Original Article

Immunomodulatory Effects of Aloe Vera on Skin of BCG Vaccinated Albino Rats

Effects of Aloe Vera on Skin

Huma Riaz[1], Aisha Akbar[2], Maria Ilyas[1] and Mariam Saad[3]

ABSTRACT

Objective: To determine the **Immunomodulatory** and anti- inflammatory effects of Aloe Vera gel by observing the changes in scar of Mantoux reaction on rat skin.

Study Design: Randomized Control Trial Study

Place and Duration of Study: This study was conducted at the Anatomy Department of PGMI, Lahore in March 2016 till February 2017.

Materials and Methods: The randomized experiment was performed on 24 albino rats (divided in 4 groups) of either sex. Group A was control. Group B,C and D were vaccinated by BCG intra dermally on day one. Group B was on placebo for thirty days. Group C and Group D were on different doses of *Aloe Vera (*For comparative study) orally by 5 cc disposable syringe for thirty days. On 30th day, Mantoux injection was given to Group B,C and D. After 72 hours the Mantoux injection site was observed grossly. Rats were sacrificed and specimen of skin were taken from that injection site, fixed and stained with H& E for microscopic analysis to observe the inflammatory and immune response.

Results: Statistically significant decrease in scar size of Mantoux reaction was observed in experimental groups (C and D) given variable doses of *Aloe Vera* (*p<0.001*) as compared to the group B. Leukocyte infiltration in *Aloe Vera* administered groups increased significantly (p=0.047) as compared to group B showing their role in anti-inflammatory response.

Conclusion: The results proved that *Aloe Vera* reduces the inflammation and promote the growth of collagen fibers when orally taken.

Key Words: Aloe Vera, Inflammatory reaction, Immune response.

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INTRODUCTION

Aloe Vera is a succulant plant with fleshy leaves belongs to a family called Aloaceae. It has more than 400 species but only two species Aloe barbedensis Miller and Aloe aborascens are used for trade in the world.[1] It has polysaccharide that haspotential to have effects on innate immune system. The carbohydrate derived polysaccharide has immune mediator capacity.[2] Aloe Vera traditionally used to cure skin cancer, arthritis, eczema, psoriasis, minor burns and digestive problems.[3] It has a mannoprotein containing several amino acids called wound healing hormones.[4]

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Gel part of the leaf containacemannan a large polysaccharide have anti- inflammatory, immunomodulatory and antioxidant properties. Immune modulators means that these macro molecules have the ability to slowdown or speed up the immune system response thus protecting the body against itself. [5]

BCG is an attenuated vaccine recommended in healthy babies as close to the time of birth as possible with dose 0.1mg/ml percutaneous in upper part of right arm to enhance the immunity against tuberculosis.[6] BCG induces CD4+ and CD8+ T lymphocytes that have cytotoxic potential in rats when given intradermal.[7]

Mantoux test is tuberculin sensitivity test and is screening tool for tuberculosis. A standard dose of 5 tuberculin units (TU) 0.1ml is injected intradermal and read 48 to 72 hours later.[8] The reaction to intra-cutaneous injected tuberculin is the classic example of delayed (cellular) hypersensitivity reaction. T-Lymphocytes sensitized by prior infection are recruited to the skin where they release lymphokines.[9]

These lymphokines induce in duration through local vasodilation, edema, fibrin deposition and recruitment of other inflammatory cells to the area. The reaction after Mantoux test is the response of the area of skin through inflammatory phase, fibroblastic phase and remodeling phase.[10] Aloe Vera gel was extracted with the help of department of PCSIR and dose of 300 mg/kg was calculated.[11]

MATERIALS AND METHODS

A total of 24 albino rats of average weight 100-150 grams were obtained from animal house of Post Graduate Medical Institute Lahore. This animal house had a setup according to international standards for breeding and housing of experimental animals. After acclimatization divided into four groups randomly.

Group A was control. Group B was vaccinated with BCG .Group C and Group D were after BCG vaccination given doses of Aloe Vera 300 mg and 600 mg respectively by disposable syringe orally daily for one month. After 30 days Groups B,C and D were given Mantoux test percutaneous at abdominal region. After 72 hours the reaction site is observed grossly and then skin specimen were taken from the site for histological observations. For hematological parameters blood samples were taken from heart chambers of control and experimental animals group.

Analysis of Samples: Analysis of blood for hematological parameters were done from Indus lab Shadman Lahore. (Lymphocyte count and Immuno-globulins IgG and IgM was analyzed. Histological slides were made by Pathology department of Lahore General Hospital Lahore. Micrometry and photography done on teaching microscope of Anatomy department of PGMI Lahore.

Data Analysis: Data was analyzed using SPSS 21.0 (Statistical Package for Social Sciences).

Quantitative data like weight, inflammatory cell infiltration count, number of fibroblast and hematological parameters were described in form of Mean +/- SD. Analysis of variance was done with Krushkal Wallis H test for quantitative variable. Pair wise comparison was made by a post hoc Tukey's test.

The qualitative data such as vascular congestion and fibrosis was described in form of frequency and percentages. Chi square was applied to observe association between categorical variables within groups. A p-value <0.05 was considered as statistically significant.

RESULTS

The data was collected from 24 albino rats of either sex. The observations of all studies subjects were recorded on various parameters, after giving Aloe Vera for one month to experimental groups C and D. At the end of study Mantoux reaction size was 0.68 mm+_0.12 in experimental group C treated with Aloe Vera 300mg/kg and 0.69 mm +- 0.15 in group D with Aloe Vera 600 mg / kg for one month orally. While in group B (without Aloe Vera) area of inflammation was found bigger i.e. 0.89mm +- 0.13. Significant p-value of 0.001 was fond when ANOVA was applied. For comparative study post hoc Tuckey test was used.

The mean leukocyte infiltration in all groups was observed. Data was not normally distributed as assessed by Shapiro-Wilk test. Kruskal Wallis test was applied to compare the leucocyte infiltration among groups. It was found that the meanleucocyte infiltration count were significantly different.

p- Value=0.0047

Group C with Aloe Vera dose 300 mg/kg was found to have leukocyte/white blood cells 5.2mm per square per field. Group D with Aloe Vera dose 600mg/kg also gave the similar result i.e. increase number of leucocyte with value of 4.5 mm per square per field. Group B without Aloe Vera gave the value of 3.0 mm per square per field.

This anti-inflammatory action is due to decreased prostaglandin E2 synthesis from arachidonic acid by inhibition of cyclooxygenase pathway.5

Table No.1: Comparison of size of Mantoux reaction among groups

Parameters	Group A (Control)	Group B (Only PCG Vaccination)	Group C (Aloe Vera 300 mg/kg	Group D (Aloe Vera 600 mg/kg	P Value#
Size of Mantoux Reaction	0.00±0.00	0.98±0.13	0.77±0.12	0.78±0.15	<0.001

*p value is significant = 0.001

Table No.2: Comparison of Leucocyte Infiltration among groups.

Parameters	Group A (Control)	Group B (Only PCG Vaccination)	Group C (Aloe Vera 300 mg/kg	Group D (Aloe Vera 600 mg/kg	P Value#
Leucocyte Infiltration count (mm2 per field)	4.5±0.5	3.0±1.1	5.2±1.3	4.5±2.3	<0.047

*p value is significant = 0.047

Table No.3: Comparison of Neutrophil Count among groups.

Parameters	Group A (Control)	Group B (Only PCG Vaccination)	Group C (Aloe Vera 300 mg/kg	Group D (Aloe Vera 600 mg/kg	P Value#
Neutrophil Count	1.0±0.6	1.2±0.4	1.5±0.5	2.0±0.0	<0.13*

P-value:0.013

Table No.4: Comparison of number of fibroblasts among groups.

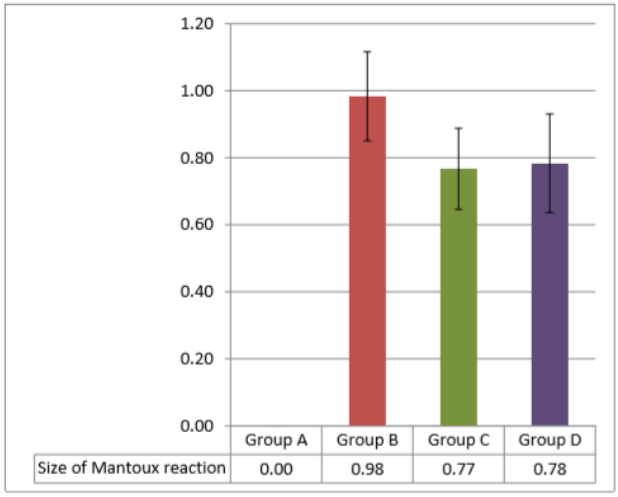
Parameters	Group A (Control)	Group B (Only PCG Vaccination)	Group C (Aloe Vera 300 mg/kg	Group D (Aloe Vera 600 mg/kg	P Value#
No. of fibroblasts (mm2 per field)	1.8±0.4	3.3±0.8	4.8±1.0	5.2±1.2	<0.001*

As p- value: 0.001 for number of fibroblast was found highly significant in groups C and D with Aloe Vera.

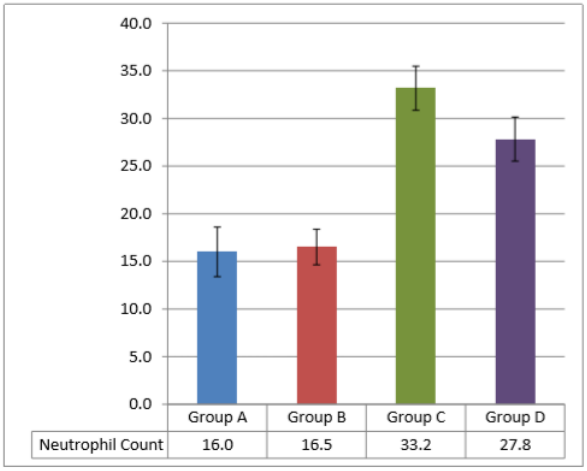
Hematological Parameters

Table No.5: Comparison of IgG level among groups.

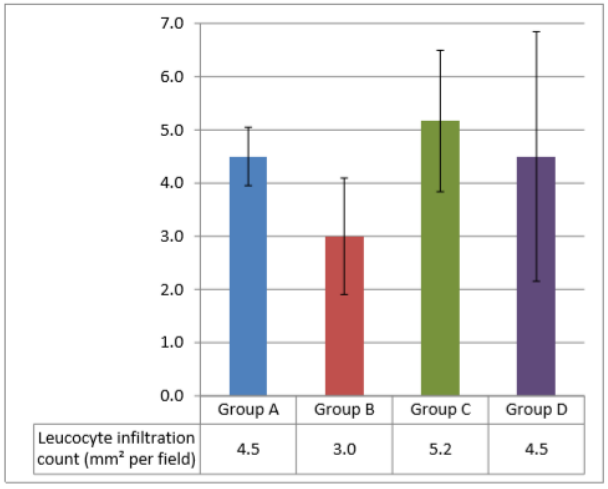
Parameters	Group A (Control)	Group B (Only PCG Vaccination)	Group C (Aloe Vera 300 mg/kg	Group D (Aloe Vera 600 mg/kg	P Value#
IgG level	4196.7±81.6	4306.8±64.3	4373.5±57.1	3895.5±59.0	<0.001



Graph No.1: Comparison of size of Mantoux reaction among groups.

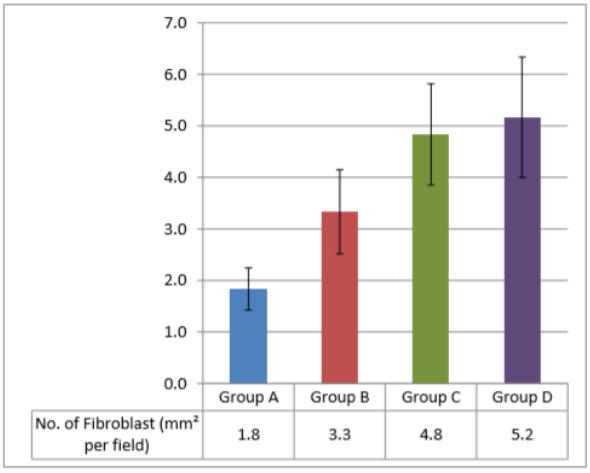


Graph No.3: Bar chart showing comparison of Neutrophil count among groups.



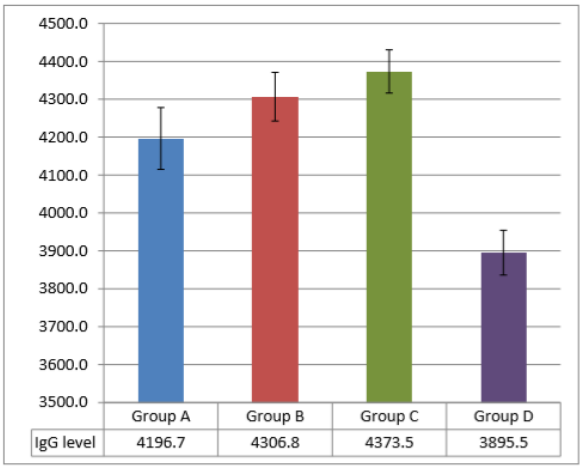
Graph No.2: Bar chart showing comparison of leucocyte infiltration among groups.

There was significant difference among experimental group B and C. There was no remarkable difference was found in group C & D.



Graph No.4: Bar chart showing comparison of number of fibroblasts among groups.

As p- value: 0.001 for number of fibroblast was found highly significant in groups C and D with Aloe Vera.



Graph No.5: Comparison of IgG level among groups.

IgG levels was found increased in group C (Aloe Vera 300mg/kg) but decreased in group D (Aloe Vera 600mg/kg). These results indicates that higher doses of Aloe Vera extract deliver immunosuppressive effect as research by GhasemVahedi.

DISCUSSION

In our study albino rats were used as experimental animal model. As their resemblance with human characteristics, availability and low price. Animal models have been the holy grail of vaccine research for many years.

Aloe Vera, a traditional herb and a succulent plant has been used for centuries for treatment of wounds and minor burns. Aloe Vera industry is gaining lots of attention as latest research proved lots of benefits of this magical herb.

Aloe Vera contains 75 potentially active constituents, vitamins, enzymes, minerals, sugar, lignin, saponin, salicylic acids and amino acid.[12]

Term immune modulation means increase or decrease responsiveness to immune system. Latest research on Aloe Vera demonstrated that immune stimulating effects are dependent on the activation of the innate immune cells like neutrophils, macrophages, lymphocytes and NK (Natural Killer) cells by production of tumor necrosis factors and leukotrienes.[13]

Herbs that contain ascorbic acid, poly phenols and tannis act as antioxidant. They act by scavenging of reactive oxygen and nitrogen free radicals.[2]

Most important constituent of this medicinal plant is carbohydrate containing polysaccharides like acemannan and glucomannan. Acemannan is sometimes referred to as the foundation of other gradients.[14]

These polysaccharides have property to activate various toll like receptors and involve several innate immune stimulator that result in ensuring the immune response.[15]

Aloeride in gel part of Aloe Vera act as an immunostimulant, either alone or in combination with other aloe components, may have significant potential for wound healing and immunotherapy.[16]

Many immune modulatory activities including stimulation of cytokine secretion, histamine release, immunoglobulin release, macrophage stimulation.[17]

This study is about anti-inflammatory action and immune response of Aloe Vera on skin of albino rats by giving them vaccination and after that applying injury to skin by giving Mantoux test which is delayed type IV sensitivity reaction. Acute wound healing take four stages, these are hemostasis, inflammation, proliferation and remodeling.[18]

There are thousands of articles on Aloe Vera published during last few decades that proved that it has positive role in inflammation. Inflammation depends on leukocyte infiltration and their role in infection and inflammation.[19]

High poly saccharides and anthroquinones present in Aloe Vera can act as pro-oxidant and pro-inflammatory product. Polysaccharides with high molecular weight were found to exhibit the most potent macrophage activity, due to increase cytokines production, nitric oxide (NO) release expression of surface markers and phagocytic activities.[20]

A study by Langmead in 2004 proved that Aloe Vera reduces the inflammatory response by its ingredient mannose-6-phosphate.[21]

Neutrophils adhere to endothelium of blood vessels and block the micro vessels and cause a local decrease in blood flow. Aloe Vera decreases the cell mediated immunity by decreasing the secretion of lymphokienes and reduce the levels of interleukins (IL-5 and IL-10).[22]

Aloe Vera mucilage includes some ingredients like vitamin C and vitamin E which play crucial role in acceleration of wound healing with production of collagen fibers.[23]

Lysle oxidase levels are associated with degree of cross linking of collagen fibers that are newly synthesized. A recent study of Aloe Vera used topically and orally in rats showed increased levels of this enzyme, along with increased levels of this enzyme, along with increased amount of collagen.[24]

Oral administration of Aloe Vera affected the composition of lymphocyte subsets and immunoglobulins positively. It stimulated both cellular and humoral immune responses after immunization. IgG levels was found increased in group C (Aloe Vera300mg/kg) but decreased in group D (600 mg/kg).These results indicates that higher doses of Aloe Vera extract deliver immunosuppressive effect as research by GhasemVahedi.[25]

The data of our study has revealed encouraging results when Aloe Vera taken orally which could help to evolve new strategy of treatment for reduction of inflammation and lowering the immune response. Aloe Vera is easily available in Pakistan and could be used to alleviate the symptoms of auto-immune diseases like rheumatoid arthritis, ankylosing spondylosis, systemic lupus erythematosus, vasculitis and many more.

CONCLUSION

Aloe vera decreases the inflammatory response as size of Mantoux test was reduced. Its role on fibroblast and collagen fiber formation proved the wound healing capacity of Aloe Vera.

Author's Contribution:

Concept & Design of Study:	Huma Riaz
Drafting:	Aisha Akbar
Data Analysis:	Maria Ilyas, Mariam Saad
Revisiting Critically:	Huma Riaz, Aisha Akbar
Final Approval of version:	Huma Riaz

Conflict of Interest: The study has no conflict of interest to declare by any author.

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