ORIGINAL ARTICLE

Immunomodulatory properties of *aloe vera* gel in mice

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Administration of *Aloe vera* extract to swiss albino mice (300 mg/kg i.p.) daily for five days, significantly ($P < 0.01$) increases the total white blood cells count. Further, it increases humoral immune response, as demonstrated from the increase in plaque-forming cells in the spleen and circulating antibody titre.

Key words: *Aloe vera*, immunomodulatory activity, phagocytic activity

INTRODUCTION

The natural resistance of the body against infection can be enhanced by the use of herbal drugs.[1] Several herbal preparations that can enhance the body's immune status are extensively being used in the indigenous system of medicines. There is an upsurge in the clinical usage of indigenous drugs as they are free from serious side effects. Dua *et al*,[2] reported a large number of plants having known immunomodulatory activity.

Aloe vera (L.) Burm. (Liliaceae) (*synonym: Aloe barbadensis)* is one of the most widely used healing plants in the history of mankind. *A. vera* is used in traditional medicine of many cultures and said to be beneficial in the treatment of disorders such as arthritis, gout, dermatitis, peptic ulcer and burns.[3] *Aloe vera gel* (AVG) is one of the few substances known to effectively decrease inflammation and promote wound healing.[4] It has also been investigated for its antioxidant property.[5] Therefore, the present study was conducted to investigate the immunomodulatory activity of an AVG extract.

MATERIALS AND METHODS

Plant Material

A. vera (Fresh) were collected and authenticated from Medicinal plant Research and Development Centre, Govind Pant University of Agriculture and Technology, Pantnagar (Uttarakhand), India. A voucher specimen (AV-8) has been retained in our museum for future reference.

Extract Preparation

Collected leaves of *A. vera* were washed with water and cut transversely into pieces. The thick epidermis was selectively removed and solid gel in the center of the leaf was separated and homogenized. The resulting mucilaginous homogenate was lyophilized and further extracted with ethanol (95%). The filtrate was collected and evaporated to dryness under reduced pressure in a rotary evaporator. The residue was stored in dry sterilized containers at 4° until further used.[5] The extract was resuspended in distilled water for further use.

Animals

Swiss albino mice of either sex weighing 25 ± 2 g were obtained from Indian Veterinary Research institute, Izatnagar, Bareilly. The animals were housed in controlled environment (temperature 25 ± 2° and 12 hr dark/light cycles) and fed with standard pellets diet (Hindustan lever pellets, Bangalore India) and tap water *ad libitum*. The study was conducted after obtaining Institutional Animal Ethical Committee approval.

Effect on Total WBC Count

Mice were divided randomly into 3 groups with 6 mice in each group. Mice in group A served as a control and were given saline (5 ml/kg, i.p). Mice in group B and C were given AVG extract i.p. in the dose of 150 mg/ kg and 300 mg/kg respectively for 5 days. Blood was collected from the tail vein before the first treatment and then every 3rd day after the 5th dose of drug administration till one month. The total

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WBC (white blood cell) count was determined using a Hemocytometer.[6]

Effect on Antibody Production

Three groups of six swiss albino mice each were immunized with 2.5 x 10^8 sheep red blood cells (SRBC) intraperitoneally. The animals in group B1 and C1 were given extract 150 mg/kg, i.p and 300 mg/kg, i.p respectively, daily for 5 days prior to the immunization. Blood was collected from the caudal vein before the first dose and on every 3rd day after the fifth dose till one month. Antibody titre was determined by the hemagglutination method.[7] The animals in control group A1 received saline (5 ml/kg, i.p).

Effect on Plaque-Forming Cells

Three groups of nine mice each were immunized with 2.5 x 10^8 SRBC i. p. The mice of group B2 and C2 were given extract 150 mg/kg, i.p and 300 mg/kg, i.p respectively, daily for five days prior to the immunization. The animals were killed on various days, the spleen was processed and the numbers of plaque-forming cells were determined by the method of Jerne and Nordin.[9] The animals in control group A2 received saline (5 ml/kg, i.p).

Effect on the Phagocytic Activity of Peritoneal Macrophages

Peritoneal macrophages were elicted with sodium caseinate in three groups of swiss albino mice treated with AVG extract (150 mg/kg i.p or 300 mg/kg, i.p) daily for five consecutive days, while control animals received only saline. Macrophages were harvested on the 5th day and the phagocytic activity was assessed by the method of Mehara and Vaidya[9] using opsonized SRBC.

Statistical Analysis

Results are expressed as mean $\pm$ S.D. Data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnet's test to determine statistical significance.

RESULTS

Effect on Total WBC Count

In Group B (treated with 150 mg/kg, i.p AVG extract), there was no increase in the total count of leukocytes while a significant increase ($P < 0.01$) in WBC count was observed in Group C (treated with 300 mg/kg, i.p extract). The maximum count (13537 cells/ml) was obtained on the 15th day [Fig. 1]

Effect on Antibody Production

AVG extract in the dose of 300 mg/kg, i.p was found to enhance the production of circulating antibody titre [Table 1]. The highest antibody titre of 266 was observed on the 18th day for the dose of 300 mg/kg, i.p, whereas control animals showed a maximum antibody titre of 34 on the same day. The animals treated with the dose of 150 mg/kg, i.p did not show any enhancement in antibody production.

Effect on Plaque-Forming Cells

A significant increase ($P < 0.01$) in the number of plaque-forming cells (PFC) was observed after administrationof AVG extract (300 mg/kg, i.p). The highest number of PFC (1604 PFC/10^6 spleen cells) were observed on the 5th day after immunization [Fig. 2]. However the mice treated with AVG extract 150 mg/kg, i.p did not show any increase in the number of PFC.

Effect on the Phagocytic Activity of Macrophages

The phagocytic activity of peritoneal macrophages was enhanced in animals treated with AVG extracts (300 mg/kg, i.p). The number of macrophages with the engulfed SRBC [Table 2] were significantly ($P < 0.01$) higher (78.4/200 cells) in comparison to control animals (37.35/200 cells).

DISCUSSION AND CONCLUSIONS

Immunomodulators are used as an adjuvant in conditions

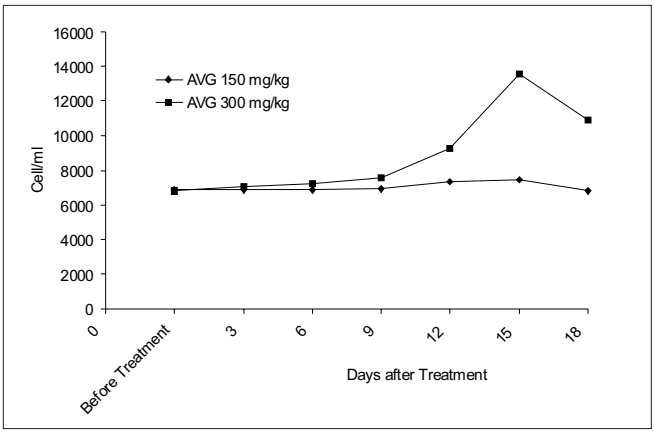

Figure 1: Effect of *Aloe vera* gel (AVG) extract on total WBC count; $n = 6$; $P < 0.01$ *vs* control

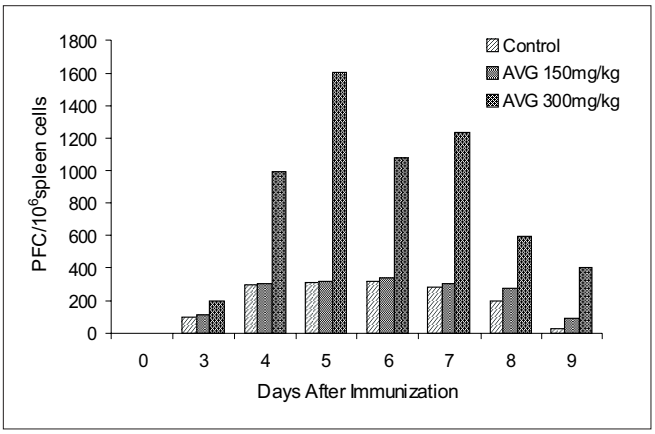

Figure 2: Effect of *Aloe vera* gel (AVG) extract on plaque forming cells (PFC); $n = 9$; $P < 0.01$ *vs* control

Table 1: Effect of *Aloe vera* gel (AVG) extract on circulating antibody titre in mice

	Dose[a]	Antibody titre at days after immunization									
		3	6	9	12	15	18	21	24	27	30
Control (saline, 5 ml/kg, i.p)	-	8	9	16	18	33	34	33	16	17	17
AVG extract	150	8	9	18	16	33	33	33	17	15	17
AVG extract	300	16	32	32	66	132	266	133	68	68	31

[a]Daily for 5 consecutive days; along with the fifth dose, 2.5 x 10^8 SRBC was administered

Table 2: Effect of *Aloe vera* gel (AVG) extract on macrophage phagocytic activity

Treatment	Dose[a] (mg/kg, i.p)	Phagocytised/200 macrophages[b]
Control (saline. 5 ml/kg, i.p)	-	37.35±11.2
AVG extract	150	35.3±5.1
AVG extract	300	78.4±5.5*

[a]Daily for 5 consecutive days; [b]Values are mean + S.D.; $n = 6$; $P^* < 0.01$ extracts vs. control

of immunodeficiency in cancer and to a limited extent in acquired immunodeficiency syndrome. Immunomodulatory agents of plant and animal origin enhance the immune response of an organism against a pathogen by activating the immune system. However these agents should be subjected to systematic studies to substantiate the therapeutic claims made with regard to their clinical utility.[10] The present experiments revealed that AVG extract (300 mg/kg, i.p) has immuno-stimulatory action. However negligible or no effects were observed at a dose of 150 mg/kg. The higher dose stimulates the proliferation of stem cells, as seen from an increase in total white blood cells. Further increase in PFC and circulating antibody titre, suggests that AVG extract may stimulate the humoral immunity. More over the extract was found to stimulate phagocytic activity. Hence it can be concluded that the AVG extract may be a potential candidate in several immuno-suppressed clinical conditions.

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