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Effect of Methanolic Extract of *Aloe vera* on Immunomodulatory Activity in Wistar Rats

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ABSTRACT

Background: To evaluate the Immunomodulatry activity of methanolic extract of *Aloe vera* in Wistar rats.

Methods: Eighteen clinically healthy wistar rats were divided into three groups consisting of six rats in each group. Group I treated with normal saline, Group II with 0.8% SRBC as antigen 5th day as sensitizing and 10th day as challenging dose, Group III antigen with methanolic extract of *Aloe vera* Orally @ 200mg/kg.body wt. consecutive for 5 days during sensitizing and challenging dose. The immunological parameters were evaluated with haemagglutination (HA) test for humoural immune response (HIR) whereas delayed type hypersensitivity (DTH) for evaluating cell mediated immune response (CMIR). DTH was assessed by injecting 1-chloro-2,4-dinitrobenzene (DNCB) in the foot paw region as well as by absolute lymphocyte count.

Result: Methanolic extract of *Aloe vera* gel showed significant effect on humoural immune response as assessed by HA test. Antibody titre were found to be significantly ($p<0.01$) higher in *Aloe vera* treated group (1.35402 ± 0.205) as compared to antigen treated (0.60201±0.076)group.DTH assessed by injecting 1-chloro-2,4-dinitrobenzene (DNCB) mitogen was also found to be significantly ($p<0.01$) higher in aloe vera treated group (3.012 ± 0.15* mm) as compared to antigen treated (0.482 ± 0.04 mm). Methanolic extract of Gel of *Aloe vera* exhibited immunomodulatory activity with respect to cell mediated immune response in wistar rats.

Key words: *Aloe vera*, Humoral antibody delayed type hypersensitivity, Immunomodulation, Wistar rat.

INTRODUCTION

Natural products that are used traditionally will help in the rediscovery of useful drugs. Herbal products are widely used in the treatment of various human diseases for centuries. Mother Nature has gifted numerous plants containing therapeutic activity. Medicinal plants have played an important role in primary health care system among the people as surrounding in resident. People use these plants traditionally available for betterment of the health, because these are easily available, less expensive and have lesser side effects as compare to modern medicines. The primary cure of diseases by medicinal plants is based upon deep observation of nature and the understanding of traditional knowledge of medical practices. Herbal therapies are based on indigenous theories, beliefs and experiences that are handed down from generation to generation (WHO, 2000). Traditional medicine has developed in accordance with the life style and cultural practices of the society throughout the centuries. In Ayurvedic system of medicine considers immunostimulation as an alternative to conventional chemotherapy under the concept of 'Rasayana', where the host defense mechanism has to be activated under conditions of impaired immune responsiveness. True immunomodulation includes both stimulation and suppression of the immune system. Certain drugs that normalize or modulate pathophysiological processes are called immunomodulatory agents.

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.(Lay DG 1986). Aloe vera gel (AVG) has antioxidant properties, effectively reduces inflammation and promotes wound healing (Davis *et al.,* 1989 and Rajasekaran 2005). Aloe vera is well known for its considerable medicinal properties. *Aloe vera* has been described as a "magic bullet" in various literatures but when examined closely and thoroughly it is not one but many magic bullets that combine to create the magic of the *Aloe vera* (Ronald 1997). This plant is one of the richest natural sources of health for human beings coming. The chemistry of the plant has revealed the presence of more than 200 different biologically active substances. Many biological properties associated with Aloe species are contributed by inner gel of the leaves.

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Most research has been centralized on the biological activities of the various species of Aloe, which include antibacterial and antimicrobial activities of the nonvolatile constituents of the leaf gel. Aloe species are widely distributed in the African and the eastern European continents and are spread almost throughout the world. A. vera has various medicinal properties such as antitumor, antiarthritic, antirheumatoid, anticancer and antidiabetic properties. In addition, A. vera has also been promoted for constipation, gastrointestinal disorders and for immune system deficiencies. Hence The present study was aimed at screening the Gel of *Aloe vera* for immunomodulatory activity in wistar rat and work has been approved by CPCSEA Institutional Animal Ethical committee no. IAEC/BVC/20/17.

MATERIALS AND METHODS

Plant material and extract preparation

The leaves of *Aloe vera* were washed with distilled water and were subjected to surface sterilization with 70% ethyl alcohol followed by 0.1% H_gCl_2.The parenchymatous covering of the leaves were peeled and the gel drained out and fresh leaf gel was dried in the oven at 80°C for 48 h. (Rubina *et al.,* 2009.*)* and then powdered dried powder were used for Soxhlet extraction with methanol for 8 h. The contents was filtered through Whattman filter paper no. 1 and the filtrate was evaporated to dryness. This dried extract was further powdered and then dissolved in distilled water with concentration of 50 mg/ml.

Experimental animals, drug and designed

18 (Eighteen) healthy male wistar rats (150-200 g) were bought from Chakrworty enterpirses Pvt. Ltd Kolkata CPSEA approved experimental animal supplier. The rats were kept for two weeks to adapt the environmental condition. They were housed in steel grill cages in a room with controlled temperature of 20±22°C. The rats were fed standard diet and provided with fresh water *ad libitum*. The study was approved by Institutional Animal Ethics Committee, Bihar Animal Sciences University which is registered by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. Vide no. IAEC/BVC/20/17 For conducting immunological study, clinically healthy rats were divided into three groups consisting of six rats in each group. Details of treatment given to different groups are given below.

Group I: Saline control.

Group II: Antigen control- 0.1 ml of sheep red blood cell. (SRBC) suspension (1×10^8) given intra peritoneal in each rat on the 5st day of Experiment (sensitizing dose) and on the 10th day of experiment (challenging dose). described by Suja *et al.* (2009).

Group III: *Aloe vera* + Antigen – Apart from antigen (SRBC) given as in Group II, methanolic extracts of *Aloe vera* was administered @ 200 mg/kg, orally daily for consecutive 5 days during sensitizing and challenge period with the help of oral gavage needle.

Assessment of immune response after different treatment

Humoral immune response (HIR)

For assessing HIR, HA test was done as described below:

Haemagglutination (HA test)

Humoral immune response (HIR) was assessed by HA test. Antigen used for haemagglutination reactions was sheep red blood cell (SRBC). Haemagglutination test was performed in the test and control rats of sera. The anti-SRBC antibody titres were measured using micro-titration technique by the procedure described by Beared (1980).

HA test was performed in 'U' shaped micropersplex plate. By taking 0.5 ml of serum, two fold serial dilutions were made in PBS, except in control well in which only PBS (0.25 ml) was added. In next step 0.25 ml of 0.8% SRBC suspension was added to all the wells. A known positive and negative control was also included. The plate was stirred gently for mixing and uniform distribution of erythrocytes and left at room temperature for 40 min. The rat's serum produced a diffused sheet of agglutination RBC covering the bottom of the wells. On the other hand, negative on control well showed circumscribed compact bottom at the bottom. The HA pattern was read with the aid of reading mirror and result of HA titre was recorded as reciprocal of the highest dilution showing 100% HA (complete agglutination of erythrocytes) and expressed as $\log_2$HA titre/0.5 ml of rat's serum.

Cell-mediated immune response (CMIR)/Delayed type hypersensitivity (DTH)

Cell-mediated immune response was assessed by DTH reactions

Mitogens used for cutaneous basophilic hypersensitivity reactions are as follows:

DNCB (1 chloro-2, 4-diritrobenzene)

DNCB skin sensitivity test

The test was done as per the described method by Chauhan 1983**.** Six rats from each group were taken. Area of about 0.5-1 cm^2 was cleaned with acetone and dried. 0.1 ml of DNCB (10 mg/ml) in acetone vehicle was applied on right side paw. On left side, 0.1 ml of acetone was applied which served as control. DNCB was applied on 5th day and challenged on 10th day of experiment by applying 0.1 ml of DNCB (10 mg/ml) in acetone on right side and 0.1 ml acetone on the left side at the same site of first application. The skin thickness was measured with the help of slide caliper at 4, 8,12,24 and 48 h during pre-and post-challenge. The CMI response was calculated by subtracting the thickness of right side from left side.

Measurement of skin thickness by slide caliper

Main scale×Vernier scale×limit factor (0.01). Diameter measured on three different positions.

Statistical analysis

Comparison of effects of different extracts on immune response in Wistar rats at different time intervals and on various days of post treatment in all five groups and data of the parameters were subjected for statistical analysis using Ms office-excel (2010). (Savala *et al*., 2012)

RESULTS AND DISCUSSION

Humoral immune response (HIR)

Effect of methanolic extracst of *Aloe vera* on humoral immune response in Wistar rats were recorded using sheep red blood cell (SRBC) as an indicator of humoral immunity.

Table 1 shows humoral immune response of different group against SRBC antigen (mean ± S.E.) to HA antibody titre (log 2 value) in Wistar rats. The study revealed that significant ($p>0.05$) immunomodulatory effect on humoral immunity by *Aloe vera* treated group as compared to saline and Antigen control. The HA antibody titre were recorded in *Aloe vera* treated group (0.652222 ± 0.090223*) produced significantly higher titer followed by Antigen Treated group (0.411398 ± 0.038272). The antibody forming responses against SRBC were gradually increased from 7th day and highest HA titre was observed on 14th day and then declining trends noted

Cell-mediated immune response (CMIR)

Effect of extracts of *Aloe vera* was shown on cell-mediated immune response in Wistar rats after multiple oral administrations were observed by using mitogen dinitrochlorobenzeene (DNCB) as indicator of cell-mediated immunity.

Table 2 and 3 depict cell-mediated immune response of extracts to DNCB mitogen (mean ± S.E.).The treatment caused increased in skin thickness (mm) in rats after multiple oral administration. Table 2 indicated that there was significant increase in skin thickness in *Aloe vera* treated group (1.364±0.0572 mm) as compared to both the control groups (0.362±0.0248 mm-saline group and 0.406±0.038 mm-antigen group) in pre-challenging period *i.e.*, fifth day of experiment. Table 3 indicated that there was significant increase in skin thickness in at 12 hr in *Aloe vera* treated group (3.012±0.1512 mm) as compared to both the control groups (saline and antigen group) in post-challenging period (10th day) of experiment. Thus, the present study indicated that there was immunomodulatory action on cell-

Table 1: Humoral immune response (HIR) of different extracts of *Aloe vera* against SRBC antigen (Mean ± S.E.) to antibody titre (Log 2 value) in Wistar rats.

Days	Saline control	Antigen control	*Aloe vera* + Antigen
0	0.60204±0.0776	0.30101±0.01	0.30102±0.01
7	0.30101±0.01	0.30101±0.01	0.30102±0.01
14	0.30101±0.01	0.60205±0.0777	1.35462±0.2019
21	0.30101±0.01	0.50170±0.0634	0.80273±0.1488
28	0.30101±0.01	0.35119±0.05017	0.50170±0.1003

Overall effect

Group	Mean±S.E.
1. Saline control	0.361226± 0.01552
2. Antigen control	0.411398± 0.038272
3. *Aloe vera* + Antigen	0.652222±0.090223*

It denotes level of significant variation ($P<0.01$).

Table 2: Cell-mediated immune response (CMIR) of *Aloe vera* (Mean ± S.E.) to DNCB mitogens in wistar Rats.

Hour (h)	Saline control	Antigen control	*Aloe vera* + Antigen
4	0.38±0.023	0.42±0.025	0.92±0.038
8	0.37±0.039	0.41±0.031	1.11±0.042
12	0.41±0.022	0.53±0.085	1.99±0.091
24	0.32±0.015	0.36±0.021	1.46±0.086
48	0.31±0.025	0.31±0.028	1.31±0.029

Overall effect

Group	Mean±S.E.
1. Saline control	0.362±0.0248
2. Antigen control	0.406±0.038
3. *Aloe vera*+Antigen	1.363±0.0572

Pre-challenge (5th day).

Table 3: Cell-mediated immune response (CMIR) of *Aloe vera* (Mean ± S.E.) to DNCB mitogens in Wistar Rats.

Hour (h)	Saline control	Antigen control	*Aloe vera* + Antigen
Post-challenge (10th day)			
4	0.41±0.023	0.44±0.029	2.25±0.152
8	0.49±0.039	0.47±0.046	2.53±0.187
12	0.53±0.022	0.69±0.079	4.66±0.201*
24	0.38±0.015	0.45±0.028	3.05±0.117
48	0.31±0.025	0.36±0.021	2.57±0.099
Overall effect			

Group	Mean ± S.E.
1. Saline control	0.424±0.0248
2. Antigen control	0.482±0.0406
3. *Aloe vera* + Antigen	3.012±0.1512*

It denotes level of significant variation ($P < 0.01$).

mediated immunity by these extracts. Increase in cutaneous basophilic hypersensitivity (CBH) responses to DNCB was gradually increased from 4 h with the highest CBH reaction at 12 h and thereafter declining trend was observed upto 48 h during both pre-challenge period (5th day) and post-challenge (10th day) of experiment.

All vertebrate animals have two arms of immune response *i.e.* humoral immune response (HIR) and cell mediated immune response (CMIR). The efficacy of both the arms can be judged. The humoral immune response can be judged by the demonstration of antibody titre against particular antigen where as CMIR can be judged by delayed type hypersensitivity (DTH). Sheep red blood cell (SRBC) is more often used as an antigen for the evaluation of HIR in different experimental animals by assessing the antibody titre in the sensitized host. In the present study, the serum antibody titre to SRBC was evaluated using haemaglutination test for the assessment of HIR (Chauhan, 1983). Like wise, CMIR was determined by DTH (Beard, 1980) reaction. *Aloe vera* is reported to be beneficial in the treatment of respiratory, cardiovascular and rheumatic diseases as well as in diabetes. Various experimental studies also have suggested antioxidant (Gopa *et al*., 2012 and Bhattacharya, 1999).

In the present investigation, the haemagglutination antibody titre recorded in *Aloe vera* treated group produced significantly higher titre (2.35805±0.1633) as compared to saline control (0.361226 ±0.01552) and antigen (0.411398 ±0.038272) treated group. Therefore, it is suggested that significant immunomodulatory effect has occurred on humoral immune response by simultaneous administration of *Aloe vera* The above findings are in agreement with the supportive results who observed that the administration of *Aloe vera* increases antibody titre resulting in potentiation of humoral immune response. In the present study, the antibodies formation was observed on 7th days of antigen exposure and highest HA titre was observed on 14th day followed by declining trend thereafter upto 28th days. The above findings are also in agreement with findings of Jyotsana (2008), who observed more or less similar observation and reported antibody titter that alter the humoral immune response of mice, the highest titter was recorded on 18th day after the administration of extracts given intra peritoneal @300mg/kg in mice.

The DNCB skin sensitivity tests suggest significant immunomodulatory effect on cell mediated immune response by the methanolic extracts used in the present study (Table 2 and 3)as compared with other antigen treated group skin sensitivity test showed significant immunomodulatory effect on cell-mediated immune response in post challenge period of experiment. Halder (2012) was also observed significant increases DTH responses in post Challenged of experiment. The result also supported by Narayanan *et al*. (2021).

Cutaneous basophilic hypersensitivity (CBH) responses to DNCB was gradually increased from 4 h and thereafter with the highest CBH reaction was found at 12 h and thereafter declining trend was observed upto 48 h during both pre-challenge period (5th day) and post-challenge (10th day) of experiment and also observed the increases skin thickness after simultaneously administration of *Aloe vera* in mice (Chandua *et al.,* 2011 and Halder *et al*., 2012). In immunomodulatory study, *Aloe vera* gel extract produce immunostimulant activity. It could be due to chronic use of *Aloe vera* gel extract produce immunostimulation (Bhalsinge *et al*.2018). Other finding on fraction of aloe vera showed immunomodulatory activity by inducing macrophage cell viability (Farahnejad *et al*., 2011 and Purnamasari *et al*., 2024). Effect of Aloe vera extract on humoral and cellular immune responses in rabbit showed immunopotentiation including lymphocytes and serum immunoglobulin (Vahedi, 2011). The immunomodulatory finding of the study also supported by Subhasis *et al.* (2024) and Patel *et al.* (2017).

CONCLUSION

The study revealed immunomodulatory effect on humoral immunity as well as cell mediated immune responses by *Aloe vera* and it showed highly Immunopotentiating activity as compared to antigen Control and saline Control group, Hence it will be useful after immunization and other immunocompromising conditioned.

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Conflict of interest

We certify that there is no conflict of interest discussed in the manuscript.

REFERENCES

Beard, C.W., (1980). Serological Procedures. Isolation and Identification of Avian Pathogens. 2nd edn. Creative Printing Company, New York. pp 129-135.

Bhalsinge, R.R., Rajbhoj, S.R., Limaye, M.V., Vaidya, M.U., Rane, P.S. and Tilak, A.V. (2018). Anti-Inflammatory and Immunomodulatory activity of ethanol extract of *aloe vera* gel. International Journal of Pharmaceutical Sciences and Research. 9: 832-835.

Bhattacharya, A., Chatterjee, A., Ghosal, S., Bhattacharya, S.K. (1999). Antioxidant of *Emblica officinalis.* Indian Journal of Experimental Biology. 37: 676-680.

Chandua, A.N., Santhosh, K., Bhattacharjee, C., Debnath, S., Kamala, K. (2011). Studies On Immunomodulatory activity of *Aloe vera* Int. J. Appl. Biol. Pharm. Technol. 2: 19-22.

Chauhan, H.V.S., Verma, K.C. (1983). Evolution of cell mediated immunity to Marek" disease Br. Vet. J. 139: 57-65.

Davis, R.H., Leitner, M.G., Russo, J.M., Byrne, M.E. (1989). Wound healing, Oral and topical activity of Aloe vera. J. Am. Podiatr. Med. Assoc. 79(11): 559-562.

Farahnejad, Z., Ghazanfari, T., Yaraee, R. (2011). Immunomodulatory effects of *Aloe vera* and its fractions on response of macrophages against *Candida albican*. Immunopharmacology and Immunotoxicology. 33: 676-681.

Gopa, B., Bhatt, J.K., Hemavathi, K.G.A. (2012). Comparative clinical study of hypolipidemic efficacy of Amla (*Emblica officinalis*) with 3 hydroxy 3 methylglutaryl coenzyme A reductase inhibitor simvastatin. Indian Journal of Pharmacology. 44: 238-242.

Halder, S. Mehta, A.K., Mediratta, P.K. (2012). Augmented humoral immune response and decreased cell mediated immunity by aloe vera in rats. Inflammopharmacol. 20: 343-346.

Madan, J., Sharma, A.K., Inamdar, N., Rao, H.S., Singh, R. (2008). Immunomodulatory Properties of *aloe vera* gel in mice International Journal of Green Pharmacy. pp 152-154.

Purnamasari, L., dela Cruz , J.F., Lee , D.B., Choi, Y.J., Yi , J.K., Hwang, S.G. (2024). *In vitro* study on the immunomodulatory and antiinflammatory effect of soregen® technology water in broiler and layer hens peripheral blood mononuclear cells. Indian Journal of Animal Research. doi: 10.18805/IJAR.BF-1798.

Lay, D.G., Reynolds, T. (1986). The Aloe vera phenomenon: A review of the properties and modern uses of the leaf parenchyma gel. J. Ethnopharmacol. 16(2-3): 117-51.

Narayanan, K.R, Dhasarathan, P., Manujula, M., Thenmozhi, M. (2021). Immunomodulatory efficiency of *Abelmoschus esculentus* in swiss albino mice. Indian Journal of Animal Research. 55(4): 401-406.

Patel, P., Singh, H.S., Mishra, A., Sahina, P., Ansari (2017). *Emblica officinalis* and *Tinospora cordifolia* supplementation possess immunomodulatory and adaptogenic properties in murrah buffalo calves. Indian Journal of Animal Research. 51(3): 506-509. doi: 10.18805/ijar.v0iOF.7654.

Rajasekaran, S., Sivagnanam, K., Subramanian, S. (2005). Antioxidant effect of Aloe vera gel extract in streptozotocin-induced diabetes in rats. Pharmacol Rep. 57(1): 90-96.

Ronald, P.P. (1997). *Aloe vera* as a magic bullet". Aloe Science Council, Inc. reprint of articles, Jan (www.google.com).

Lawrence, R., Tripathi, P., Jeyakumar, E. (2009). Isolation, Purification and Evaluation of Antibacterial Agents From *Aloe Vera* Brazilian Journal of Microbiology. 40: 906-915.

Savala, N.K., Parvathaneni, N.H. and Mangamoori, L.N. (2012). Haematological studies of *Emblica officinalis* formulation on Wistar Rats. International Journal of Medical and Health Sciences. 2: 29-34.

Suja, R.S., Nair, A.M.C., Sujith, S., Preethy, J., Deepa, A.K. (2009). Evaluation of immunomodulatory potential' of *Emblica officinalis* fruit pulp extract in mice Indian Journal of Animal Research. 43(2): 103-106.

Subhasis, S., Pradhan, R., Upadhyay, R., Nipa, S., Ratha, B., (2024). Evaluation of immunomodulatory effect of aqueous extract of *Aloe vera* in wistar albino rat models. Panacea Journal of Medical Sciences 14(1): 249-254.

Vahedi, G., Taghavi, M., Maleki, A.K., Habibian, R. (2011). The effect of Aloe vera extract on humoral and cellular immune response in rabbit. African Journal of Biotechnology. 10: 5225-5228.