

IMMUNOMODULATORY EFFECT OF ALOE VERA GEL ON PRO-INFLAMMATORY GENE EXPRESSION IN SPRAGUE DAWLEY RATS

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INTRODUCTION

- Immune response can be divided into humoral, mediated by B lymphocytes and cell mediated involving the activities of T lymphocytes.
- In the case of immune response against intracellular pathogens responsible for diseases like malaria and tuberculosis, humoral immune response involving immunoglobulins have been found to be ineffective.
- Therefore, there is a need for the body to mount cell mediated immune response of sufficient intensity to prevent diseases caused by intracellular pathogens.
- Vaccines used against human and veterinary diseases normally contain antigens of interest along with immunomodulators known as adjuvants
- The adjuvant serves as an immune response booster to promote the immunogenicity of poorly immunogenic recombinant antigens and possibly induce a qualitative alteration in the type of response elicited.
- Aloe vera* (*Aloe barbadensis*) is known for its anti-microbial, and immunomodulatory activities. Though the immunomodulatory activity of *Aloe vera* through oral route is reported, studies on the intramuscular route of administration are limited.

OBJECTIVES

The aim of the study was to assess the ability of freeze-dried *Aloe vera* (FDAV) gel to prime immune response towards Th1 phenotype when administered intramuscularly.

- To assess the potentials of crude FDAV gel to serve as an adjuvant when administered along with Bovine Serum Albumin (BSA) as the experimental antigen.
- To assess the quantitative and qualitative effect of the gel on antigen-induced immune response.

METHODOLOGY

- Fresh aerial parts of *Aloe barbadensis* were collected, identified and authenticated at the Department of Biotechnology, Faculty of Applied Sciences, AIMST University

(No: AUFOM/HERB/2013/002).

Thirty-two (32) female Sprague Dawley rats, aged seven (7) months and weighing between 180g to 200g

- Group 1: 100µl of PBS
- Group 2: 100µl of a 1% (w/v) solution made up of 1g of FDAV dissolved in 100ml of PBS
- Group 3: 100µl of a 0.005% (w/v) solution made up of 5mg of BSA dissolved in 100ml of PBS
- Group 4 was administered with 100µl of a 0.005% (w/v) solution made up of 5mg of BSA dissolved 100ml of 1% (w/v) freeze dried *Aloe vera* gel.

Blood samples were collected sixty-six (66) hours after treatment

- Assessment of quantitative effect was done through evaluating the level of serum NO, expression level of iNOS gene, total WBC count and differential WBC count.
- Qualitative effect of the gel on immune response was assessed through evaluating the expression levels of the Th1-associated proinflammatory cytokines, IL-12 and IFNγ.

AIMST University Human and Animals Ethics Committee (AUHAEC/3/FOM/2014).

RESULTS

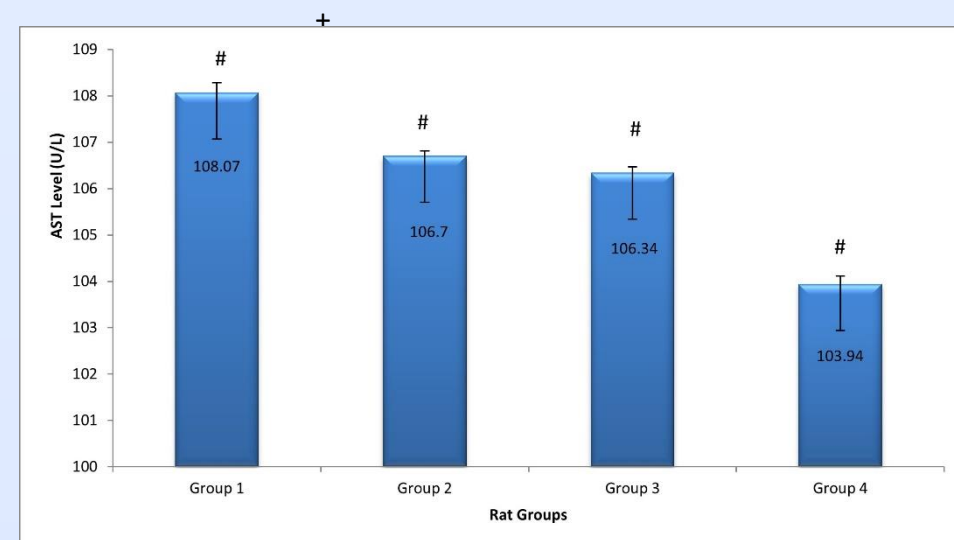


Figure 1: Plasma Aspartate Transaminase Levels

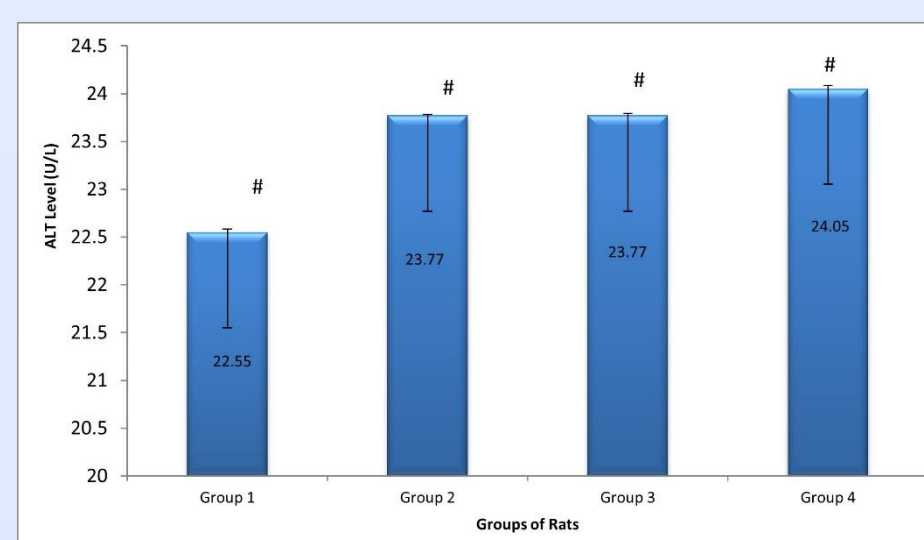


Figure 2: Plasma Alanine Transaminase Levels

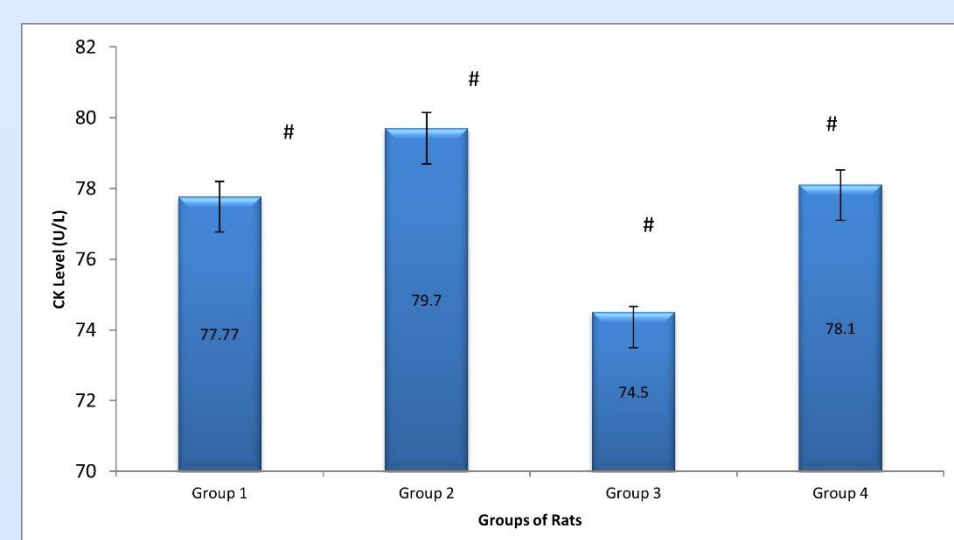


Figure 3: Plasma Creatine Kinase Levels

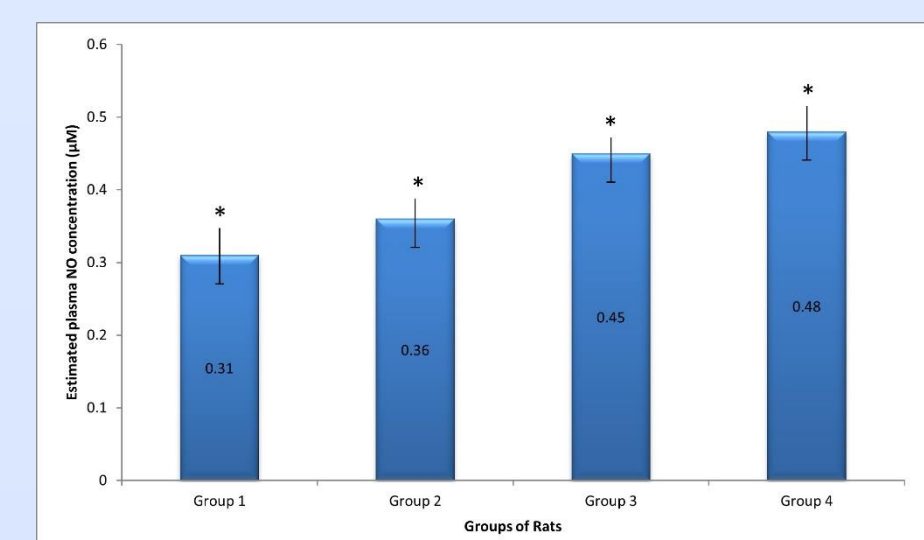


Figure 4: Plasma NO Levels

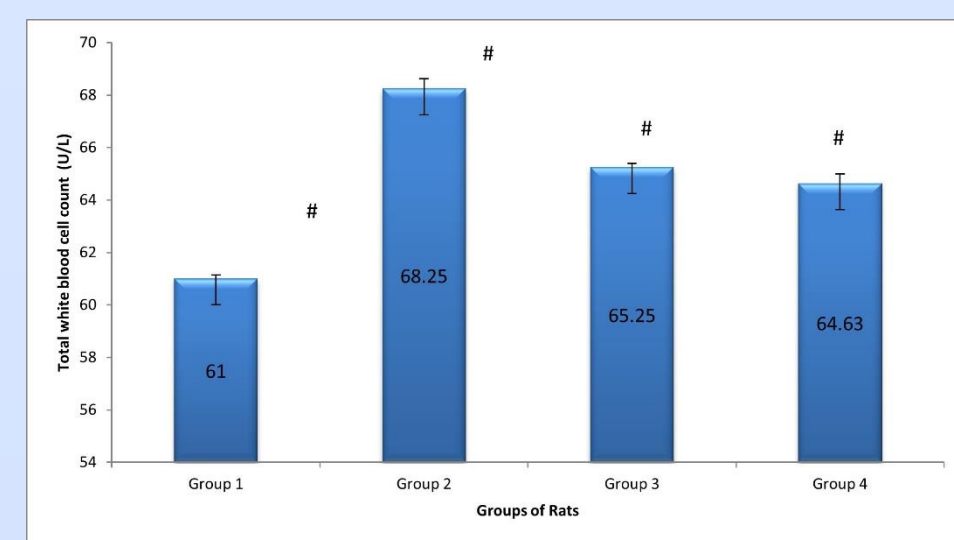


Figure 5: Total WBC Counts

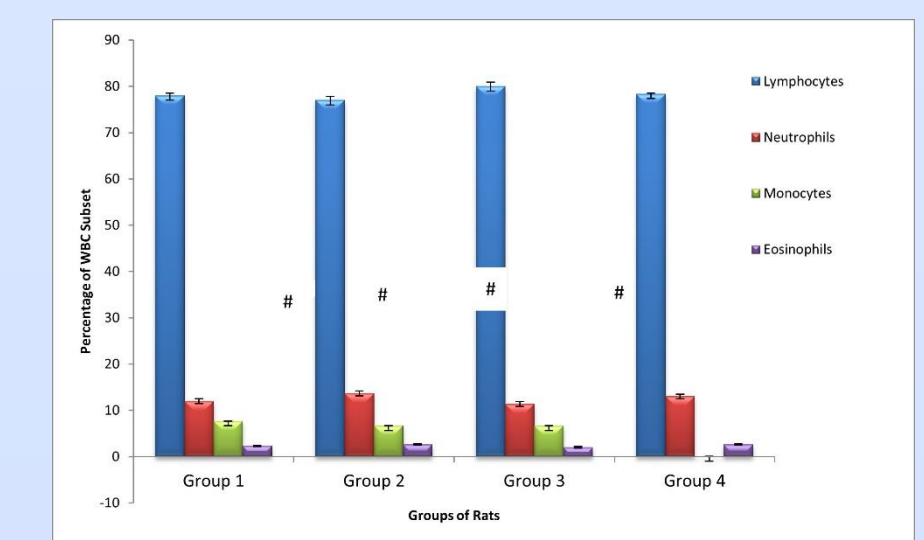


Figure 6: Differential Count

Primer	Sequence (5' 3')	Size	Accession Number
*INFγ(F)	GCCCTCTCTGGCTGTACTG	147	NM_158880.2
*INFγ(R)	CGTGTACCGTCTTTTGCC		
IL-12 (F)	AGTCTCTGTCGGCATCCAG	158	NM_022611.1
IL-12 (R)	GCGCTTGACGACGAGATTC		
iNOS (F)	GACCAGAAACTGTCTCACCTG	157	NM_012611.3
iNOS (R)	CGAACATCGAACGCTCACA		
β-actin(F)	AAGTCCCTCACCTCCAAAAG	97	J00691
β-actin (R)	AAGCAATGCTGCACCTCCC		

Table 1: Primers used for RT-PCR

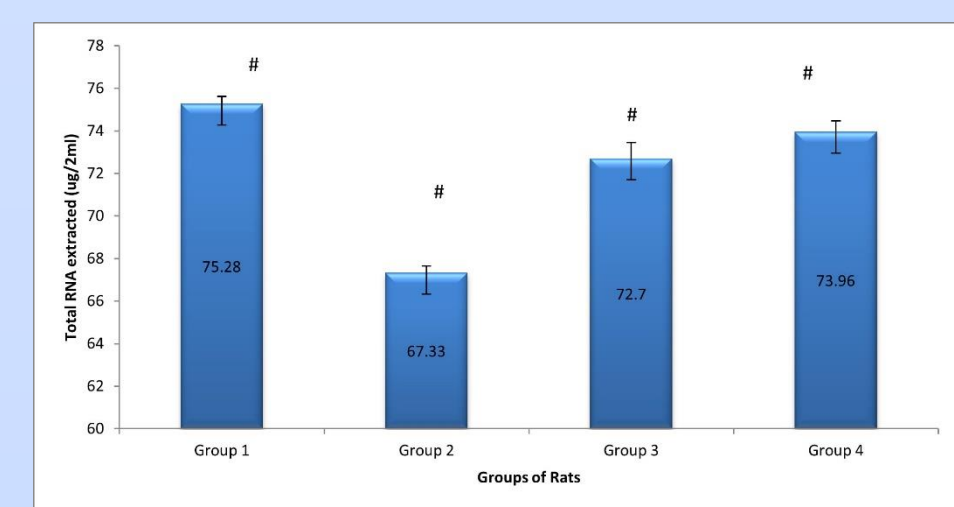


Figure 7: Total RNA Levels

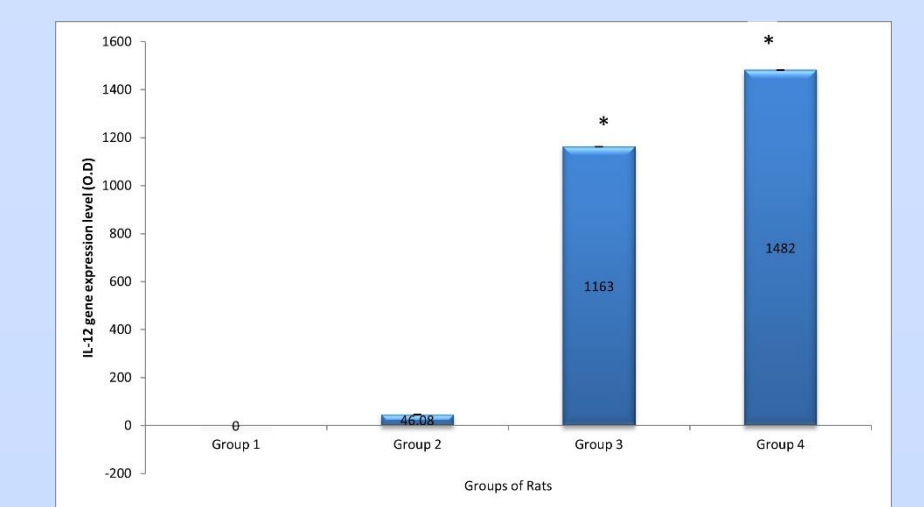


Figure 8: IL-12 Expression Levels

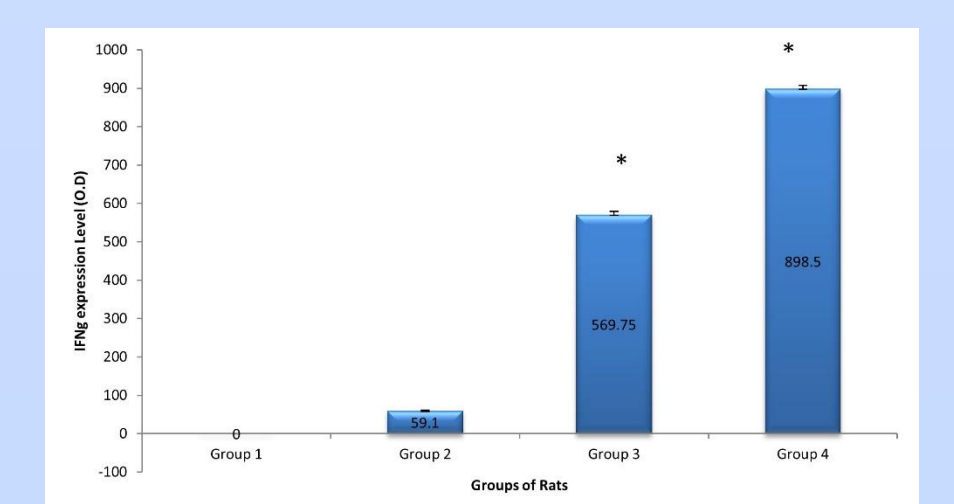


Figure 9: IFNγ Expression Levels

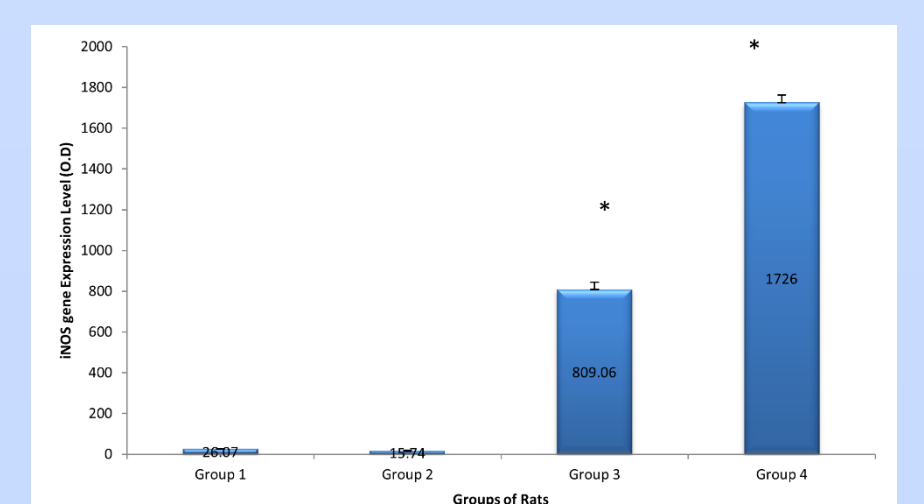


Figure 10: iNOS Gene Expression Levels

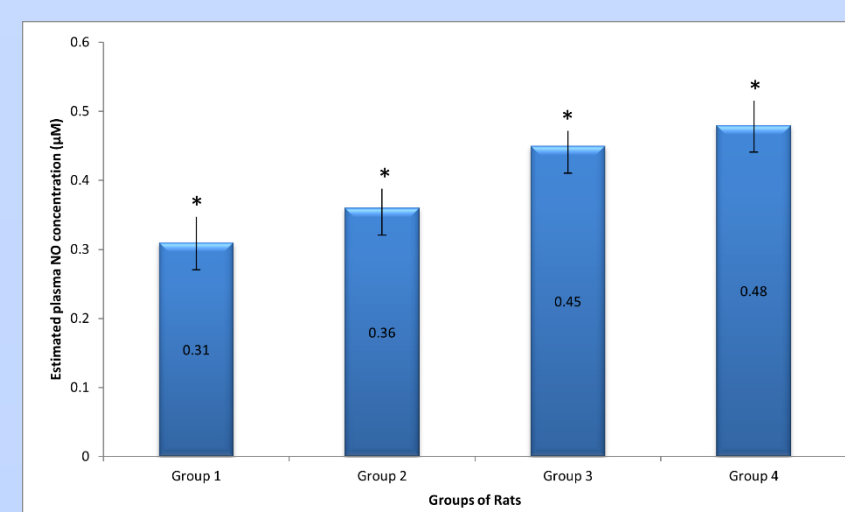


Figure 11: Plasma NO Levels

- AST, ALT, and CK levels showed no significant change between the 4 groups.
- No significant difference was observed for the total WBC and differential count in all the 4 groups.
- No significant difference was observed in the quantity of RNA extracted from all the 4 groups.
- Group 2 showed a significantly higher ($p < 0.05$) expression of IL-12 and IFNγ than group 1.
- Group 3 showed a significantly higher ($p < 0.05$) expression of IL-12 and IFNγ than group 1 and 2.
- Group 4 has a significantly higher ($p < 0.05$) expression of these cytokines than the other 3 groups.
- Unlike IL-12 and IFNγ, iNOS had a detectable expression in all the four groups.
- Expression level significantly increased from group 2 to 4.
- No significant difference was observed ($p < 0.05$) between group 1 and 2.

Statistical Analysis

- Data were expressed as mean \pm SD.
- Statistical analyses was carried out using one-way ANOVA, followed by Tukey's post-hoc test.
- SPSS version 22.0 was used for all statistical analyses.
- $p < 0.05$ was considered statistically significant.

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DISCUSSION

- No significant changes in the enzyme levels between the 4 groups - the dose (100µl of 1% w/v FDAV gel) used was non-toxic within the duration of the study.
- Activation of the immune system normally results in WBC proliferation. Differences were not observed in this case probably because effect of gene activation is basically felt after mRNA translation which may be at a preliminary stage when animals were sacrificed.
- IL-12 exerts multiple biological activities mainly through T and NK cells by inducing the production of IFNγ. It is suggested that Th1-type cytokines inducing IFNγ are required to direct cell-mediated immune responses leading to eradication of intracellular pathogens and tumour cells.
- Expression of these cytokines may be the result of induction by components of the FDAV gel. Several studies have reported the induction of cytokine genes by polysaccharide component of the gel.
- It is reported that the upregulation of iNOS mRNA by murine macrophages is strictly associated with their capacity to produce NO and consequent microbicidal activity *in vitro* and *in vivo*.
- Of the three isoforms, iNOS is generally associated with immune response and produces NO for prolong period of time in a calcium-independent manner. The significant difference may be due to NO from other sources as the molecule is not only restricted to the immune system.
- The observed upregulation of iNOS gene expression and significantly higher NO level recorded in group 4 is an indicator of the ability of crude FDAV gel to boost antigen-mediated immune response. The concurrent upregulation of iNOS gene expression, NO level, IFNγ and IL-12 gene expression appears to support this assertion.

CONCLUSION

From the results of the study, it could be concluded that FDAV gel administered intramuscularly to SD rats along with BSA, had promoted the effectiveness of the antigen with respect to overall immune response as seen in the elevation of iNOS gene and NO and also promoted Th1-mediated immune response as seen in elevation in the expression levels of IL-12 and IFNγ.

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