and oxidative system in blood and mucosa of rats with oral ulcer by measuring intracellular related parameters: CD⁴⁺, CD⁸⁺ and CD⁴⁺/CD⁸⁺, IL-2 and IFN- γ level, ET-1 level, IgG, IgA and IgM levels, superoxide dismutase (SOD), and malondialdehyde (MDA).

2. Materials and methods

2.1. Materials

Fresh *A. vera* was purchased from the local market of Lanzhou, Gansu Province, China.

2.2. Preparation of *Aloe vera* polysaccharides

Aloe vera polysaccharides were prepared according to the method described by Wu, Xu, Shan, and Tan (2006). In brief, the leaves (300 g) of the title plant, after ground on an electrical grinder, were extracted thrice with 2500 ml of distilled water at 70–80 °C for 2 h. The filtrate of the obtained extract was condensed in vacuo to a syrup (ca. 500 ml), to which cold 95% ethanol (approximately 1500 ml) was added. The crude polysaccharide part was

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precipitated from the alcoholic liquor during its subsequent standing at 4 °C overnight. The precipitate that formed was collected by centrifugation at 12,000g 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 12,000g for 10 min, the supernatant was then precipitated with 3-fold volumes of 95% ethanol to obtain the crude polysaccharides.

2.3. Animal preparation and diets

Forty wistar rats (40 d old), weighing 210–250, were obtained from animal feeding center of our institute (Lanzhou, china). All studies were approved by the animal care and use committee at Lanzhou university. The rats were maintained in cages in the animal care facility. The rats were subjected to alternate 12-h periods of a dark/light cycle. During the study, all rats were fed with the standard chow and given water ad libitum. Rats were randomly divided into the following 1 control group and 3 experimental groups (each group contained 10 rats): group 1, control rats; group 2, untreated oral ulceration rats (model group); group 3, polysaccharides-treated oral ulceration rats (low-dose polysaccharides group I); group 4, polysaccharides-treated oral ulceration rats (high-dose polysaccharides group II). Oral ulceration of rats in experimental groups were induced by the method described by Jiang, Fu, Tian, Wang, and Liu (2003). Polysaccharides were dissolved in distilled water and were fed by gavage to mice once a day. Low-dose (100 mg/kg body weight) and high-dose (200 mg/kg body weight) groups were treated with 0.3 ml of polysaccharides solution by orally. Rats in control group and model group were treated with the same volume of physiological saline. The treatments lasted for 20 days. Twenty-four hours later after the last drug administration, rats were anesthetized with ether, and blood samples were collected before the rats were killed by decapitation. Blood samples were centrifuged at 3000 r/min at 4 °C for 10 min to obtain serum. Oral mucosa was excised from the animal and stored at –20 °C. Oral mucosa was centrifuged and supernatants were then subjected to the measurement of SOD activities, and ET-1, MDA levels.

2.4. Analysis

2.4.1. Flow cytometric analysis

Single cell suspensions were prepared from CILN by mechanical tissue disaggregation in cold PBS and centrifuged at 700 rpm for 5 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 (100 µl, about 10⁶ cells) were incubated with a mixture of the anti-CD3-FITC + 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. The CD4⁺/CD8⁺ T cells ratio was calculated by dividing the percentage of the CD3⁺CD4⁺ T cells by the percentage of the CD3⁺CD8⁺ T cells.

2.4.2. IL-2 and the IFN-γ content

IL-2 content was measured by ELISA (Mouse ELISA kit; Endogen, Woburn, MA). The IFN-γ content was measured using a commercially available ELISA kit (Endogen Inc., Boston, MA).

2.4.3. ET-1 content

Plasma and mucosa ET-1 were measured by specific enzyme-linked immunosorbent assay kits from Alpco Diagnostics (Windham, NH).

2.4.4. IgG, IgA and IgM content

IgG, IgA and IgM content were measured by Mouse immunoglobulin isotyping cytometric bead array kit (CBA).

2.4.5. SOD and MDA analysis

SOD activity and MDA level were determined using a commercially available kit (Randox, UK).

2.5. Statistical analysis

All the grouped data was evaluated using SPSS/10.0 software. Student *t* test was used to assess the statistical significance of the continuous variables. A value of *P* < .05 indicates statistical significance. All the results were expressed as mean ± s.d. for ten rats in each group.

3. Results and discussion

3.1. Effect of Aloe vera polysaccharide on CD4⁺, CD8⁺ and CD4⁺/CD8⁺

CD4⁺ cells are a type of white blood cell. White blood cells are important in fighting infections. A CD4⁺ count is a blood test to determine how well the immune system is working in people (Shirwan, Mhoyan, Yolcu, Que, & Ibrahim, 2003). CD8⁺ is a transmembrane glycoprotein that serves as a co-receptor for the T cell receptor (TCR). Like the TCR, CD8⁺ binds to a major histocompatibility complex (MHC) molecule, but 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 (Vecchi-one, Catchpole, D'Mello, Kanellos, & Hamblin, 2002). Table 1 represents the effect of *A. vera* polysaccharide on the CD4⁺, CD8⁺ and CD4⁺/CD8⁺ in the blood of normal and phenol induced rats. Phenol treatment produced a significant decrease in the blood CD8⁺, when compared with normal rats. But phenol treatment did not significantly affect CD4⁺. A significant decrease of CD4⁺/CD8⁺ could be found in ulcer model group compared to control group. *Aloe vera* polysaccharides-treatment dose-dependently significantly enhanced blood CD8⁺ and reduced CD4⁺/CD8⁺ in polysaccharides-treatment groups (I, II) when compared with ulcer model rats.

3.2. Effect of Aloe vera polysaccharide on IL-2 and IFN-γ level

Interleukin-2 (IL-2) is an interleukin, a type of cytokine immune system signaling molecule, that is instrumental in the body's natural response to microbial infection and in discriminating between foreign (non-self) and self. IL-2 mediates its effects by binding to IL-2 receptors, which are expressed by lymphocytes, the cells that are responsible for immunity (Kim

Table 1
Effect of *Aloe vera* polysaccharide on CD4⁺, CD8⁺ and CD4⁺/CD8⁺

Group	CD4 ⁺	CD8 ⁺	CD4 ⁺ /CD8 ⁺
Control	85.31 ± 5.51	69.13 ± 3.83	1.23 ± 0.09
Ulcer model	85.63 ± 4.91	38.91 ± 1.93 ^b	2.20 ± 0.11 ^b
Polysaccharides (I)	81.95 ± 7.02	48.92 ± 2.71 ^d	1.68 ± 0.13 ^d
Polysaccharides (II)	86.25 ± 7.49	59.22 ± 3.81 ^d	1.46 ± 0.08 ^d

^b *P* < .01, comparison between control group and ulcer model group.

^d *P* < .01, comparison between ulcer model group and *A. vera* polysaccharides-treated group.

& Lim, 2002). Interferon-gamma (IFN- γ) was originally called macrophage-activating factor. IFN- γ is secreted by Th1 cells, Tc cells, dendritic cells and NK cells. Also known as immune interferon, IFN- γ is the only Type II interferon. It is serologically distinct from Type I interferons and it is acid-labile, while the type I variants are acid-stable. IFN- γ has antiviral, immunoregulatory, and anti-tumour properties (Lin, Wei, & Bolling, 1995). It alters transcription in up to 30 genes producing a variety of physiological and cellular responses. Table 2 illustrates the effect of *A. vera* polysaccharides on the blood IL-2 and IFN- γ levels in experimental groups of rats. Phenol treatment produced a significant decrease in the blood IL-2 and IFN- γ levels (ulcer model group), when compared with normal rats. Administration of *A. vera* polysaccharides showed a significant increase in blood IL-2 and IFN- γ levels in polysaccharides-treatment groups (I, II) when compared to ulcer model rats. Moreover, there were higher blood IL-2 and IFN- γ levels in polysaccharides-treatment groups II than in polysaccharides-treatment groups I. Our experimental data supports result of Yang, Zhang, Liu, and Zhuang (2007) and Djeraba and Quere (2000).

3.3. Effect of *Aloe vera* polysaccharide on plasma and mucosa ET-1 level

Endothelin (ET-1) is a 21 amino acid peptide that is produced by the vascular endothelium from a 39 amino acid precursor, big ET-1, through the actions of an endothelin converting enzyme (ECE) found on the endothelial cell membrane. ET-1 formation and release are stimulated by angiotensin II (AII), antidiuretic hormone (ADH), thrombin, cytokines, reactive oxygen species, and shearing forces acting on the vascular endothelium. ET-1 release is inhibited by prostacyclin and atrial natriuretic peptide as well as by nitric oxide (Hültner & Ehrenreich, 2005; Pang et al., 2007).

Table 3 illustrates the effect of *A. vera* polysaccharides on the plasma and mucosa ET-1 level in experimental groups of rats. Phenol treatment produced a significant increase in the plasma and mucosa ET-1 levels in ulcer model group, when compared with normal rats. Administration of *A. vera* polysaccharides showed a significant decrease in the plasma and mucosa ET-1 levels in polysaccharides-treatment groups (I, II) when compared to ulcer model group. Moreover, there were higher plasma and mucosa ET-1 levels in polysaccharides-treatment groups II than in

polysaccharides-treatment groups I. Slomiany and Slomiany's findings (2005) found that the delay in oral ulcer healing was characterized by a increase in apoptosis and buccal mucosal expression of tumor necrosis factor- α (TNF- α) and endothelin-1 (ET-1). The oral ulcer healing was reflected in a decrease in apoptosis and a decline in buccal mucosal expression of tumor necrosis factor- α (TNF- α) and endothelin-1 (ET-1). Our findings are the first to demonstrate that *A. vera* polysaccharides leads to a decline in oral mucosal expression of endothelin-1 (ET-1) that interfere with soft oral tissue repair.

3.4. Effect of *Aloe vera* polysaccharide on plasma IgG, IgA and IgM levels

Repeat episodes of mouth ulcers can be indicative of an immunodeficiency, signaling low levels of immunoglobulin in the mucous membrane of the mouth. Chemotherapy and Human immunodeficiency virus (HIV) are both causes of immunodeficiency with which mouth ulcers become a common manifestation (Alves de Sousa et al., 2007; Drabick et al., 1997; Zhao et al., 2007). Fig. 1 illustrates the effect of *A. vera* polysaccharides on the plasma IgG, IgA and IgM levels in experimental groups of rats. Phenol treatment produced a significant decrease in the plasma IgG, IgA and IgM levels in ulcer model group, when compared with normal rats. Chen, Wang, Bao, Zhang, and Gong (2005) reported that *A. vera* polysaccharides could increase the indexes of spleen and thymus, and the level of blood TNF- α , IL-2 and IgG of the immune inhibited mice. In this study, administration of *A. vera* polysaccharides showed a significant increase in the plasma IgG, IgA and IgM levels in polysaccharides-treatment groups (I, II) when compared to ulcer model group. Moreover, there were higher plasma IgG, IgA and IgM levels in polysaccharides-treatment groups II than in polysaccharides-treatment groups I.

3.5. Effect of *Aloe vera* polysaccharide on plasma SOD activity and MDA level

The cellular antioxidant defense system operates through enzymatic and nonenzymatic components. SOD is the necessary enzymes for an effective defense against ROS (Chen, Zhong, Zeng, & Ge, 2008). SOD accelerates the formation of hydrogen peroxide (H₂O₂) by using superoxide radicals. CAT is the enzyme that accelerates the degradation of unstable H₂O₂ to H₂O and O₂ (Dugan et al., 1995; Luo & Fang, 2008; Yu, Yin, Yang, & Liu, 2009). Although there were a few studies on free radical metabolism of mucosa and serum in animals with ulceration (Hung & Wang, 2002), to our

Table 2
Effect of *Aloe vera* polysaccharide on IL-2 and IFN- γ level

Group	IL-2 (ng/ml)	IFN- γ (μ g/ml)
Control	67.83 $\pm$ 3.04	6.32 $\pm$ 0.47
Ulcer model	31.47 $\pm$ 1.03 ^b	2.27 $\pm$ 0.12 ^b
Polysaccharides (I)	42.52 $\pm$ 2.61 ^d	4.48 $\pm$ 0.22 ^d
Polysaccharides (II)	63.50 $\pm$ 4.25 ^d	6.01 $\pm$ 0.31 ^d

^b $P < .01$, comparison between control group and ulcer model group.

^d $P < .01$, comparison between ulcer model group and *A. vera* polysaccharides-treated group.

Table 3
Effect of *Aloe vera* polysaccharide on plasma and mucosa ET-1 level

Group	ET-1 (ng/l)	ET-1 (ng/g tissue)
Control	142.71 $\pm$ 5.63	413.27 $\pm$ 17.91
Ulcer model	211.56 $\pm$ 8.73 ^b	731.73 $\pm$ 21.21 ^b
Polysaccharides (I)	180.25 $\pm$ 8.62 ^d	541.18 $\pm$ 31.22 ^d
Polysaccharides (II)	157.93 $\pm$ 7.12 ^d	439.93 $\pm$ 13.12 ^d

^b $P < .01$, comparison between control group and ulcer model group.

^d $P < .01$, comparison between ulcer model group and *A. vera* polysaccharides-treated group.

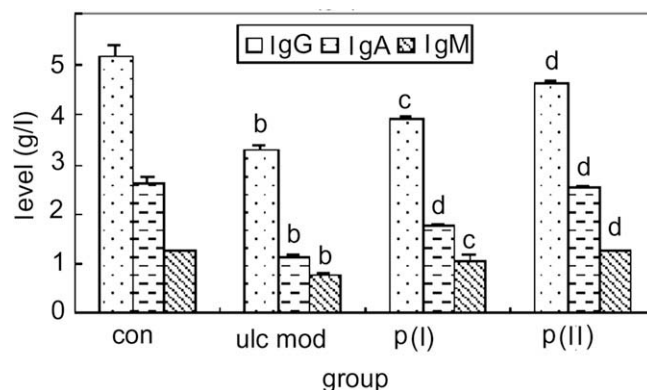


Fig. 1. Effect of *Aloe vera* polysaccharide on IgG, IgA and IgM levels. ^b $P < .01$, compared with control group; ^c $P < .05$, ^d $P < .01$, compared with ulcer model group con-control, ulc mod-ulcer model, p(I)- polysaccharides (I), p(II)- polysaccharides (II).

Table 4Effect of *Aloe vera* polysaccharide on SOD activity and MDA level

Group	SOD (U/ml)	MDA (nmol/ml)
Control	173.37 ± 11.22	11.37 ± 0.97
Ulcer model	92.41 ± 6.71 ^b	18.84 ± 1.21 ^b
Polysaccharides (I)	132.31 ± 11.14 ^d	15.03 ± 1.01 ^d
Polysaccharides (II)	169.32 ± 9.84 ^d	10.27 ± 1.13 ^d

^b $P < .01$, comparison between control group and ulcer model group.^d $P < .01$, comparison between ulcer model group and *A. vera* polysaccharides-treated group.**Table 5**Effect of *Aloe vera* polysaccharide on SOD activity and MDA level

Group	SOD (U/mg)	MDA (nmol/mg)
Control	231.38 ± 17.13	7.72 ± 0.57
Ulcer model	142.28 ± 20.82 ^b	13.74 ± 1.09 ^b
Polysaccharides (I)	179.42 ± 9.21 ^d	11.57 ± 0.84 ^d
Polysaccharides (II)	227.29 ± 14.25 ^d	6.23 ± 0.52 ^d

^b $P < .01$, comparison between control group and ulcer model group.^d $P < .01$, comparison between ulcer model group and *A. vera* polysaccharides-treated group.

knowledge, the oxidant/antioxidant status in animals with oral ulceration has not been studied up to now. In previous studies, oxygen radical production and antioxidant enzymes activities of patients with ulceration had been found to be higher than that of the controls (Ganguly, Kundu, Banerjee, Reiter, & Swarnakar, 2006; Minagawa, Okamura, Shigemasa, Minami, & Okamoto, 2007). Our data support their results.

Table 4 illustrates the effect of *A. vera* polysaccharides on the plasma SOD activity and MDA level in experimental groups of rats. Phenol treatment produced a significant decrease SOD activity and increase MDA level in the plasma in ulcer model rats, when compared with normal rats. Antioxidant activities of *A. vera* polysaccharide had been much reported (Liu, Wang, Xu, & Wang, 2007). In this study, administration of *A. vera* polysaccharides significantly enhanced SOD activity and reduced MDA level in the plasma in polysaccharides-treatment rats (I, II) when compared to ulcer model rats. Moreover, there were higher plasma SOD activity and lower MDA level in polysaccharides-treatment groups II than in polysaccharides-treatment groups I.

3.6. Effect of *Aloe vera* polysaccharide on mucosa SOD activity and MDA level

Table 5 illustrates the effect of *A. vera* polysaccharides on the mucosa SOD activity and MDA level in experimental groups of rats. Phenol treatment produced a significant decrease SOD activity and increase MDA level in the mucosa in ulcer model rats, when compared with normal rats. Administration of *A. vera* polysaccharides significantly enhanced SOD activity and reduced MDA level in the mucosa in polysaccharides-treatment rats (I, II) when compared to ulcer model rats. Moreover, there were higher mucosa SOD activity and lower MDA level in polysaccharides-treatment groups II than in polysaccharides-treatment groups I.

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