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EFFECT OF *ALOE VERA* (L). BURM. F POLYSACCHARIDE ON VARIOUS IMMUNE PARAMETERS

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ABSTRACT:
In present study, we studied the effect of *Aloe vera* polysaccharide on various immune parameters. *Aloe Vera* polysaccharide showed significant ($p<0.001$) stimulation of phagocytosis (82.9924 ± 2.4488) at a concentration 100 µM. *Aloe vera* polysaccharide at 200 µM had no significant cytotoxic effect towards human lymphocytes. The polysaccharide was examined for its antioxidant potential. Aloe polysaccharide possesses observable antioxidant activity as its EC_{50} value was found to be 3.5µgm/ml, which is significant when compared to standard solution of ascorbic acid whose EC_{50} value is 2.86µm/ml. *Aloe vera* inner gel was evaluated for its antimicrobial activity against *Escherichia coli.* In the present study, the inner gel of *Aloe vera* was tested for its antibacterial property against *Escherichia coli* (strain no: NCIM 2118) using pour plate method. The fresh gel at 50%-100% showed significant inhibition of growth of the colonies. These results indicate that *Aloe Vera* polysaccharide possess significant immunomodulatory activity and other therapeutic uses such as an antioxidant.

KEYWORDS: Aloe vera , Immunomodulation, phagocytosis, antioxidant activity, antibacterial activity

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INTRODUCTION

Polysaacharide from plants have been subject of study for a very long time, mainly for their physical properties and industrial use based on these properties. Over the last 20 years, there has been an ever-increasing interest in the biological activity of biomolecules. It is increasingly being suggested that the polysaccharides are at the core of herbal immunomodulating effects. Laboratory studies have revealed a range of impressive results, including T cell activation, anti-tumor activity, increase in certain serum proteins, non-specific activation of the complement system, stimulation of interferon production, stimulation of phagocytosis1. Im et al (2005) studied the relationship between molecular size and immunomodulatory activity of modified polysaccharide.

Immunomodulatory activities of modified Aloe polysaccharide preparations were examined on a mouse macrophage cell line RAW 264.7 cells and in ICR strain of mouse implanted with sarcoma 180 cells. The results showed that polysaccharides between 400 and 500 KDa exhibits the most potent macrophage activity as determined by increased cytokine production, nitric oxide release, expression of surface molecules and phagocytic activity[2]. Lin et al (2006) investigated the anticancer effect of Aloe-emodin (AE) in the T24 human bladder cancer cell by studying the apoptosis regulation. They found that inhibition of cell viability and induced G2/M arrest and apoptosis in T24 cells. AE induced apoptosis in T24 cells was mediated through the activation of p53, p21, Fas/APO-1, Bax and caspase. The mechanism of action mediating the acute and chronic anti-inflammatory activity of leafy exudates of *Aloe vera* in animal models of inflammation and effect of *Aloe vera* on nitric oxide production in mouse

peritoneal macrophages was studied and the results indicate that *Aloe vera* possesses acute and chronic anti-inflammatory activity, which is partly mediated by reduced production of NO, which in turn prevents the release of inflammatory mediators[3].

These important research findings of *Aloe vera* polysaccharide pertaining to its therapeutic uses such as restoration of immune system, antimicrobial activity, antitumour activity, and other miscellaneous uses, initiated us to study their effect on some immune parameters. Our aim and objective of the study was to investigate the effect of *Aloe vera* polysaccharide and ciprofloxacin on immune response. Immune parameters to be studied includes i) Phagocytosis assay, ii) Cytotoxicity test (trypan blue exclusion method) and iii) Antioxidant activity of *Aloe vera* polysaccharide and iv) Antibacterial activity of *Aloe vera* leaf gel.

MATERIALS AND METHODS

Preparation of A*loe vera* polysaccharide:

Matured leaves of *Aloe vera* were collected and the outer hard green layer was removed with a sharp knife. The inner fleshy part was homogenized in to slurry using stirrer. The slurry was filtered to get fresh gel. Polysaccharide was obtained by using solubilization and precipitation technique. Equal amount of gel and dimethyl sulfoxide was mixed well. Then, it was precipitated out using alcohol or acetone. The polysaccharide obtained was washed with acetone and air-dried. Air-dried polysaccharide was used for further experiments[4]

PHAGOCYTOSIS ASSAY:

Preparation of *Candida albicans* suspension:

Candida albicans culture of 3-7 days growth was used for this assay. The *C. albicans* cells after 3 days growth were centrifuged, so that the cell pellet was formed at the bottom. The supernatant was discarded and the pellet was resuspended in PBS and centrifuged again.

Evaluation of Phagocytosis:

Human blood (2-3 drops) was obtained by finger prick method on to a glass slide. The slide was kept on a cotton pad in a sterile petridish and incubated for 1 hr at 37°C. After incubation, the clot was removed very gently and washed with normal saline without disturbing the neutrophils, which had been adhered to the slide. The slide was then flooded with predetermined concentration of samples and incubated at 37°C for 30 mins. The slide was then flooded with suspension of *C.albicans* and incubated at 37°C for 1 hr. Following incubation, the slide was drained, fixed with methanol and stained with Giemsa stain. The mean number of phagocytosed cells on the slide was determined microscopically for 100 granulocytes using morphological criteria. This number was taken as the phagocytic index (PI) and was compared with basal PI of control[5]

Immunostimulation in % was calculated by using following formula:

$$Stimulation = PI_{(test)} - PI_{(control)} \times 100 / PI_{(control)}$$

Cytotoxicity Assay:

Cell separation:

Human lymphocytes can be isolated using a density gradient centrifugation. Histopaque of 1.077 specific gravity was used in this assay. Equal volume of blood and PBS was mixed before under laying it with Histopaque, followed by centrifuging at 2000 rpm for 20 mins. Lymphocytes get separated as an interface in between plasma layer and RBC layer. Lymphocytes at the interface was removed and washed for 15, 12, &10 mins respectively in PBS and cell number were determined with Neubauer improved haemocytometer. The lymphocytes were diluted to get a final concentration of $5x10^5$.

Procedure:

One ml of cell suspensions were incubated with different concentrations of samples (50-200μM) for 30 mins at 37°C in an incubator, together with untreated control samples. The samples were centrifuged at 500 rpm. Resuspend the lymphocytes in RPMI 1640 & 0.4% trypan blue[8]. The number of viable and dead cells was scored by using following formula [6]

$$Stimulation = PI_{(test)} - PI_{(control)} \times 100 / PI_{(control)}$$

Antioxidant Activity:

Hydrogen donating ability of extract was examined in the presence of DPPH stable radical. Sample stock solutions were diluted to final concentration of 0.5-5 μg/ml in ethanol.1 ml of a 0.3mM DPPH solution was added to 2.5 ml of sample solution of different concentration and allowed to react at room temperature. After 30 minutes, the absorbance values were measured at 518 nm and converted in to percentage antioxidant activity using the following formula.

$$AA\% = 100-\{[Abs_{sample} - Abs_{blank} \times 100]/Abs_{control}\}$$

Ethanol (1.0 ml) plus plant extract solution was used as blank. DPPH solution (1.0 ml; 0.3 mM) plus ethanol (2.5 ml) was used as negative control. The positive controls were those using the standard solutions.

The EC50 values were calculated by linear regression of plots where the abscissa represented the concentration of tested plant extract and the ordinate the average percent of antioxidant activity from three separate test [7]

ANTIBACTERIAL ACTIVITY:

Preparation of Media:

Nutrient agar media which is a common microbiological growth media was used for antibiotic susceptibility testing. It is prepared (pH 7.4) as follows.

Sr. No.	Ingredients	Quantity
1	Peptic digest of animal tissue	5.00
2	Sodium chloride – 5.00	5.00
3	Beef extract	1.50
4	Agar 2%	2%
5	Yeast extract – 1.50	1.50

Preparation of *Aloe vera* leaf inner gel:

Fully expanded leaves of *Aloe vera* were selected and inner gel was extracted by peeling off the green hard layer with the help of sharp knife. Then gel was homogenized in to slurry and filtered. Different percentage of the gel was prepared and used for the experiment.

Procedure:

Bacterial strain of *Escherichia coli* (strain no: NCIM-2118) was collected from National Collection of Industrial Microorganisms, National Chemical laboratory, Pune and stored at Pharmaceutical Biotechnology Laboratory, College of Pharmacy, SRIPMS, Coimbatore. Quantitative screening for the gel was done by Pour Plate method. Different percentage of gel prepared was incorporated in to the medium and autoclaved at a pressure of 15 Psi (121^{0}C) for 15 minutes. Following sterilization, the medium was cooled to 40-45^{0}C and 1 ml of 10^6 diluted culture was inoculated in to the medium. The medium containing various percentage of gel was poured in to sterile petridish and allowed to solidify. Then plates were incubated at 37 ^{0}C for 18-24 hrs. After incubation, number of colonies was counted and percentage inhibition of growth was calculated[8]

RESULTS AND DISCUSSION

Polysaccharides isolated from *Aloe vera* have been known to have diverse immunomodulatory activities *in vivo* as well as *in vitro*. Different assays are used for testing the biological activity of polysaccharides. Most of them focus on parameters that may have influence on the immune systems. In this study, the *Aloe vera* polysaccharide have shown significant immunostimulation at a concentration of 100 μM with percentage immunostimulation of 82.9924±2.4488 ($p<0.001$). Neutrophils kill ingested microorganisms by subjecting them to high concentrations of highly toxic reactive oxygen species (ROS) and bringing about myeloperoxidase – catalysed halogenations[9]. Study by Im *et al.* (2005) showed that the protein free modified aloe polysaccharide increased phagocytic activity in cultured RAW 264.7 cells at a concentration of 100 μm/ml using flow cytometry analysis.

Actions of acemannans may be attributed to the residual presence of aloerides[2] and they have been shown to enhance transcription of cytokines. High concentration of aloeride seemed to enhance macrophage activities which may be a contributing factor for the increased phagocytic stimulation by acemannan.

In the present work, *Aloe vera* polysaccharide showed that the viability of the lymphocyte cell is higher at 50 μM, indicating no toxicity toward the lymphocyte cells. *Aloe vera* polysaccharide had shown insignificant cytotoxicity at concentrations 100 μM and 200 μM. Percentage cell viabilities with *Aloe vera* polysaccharide were 72.9791±1.7175 ($p<00.1$), 69.7472±0.9548 ($p<00.1$) and 53.0386±15.1022 ($p<0.5$) at 50 μM, 100 μM and 200 μM respectively. Most of the active constituents of *Aloe vera* were evaluated for its antioxidant potential. The extract exhibited the strongest radical scavenging activity of 72.19 %, which is significantly higher than that of BHT (Butylated hydroxytoluene) at 70.52 %[7]. The EC_{50} value of the Aloe polysaccharide was 3.5 μm/ml, which is significant when compared to standard solution of ascorbic acid whose EC_{50} is 2.86 μgm/ml. This indicates that the Aloe polysaccharide possess observable antioxidant activity.

In order to interpret the effectiveness of the drug, the basic toxicity study is necessary to ensure their safety. The crude polysaccharide was tested for its toxicity towards human lymphocytes. The results observed in this study showed that polysaccharide had no significant effect on lymphocytes.

Aloe vera leaf and gel was evaluated for its antimicrobial activity against various pathogens Antimicrobial susceptibility test by well plate method showed that both the gel and the leaf inhibited the growth of *Staphylococcus aureus*. Only the gel inhibited the growth of *Trichophyton mentagrophytes*, while the leaf possesses inhibitory effects on both *Pseudomonas aeruginosa* and *Candida albicans* [10] Inner gel of Aloe contains many active constituents, which was reported to possess antimicrobial property against various pathogenic microbes. In the present study, the inner gel of *Aloe vera* was tested for its antibacterial property against *Escherichia coli* using pour plate method. The fresh gel at 50%-100% showed significant inhibition of growth of the colonies.

The results observed in this study showed that further investigation of the different constituents of the plant parts such as inner gel of *Aloe vera* and exudates of leaf is needed to exploit the hidden therapeutic uses of the whole plant which will be helpful in treating many infectious diseases. In addition the mechanism of action by which the constituents of the plant acts should be studied which will lead to new drug discovery.

Table 1: Effect of A*loe vera* polysaccharide on neutrophil phagocytosis by slide method

S. No	Concentration (μM)	Immunostimulation %
1	50	62.0022±10.5427 ($p<0.02$)
2	100	82.9924±2.4488 ($p<0.001$)
3	150	43.9653±4.4150 ($p<0.01$)

The effect of Aloe polysaccharide on phagocytosis was studied at 50 μM, 100 μM and 150 μM

Table 2: Cytotoxicity of A*loe vera* polysaccharide

S. No	Concentration (μM)	Cell viability (%)
1	Control	77.8459±0.6894
2	50	72.9791±1.7175 (p<00.1)
3	100	69.7472±0.9548 (p<00.1)
4	200	53.0386±15.1022 ($p<0.5$)

The viability was calculated from [nonstained cells/ (stained +nonstained)] X100. Data represents mean ± S.D. of 3 in vitro experiments carried out separately with peripheral blood lymphocytes of 3 different individuals. The p value was calculated using paired t test in relation to control.

Table 3: Antioxidant activity of A*loe vera* polysaccharide

S.No	Concentration (gm/ml)	Absorbance at 518 nm	Antioxidant activity %	EC_{50} value of standard (Ascorbic acid)
1	0.5	0.8012	10.7588±0.7132	2.86μg/ml
2	1	0.7574	15.7616±0.5232	
3	1.5	0.7052	21.6863±0.2600	
4	2	0.6120	32.2653±2.8408	
5	2.5	0.5850	35.4244±0.08831	
6	3	0.5472	39.7600±2.3734	
7	3.5	0.4590	49.8369±1.1940	
8	4	0.3972	57.0320±1.7855	
9	4.5	0.3390	63.7099±0.9457	

The antioxidant activity of Aloe polysaccharide was compared to standard solution of ascorbic acid (EC_{50})

Table 4: Antibacterial activity of A*loe vera* leaf gel

S.No	Organism	Percentage of the gel	Number of colonies	Percentage inhibition
1	*E.coli*	Control	367	-
2		50	222	38.50
3		60	203	43.76
4		70	167	53.73
5		80	158	56.23
6		90	147	59.27
7		100	112	68.97

Aloe vera inner gel of tested for its antibacterial property against *Escherichia coli* using pour plate method.

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