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Evaluation of immunomodulatory effect of aqueous extract of aloe vera in Wistar albino rat models

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ABSTRACT

Objectives: Aim of the study was to evaluate the immunomodulatory effects of Aqueous extracts of Aloe vera in Wistar albino rats using humoral immune response (antibody titre), a cellular immune response, and a neutrophil adhesion test after oral administration.

Materials and Methods: 24 healthy Wistar albino rats of either sex were divided into 4 different groups containing 6 rats for each immunomodulatory model. Group I ,the control group received gum acacia suspended in normal saline; Group II rats were treated with dexamethasone (1 mg/kg bw)whereas Groups III & IV received AloeVera aqueous extract at a dose of 125 mg/kg and 250 mg/kg, respectively. Each rat was antigenically challenged by injecting 0.1 ml of 0.5×10^9 SRBCs (suspended in normal saline) intraperitoneally. The study of humoral immune response was seen by measurement of antibody titre obtained on 20^{th} day (7^{th} day of Ag challenge) and 27^{th} day (14^{th} day of challenge) with sRBC considered the primary and secondary humoral immune response, respectively. For the cellular immune response, foot pad edema was calculated due to a hypersensitivity reaction after injection of sRBC into the .rat hind paw. A test of neutrophil adhesion is used to evaluate immunomodulatory activity. Aloe vera aqueous extracts at 250 mg/kg significantly improved a significant increase in paw edema volume indicated increased cell-mediated immunity, and elevated Ab titre on the 20th and 27th days served as a marker of increased humoral immune response. Additionally, AVE 250 mg/kg caused an increase in neutrophil adhesion, demonstrating its immunomodulatory effects. Aloe vera aqueous extracts at a dose of 250 mg/kg demonstrated immunomodulatory activity in a model using Wistar albino rats.

Results: Aloe vera extract at a dose of 250 mg/kg significantly increased hemagglutination antibody titers compared to the control group, indicating enhanced humoral immunity.

Conclusion: This study demonstrates the immunostimulant properties of orally administered Aloe vera aqueous extract in Wistar albino rats. The extract increased antibody production, enhanced cell-mediated immunity, and improved neutrophil function. These findings support the potential of Aloe vera as a natural immunomodulator and warrant further exploration of its therapeutic applications.

1. Introduction

Immune system modulation is necessary for the treatment of inflammatory and immune-related diseases caused

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by immune system flaws or disorders When the immune system is activated, it responds right away by stimulating immune component cells and producing a variety of cytokines, chemokines, and inflammatory mediators.[1–3] Numerous medications and herbal remedies known as "immunomodulators," which either work by achieving immunostimulation, as in the case of HIV, or immunosuppression, as in the case of autoimmune diseases, target the system in a number of conditions Modulation of immune response to alleviate disease conditions is recognized as a key component of effective disease control. There are several herbs that have been used for a long time and are known to strengthen the immune system, including Caesalpinia bonducella Flem. (Caesalpiniaceae), Rhododendron spiciferum Franch. (Ericaceae), Curcuma longa Linn. (Zingiberaceae), Azadirachta indica A., Juss. (Meliaceae), and Boerhaavi (Lamiaceae).Plant extracts have been recently investigated for their immunomodulatory role,[4] so that they can be helpful in the prevention of infectious disease or acquired immunodeficiency.[5] Plants like. Aloe vera (L.) (Liliaceae), also known as Aloe barbadensis, is a plant that is frequently used in traditional medicine to treat conditions like burns, dermatitis, gout, arthritis, and gouty arthritis.[6] Aloe vera gel (AVG) has antioxidant properties, effectively reduces inflammation, and promotes wound healing.[7,8] In light of this, the current study was carried out to examine the immunomodulatory effects of an oral aqueous aloe vera extract in Wistar albino rat models

2. Materials and Methods

2.1. Animals

Healthy adult Wistar albino rats of either sex weighing between 100 and 200 grams were used in the study. They were housed under standard laboratory conditions, including a temperature range of 25±1 °C, a 12-hour light cycle, and free access to rat food pellets and water. Before the experimental study was conducted, the animals underwent a two-week acclimatization period.[9] On the day before the experiment, the rats were given a free overnight access to water. Institutional Animal Ethical Committee, M.K.C.G. Medical College, Berhampur, approved the study protocol.

2.2. Plant material

From Indichem Pvt. Ltd. in Pune, we obtained an aqueous extract of aloe vera (batch number WL-243/09).

2.3. Drugs and chemicals

Indomethacin & Dexamethasone were purchased from local pharmacy.

Ether, Gum acacia, normal saline, EDTA, Leishman stain were procured from Merck pvt ltd.

Alsevers's solution, WBC diluting fluid, Phosphate buffered saline was prepared in a laboratory using required ingredients. Alsever's solution was prepared using dextrose (20.5 g), soda citrate (8.0 g), citric acid (4.2 g), water (1 L), a Seitz filter, and pH adjusted to 6.1. Phosphate-buffering saline was prepared in the laboratory using Sod dihydrogen phosphate (24.6 g/L), Disodium hydrogen phosphate (22.4 g/L), and Sod chloride (80.4 g/L), with the pH adjusted to 7.2 Fresh blood from sheep was collected from departmental animal house.

2.4. Instruments

Mercury plethysmometer**,** Oral feeding tube, syringe &needle, test tubes, WBC diluting pipette, hemagglutination titre plate, nylon fiber, sterile cotton, capillary tube, glass seeker, foreceps & a pair of scissors, Neubauers "counting chamber," sterile glass slides. Screw gauze were used.

2.5. Expermental protocol

2.5.1. Collection of sheep RBC

Sheep blood was collected from jugular vein in a sterile bottle containing sterile Alsevers' solution in 1:1 proportion aseptically and stored at 4^0C for further use.[10]

2.5.2. Antigen preparation[9]

On the day of treatment, sheep blood was centrifuged at 4000 rpm for 10 minutes to enable red blood cells to settle at the bottom of the test tube. The supernatant was discarded, leaving sheep red blood cells (SRBC) pellets that were washed three times with pyrogen-free normal saline (0.9% w/v NaCl). The sheep RBC was suspended in Phosphate-buffered saline (pH 7.2) for further use. The cell count of sheep RBC was done using Neubauer's" counting chamber and adjusted to approximately0.5×10^9 cells/mL for immunization and & challenge.

Each rat was antigenically challenged with sheep RBC (0.1ml) intraperitoneally 1^{st} at 14th day, 2nd at 20^{th} day.[11]

2.5.3. Study plan

The rats were grouped and fed with control, standard & test drug as perTable 1.

2.5.4. Humoral antibody response to SRBC (Hemagglutination antibody titer test[12]

On day 0 and for the following 14 days, the rats received an intraperitoneal injection of 0.5×10^9 sheep red blood cells (SRBCs) as a vaccination.[13] After the drug treatment was complete, blood samples from the rats were taken, and the Ab titre value was calculated by titrating serum dilutions of SRBC (0.025×10^9 cells) in microtitre plates.

Table 1: Grouping & treatment shedule of animals

Drug & doses	Humoral immune response	Cell mediated immune response	Neutrophil adhesion test	Route
Gum Acacia (1ml/rat)	I	V	IX	Oral
Dexamethasone (0.1mg/kg)	II	VI	X	Oral
AVE (125mg/kg)	III	VII	XI	Oral
AVE (250mg/kg)	IV	VIII	XII	Oral

The plates were visually checked for agglutination after a 2-hour incubation period at room temperature. On the seventh day of the experiment, all the rats received an intraperitoneal (i.p.) injection of 0.5 mL of sRBCs to immunize them. Blood was drawn from all antigenically challenged rats' retroorbital plexus veins on days 20 and 27 and placed in a test tube with a pinch of EDTA while they were lightly sedated with ether. The test tube was then centrifuged at 5000 rpm to extract the serum. According to the procedure outlined by Gaur et al. (2009), the hemagglutination technique was used to determine antibody titers. SRBC (25 μL sRBC (0.025×10^9 cells/ml of sRBC)) was added to each of the serial two-fold dilutions of serum made with normal saline in 96-well microtiter plates. After 2 hours of incubation at 37 °C, the hemagglutination plates were checked visually for hemagglutination titre. The highest dilution causing hemagglutination was used to determine the antibody titre, which was then graded. The hemagglutination antibody titer (HA units/μL) was determined as the reciprocal of the test serum concentration at which agglutination occurred at the highest concentration. Primary and secondary humoral immune responses, respectively, were determined by the antibody titres that were obtained on days 20 and 27, or on the seventh and fourteenth days following the challenge with sRBCs.

2.5.5. Cellular immune response[14,15]

2.5.5.1. Antigen challenge. Animals from each group were divided into various groups, each of which contained 6 animals, and then sensitized with 0.1 mL of SRBC containing 1×10^9 cells intraperitoneally on day 0. Prior to injection on the seventh day, the right hind footpad thickness was measured using a plethysmometer. The rats were put to the test by receiving a 20 μL injection of 1% SRBC in the right hind footpad. The animals' footpad thickness was once again measured on the eighth and ninth days. Rats' foot pads were edematous, which was used to identify cellular immune responses. Each rat's right hind paw was subcutaneously injected with 0.1 ml of 0.025×10^9 sRBC/ml on the 27th day. By measuring the volume of the foot pad after 4, 24, and 48 hours, the foot pad's reaction to the hypersensitivity was evaluated. As a measure of the cellular immune response, the foot pad reaction was expressed as a mean percent increase in paw volume before and after an antigenic challenge.

2.5.6. Neutrophil adhesion test[9]

On the 20th day of drug treatment, blood samples were drawn from the retroorbital plexus of veins and diluted with WBC diluting fluid in a WBC pipette to lyse red cells without affecting the leucocyte population. Total leukocyte count (TLC) was calculated using an improved Neubauer's chamber. For Differential count of Leucocytes (DLC) blood smear was stained with Leishman stain Neutrophils were identified on the basis of cell size, presence of numerous violet granules and shape of nucleus per hundred leukocyte count, under an oil immersion preparation. Following initial counts, blood samples were incubated for a further 25 minutes at 37 °C in 80 mg/ml nylon fibers. TLC and DC were computed. The amount of neutrophil adhesion was determined using the formula

$$Neutrophil\ adhesion\ (\%) = \frac{NI_u - Ni_t}{NIu} \times 100$$

Where, NI_u=Neutrophil Index of untreated blood samples

Ni_t= Neutrophil Index of treated blood samples

Neutrophil Index (NI) of treated blood samples=TLC×% of neutrophil

2.6. Statistical analysis

Turkey's multiple comparison t test was used, and a P value < 0.001 was considered significant after the data were analyzed using one way analysis of variance (ANOVA) and expressed as mean±SEM using GraphPad Prism 5.

3. Result & Discussion

Immunomodulators are a group of drugs that act by modifying normal immune system. Both immunosuppressants and immunostimulants are possible. Immunostimulants are a class of medications that stimulate both the specific and nonspecific immune system, including granulocytes, macrophages, complement, some T lymphocytes, and various effector substances, while immunosuppressants lessen resistance to infections, environmental stress, or chemotherapy-related factors.[16] The immune system is found to be modulated by a number of herbs and plant extracts that are effective in balancing and maintaining immune system function.[17] By assessing the impact of the aloe vera aqueous extract

Table 2: Effect of aqueousextract of aloe vera on primary abtitre (20^{th} day) & secondary ab titre on (27^{th} day)

Animal No	Primary Ab titre(20th day)				Secondary Ab titre (27th day)			
	GA	Dexa	AVE125mg/kg	AVE250mg/kg	GA	Dexa	AVE-125	AVE-250
1	5	2	6	10	6	3	9	12
2	6	4	7	11	7	4	10	13
3	7	2	8	11	6	4	11	12
4	8	3	9	12	7	4	10	13
5	6	3	8	11	7	3	10	13
6	7	4	9	10	8	4	11	12
Mean	6.5	3b	7.83	10.5**	6.8	4.1b	10.17	12.50***
SE	0.42	0.36	0.47	0.30	0.30	0.02	0.30	0.22
KW Score			13.03				15.44	
P value		<0.01	<0.01			<0.01	<0.001	

**p<0.01, ***p<0.001 AVE vs GA (KW test with Post ANOVA Dunn"s test)
b-p<0.01 Dexamethasone vs GA (Mann-Whitney U test)

Table 3: Effect of aloe vera aqeous extract on paw edema volume on 27^{th} day

Drug & doses	Paw edema volume of rat hind paw		
	4HR	24HR	48HR
GA	0.40±0.03	0.05±0.01	0.02±0.01
DEXA	0.18 ± 0.03^{b}	0.02±0.01	0.01±0.001
AVE(125mg/kg)	0.46±0.04	0.08±0.02	0.05±0.04
AVE(250mg/kg)	0.8±0.08***	0.5±0.05***	0.1±0.05*
F	29.49	14.3	4.43
df	2,15	2,15	2,15
p	<0.001	<0.001	<0.05

Data expressed as mean±SEM (n=6)
A-p<0.05, b-p<0.01: Dexamethasone Vs Control group (unpaired t test)
*p<0.05, ***p<0.001: AVE treated group Vs control group (One way ANOVA with Turkey"s multiple comparision test

Table 4: Effect of dexamethasone & ave on % of neutrophil adhesion (neutrophil adhesion test)

Drug & doses	TLC(10^3Cmm UB	TLC(10^3Cmm) FTB	%N UB	%N FTB	NI UB	NI FTB	%Neutrophil Adhesion
GA	8.73±0.27	6.98±0.46	23.33±1.24	21.17±0.87	204.47±14.51	147.15±10.01	28±2.04
DEXA (0.1mg/kg)	6.91 ± 0.47^{a}	6.07 ± 0.28^{a}	24.67±1.44	20.33±2.02	172 ± 18.56^{a}	137.1 ± 17.4^{a}	21±2.6
AVE(125mg/kg)	10.63±0.06	8.90±0.04	30.50±2.1	22.17±1.8	324.77±29.8	196.18±15.9	39±3.9
AVE(250mg/kg)	10.47±0.4***	7.40±0.8***	28.67±1.8***	22.17±8.7***	300.1±23.3***	164.95±20.8***	45±5.1***
F	56.5	28.59	16.65	12.3	78.5	65.5	58.97
Df	2,15						
P	<0.001						

Data expressed as mean ± SEM, n=6
***p<0.001 AVE vs Control group (one way ANOVA with Tukey"s multiple comparision test
a—p<0.001 Dexamethasone Vs Control group (unpaired t test)

on hemagglutination antibody titers, DTH reactions, and neutrophil adhesion tests in Wistar albino rat models, the current study investigated the immunomodulatory activity of the aloe vera extract.

T, B Lymphocytes, and macrophages must cooperate for antibody production against the T-dependent antigen SRBC. Aloe vera extract immunostimulation was accomplished through humoral immunity, according to the high values of hemagglutinating antibody titers that were obtained in this case.[18] B cells interact with the antigen as a part of humoral immunity, and as they grow and differentiate, they produce plasma cells that secrete antibodies. Its impact on sheep erythrocyte-specific HA titre in rats was examined to gauge the impact of AVE on the humoral immune response. On the 20th and 27th days, AVE at a dose of 250 mg/kg bw significantly increased antibody titre when compared to the control group (Table 2). Selvraj et al. (2005) and Im SA et al. (2005) provide support for this.[19]

When paw edema volume increases in response to an antigenic challenge, a delayed type of hypersensitivity

response—a clear indicator of cell mediated immunity (CMI)—has occurred. The paw edema volume was found to be significant at a dose of 250mg/kg of aqueous extract of aloe vera as compared to Gum Acacia (Table 3). Sensitized T lymphocytes undergo conversion to lymphoblasts and secrete cytokines that draw additional scavenger cells to the site of the reaction during CMI responses when challenged by an antigenic sheep RBC. Thus, the infiltrating cells are activated to support the defensive response. In our study, paw edema volume increased following AVE treatment, indicating an improvement in cell-mediated immunity. Sampedro et al. (2004) reported similar outcomes.[20]

Neutrophils are crucial for cell-mediated immune reactions. When activated, they cause the removal of foreign bodies through recognition, migration toward the foreign body, phagocytosis, and the destruction of the foreign agent;[13] the process of neutrophil extravasation involves confirmational changes in LFA1 and MAC1 in the neutrophil membrane in the presence of IL-1 and MIP-1β, which increase their affinity for ICAM-1 of endothelial cells. In order to bind neutrophils carrying modified integrins, nylon fibers act as an ICAM analogue. Thus there is margination of neutrophil from the blood vessels. Percentage of neutrophil adhesion was significantly increased in Aloe vera treated group at a dose of 250mg/kg when compared with control immunized group (Table 4) whereasas treatment with dexamethasone, the percentage of neutrophil adhesion was significantly reduced as compared to control group. This might be due to enhancement of immune response by Aloe vera aqueous extract.

Acemannan, the mannose rich polysaccharide of Aloe Vera has direct effect on immune systems, by activating & stimulating macrophages, monocytes, antibodies and T cell function. The current study also supports the findings of Altug et al. (2010), who established the immunostimulatory effect of aloe vera on cellular and humoral immune responses following vaccination with polyvalent vaccines in dogs and discovered that aloe vera enhanced both cellular and humoral immune responses.[21]

4. Conclusion

The present study showed the immunostimulant property of Aloe vera aqueous extract when used orally. Aloe vera aqueous extract at a dose of 250 mg/kg showed immunostimulant properties in terms of a rise in Ab titre after antigen challenge (humoral immune response), enhancement of paw edema volume as a parameter of cell mediated immune response, and also a percentage increase in neutrophil adhesion. This study reaffirms the immunostimulant property of Aloe vera aqueous extract in Wistar albino rats.

5. Source of Funding

None.

6. Conflict of Interest

None.

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