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Research Article

Comparative Efficacy of Aloe Vera and Vitamin E on Immunity, Growth, Intestinal Histopathology and Wound Healing in Rabbits

Shakeeb Ullah*[1] | Anila Khan[2] | Faiqa Ramzan[1] | Sana Ullah[1] | Noor ul ain[3]

[1]Faculty of Veterinary and Animal Sciences, Gomal University, D.I. Khan
[2]Institute of Biological Sciences, Gomal University, D.I. Khan
[3]Institute of Food science and Nutrition. Gomal University DI Khan

Correspondence
Shakeeb Ullah
Email: Shakeebullh@gmail.com

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ABSTRACT:

Background: Aloe vera, a succulent plant belonging to the Liliaceae family, has been used for centuries in traditional medicine. **Objectives:** The prevalence of UTIs and development of resistance against the antibiotics is due to unjustified use of antibiotics. **Methodology:** This study evaluates the impact of dietary supplementation with Aloe vera extract (PE) and Vitamin E (VE) on growth performance, meat quality, immune parameters, cytokine induction, and intestinal histopathology in growing rabbits, **Results:** Results showed significant improvements in live weight (LW) and average daily gain (ADG) in the PE and VE groups, with the S+PE+VE group exhibiting the highest gains. Meat quality analysis revealed increased muscle redness and reduced water loss in the PE and VE groups. Immunological assays indicated elevated IgM and IgG levels, particularly in the S+PE+VE group, suggesting a synergistic immune enhancement. Cytokine analysis showed decreased IL-6 and increased TNF-α levels in the supplemented groups, indicating modulated cytokine activity. Histopathological examination revealed significant enhancements in intestinal morphology in the S+PE+VE group, including hypertrophy of villi, increased goblet cell count, and enhanced secretory activity, suggesting improved nutrient absorption. Wound healing studies demonstrated accelerated recovery in the S+PE+VE group, with reduced inflammation and faster wound closure. **Conclusion:** It is concluded that the Aloe vera and Vitamin E supplementation in rabbit feed significantly improves growth performance, meat quality, immune response, intestinal health, and wound healing, highlighting their potential as beneficial dietary supplements in rabbit production and other animal species.

KEYWORDS:
Aloe vera, Vitamin E, Growth performance, Meat quality, Immune response, Cytokine induction, Intestinal health

1 | INTRODUCTION

Aloe vera, a succulent plant belonging to the Liliaceae family, has been used for centuries in traditional medicine. It is prized for its extensive range of therapeutic properties, including anti-inflammatory, antimicrobial, antioxidant, and immunomodulatory effects.[1] The gel extracted from Aloe vera leaves contains numerous bioactive compounds such as polysaccharides, glycoproteins, and phenolic compounds, which contribute to its health-promoting effects. In veterinary science, Aloe vera is increasingly explored for its potential to enhance animal health, particularly in areas like immunity, growth, intestinal health, and wound healing.[2] Vitamin E, a fat-soluble vitamin, is essential for maintaining the integrity of cell membranes due to its potent antioxidant properties. It plays a critical role in protecting cells from oxidative damage by neutralizing free radicals. Additionally, Vitamin E is crucial for immune function, skin health, and reproductive performance. In animals, adequate levels of Vitamin E are necessary to support overall health and productivity, making it a common supplement in animal diets to promote growth, enhance immune response, and improve skin and tissue health.[3] Both Aloe vera and Vitamin E are known for their immunomodulatory properties. Aloe vera contains compounds that can stimulate the production and activity of immune cells, enhancing the body's ability to

fight off infections. Similarly, Vitamin E plays a vital role in maintaining and boosting the immune system by protecting immune cells from oxidative stress and improving their function.[4] Growth performance is a key economic factor in animal husbandry. Aloe vera has been shown to improve nutrient absorption and digestive health, which can contribute to better growth rates. Vitamin E, by reducing oxidative stress and enhancing overall health, can also positively impact growth performance. The health of the intestinal tract is crucial for effective nutrient absorption and overall health. Aloe vera is known for its soothing and healing effects on the gut lining, potentially reducing inflammation and promoting tissue repair. Vitamin E, with its antioxidant properties, can protect intestinal cells from damage and support healthy tissue structure.[5] Effective wound healing is essential for animal welfare. Aloe vera is widely recognized for its wound healing properties, attributed to its anti-inflammatory, antimicrobial, and tissue-regenerating effects. Vitamin E also aids in wound healing by preventing oxidative damage to skin cells and supporting the repair and regeneration process.[6]

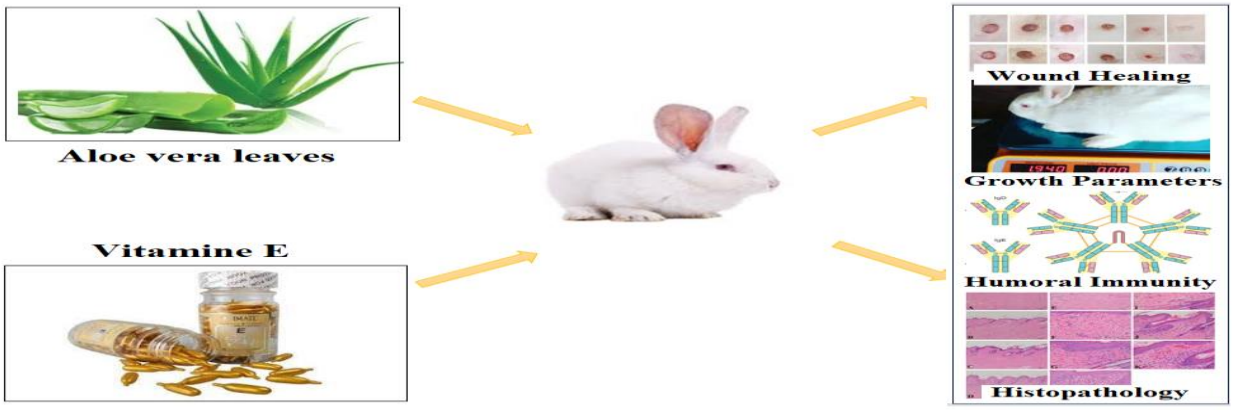

Figure 1. Experiment summary showing effects of Aloe vera & Vitamin E on Wound healing, growth, immunity & histopathology of intestine

This study aims to compare the efficacy of Aloe vera leaf extract and Vitamin E on several health parameters in Oryctolagus cuniculus (rabbits), including humoral immunity, growth performance, intestinal histopathology, and wound healing. Rabbits are an excellent model for such research due to their well-characterized immune system and their economic importance in meat production and as laboratory animals[7]. By investigating these aspects, this research seeks to provide insights into the potential benefits of Aloe vera and Vitamin E supplementation, contributing to the development of more effective and natural strategies for improving animal health and productivity. The findings could have significant implications not only for the welfare of rabbits but also for the broader field of veterinary nutrition and medicine.

2 | MATERIAL AND METHODS

This randomized controlled trial involved assigning rabbits to distinct treatment groups, considering factors such as age and weight to ensure a representative sample. Rabbits were acclimatized to the controlled environment before the study began.

2.1 | Animal Selection and Diets

Forty domesticated rabbits (7-9 weeks old, 650-800 g) were obtained from Animal Health Research Laboratories, NARC, Islamabad. They were divided into four groups; Standard diet (S): Negative control, S + Vitamin E (S+VE): Positive control, S + Aloe vera extract (S+PE) and S + Aloe vera extract + Vitamin E (S+PE+VE) respectively with five rabbits per replicate. Rabbits were housed in metal cages (60x60 cm), fed 100g of concentrate daily, and given water ad libitum. All were monitored and treated per standard management practices.

2.2 | Collection and Preparation of Aloe Vera Leaf Extract

2.2.1 | Plant Sample Collection and Authentication

Aloe vera leaf samples were taken by Habib Botanical Garden, Institute of Biological Sciences, Gomal University, D. I. Khan Pakistan in January 2024 and stored for further analysis. The plants samples were authenticated taxonomically by Dr. Naimat Baloch at the Botany Division of the Institute of Biological Sciences, Gomal University, D. I. Khan, Pakistan.

2.2.2 | Preparation of Plant Extracts

The leaves underwent a thorough washing with tap water and the gel was removed. Leaves were subsequently dried in an air-circulating oven at 50°C followed by 105°C until a consistent weight was achieved at these temperatures. Each sample was then powdered and sieved through a 300 µm mesh before being stored in an air-tight cellophane bag as a stock sample in a refrigerator for further analysis. Proximate composition analysis of the Aloe vera leaf samples was carried out following the procedures outlined by AOAC (2011) at the feed testing laboratory of the Poultry Research Institute in NARC, Islamabad, Pakistan. All analyses and determinations were conducted in triplicate.

2.3 | Growth Performance

2.3.1 | Live Body Weight

This study was a part of a thesis project and the trial was carried out at the Gomal University Zoo in the institute of biological sciences, Gomal University D.I. Khan, Pakistan. Growth performance was checked by recording Feed intake (FI) and live body weight (BW) were recorded weekly in grams throughout the experimental period (8 weeks).

2.3.2 | Meat Quality Test

The muscular pH levels of the Biceps femoris (BF) and the Longissimus dorsi (LD) muscles were assessed post-mortem at 1 hour (pH1) and 24 hours (pHu; Blasco and Ouhayoun, 1996) using a portable pH meter (ROSS, Orion Star A221, Thermo Scientific, Beverly, CA, USA) in conjunction with an Orion Kniphe pH electrode (ThermoFisher, Nepean, ON, Canada) and a temperature compensation probe (928 007 MD, micro probes ATC, Maryland, USA). Meat color was evaluated 24 hours after slaughter on the LD muscle and the exposed surface of the BF muscle using a Chromameter (Chromameter CR 300 Minolta Ltd., Osaka, Japan) equipped with a D65 light source and a 0° viewing angle geometry according to the reflectance coordinates (L*, a*, b*; CIE, 2004), after allowing the muscle surface to bloom for 20 minutes.

2.3.3 | Data Collection

Data on growth parameters were collected regularly, including weight, body length, and meat quality.

2.4 | Humoral Immunity Assessment

2.4.1 | Blood Sample Collection

Blood samples were collected at specific intervals to monitor changes in humoral immune parameters.

2.4.2 | Serum Immunoglobulin Levels

ELISA was used to measure IgG, IgM, and IgA levels in the blood samples.

2.4.2 | Cytokine Profiling

Cytokine levels (e.g., IL-6, TNF-α) were measured using ELISA.

2.5 | Statistical Analysis

Data were analyzed using appropriate statistical methods to identify significant differences between treatment groups.

2.6 | Intestinal Histopathology

2.6.1 | Euthanasia and Tissue Collection

Rabbits were euthanized, and intestinal tissue samples were collected and fixed in 10% formalin.

2.6.2 | Processing of Tissue Samples

Tissues were processed, embedded, sectioned, and examined microscopically.

2.6.3 | Sample Collection

Intestinal samples were collected, fixed, and prepared for histological examination.

2.7 | Statistical Analysis

Data were analyzed using SPSS software, with significance determined at $P<0.05$.

2.8 | Wound Healing in Rabbits

2.8.1 | Experimental Animals

Twenty healthy male rabbits (250-300 g) were assigned to four treatment groups; Standard skin ointment (A): Negative control, Ointment + Vitamin E (B), Ointment + Aloe vera extract (C) and Ointment + Aloe vera extract + Vitamin E (D). Rabbits were maintained under a uniform feeding regimen.

2.8.2 | Clinical Examination

Rabbits underwent clinical and laboratory examinations before the experiment.

2.8.3 | Skin Irritation Test

Rabbits were tested for skin irritation using standard irritants and treatments.

2.8.4 | In Vivo Studies

Approved by the Ethical Review Board, the study involved wound creation and evaluation under controlled conditions.

2.8.5 | Wound Creation and Evaluation

Wounds were created, and healing was monitored and photographed on days 0, 7, and 14. Wound closure was measured using a scale

3 | RESULTS AND DISCUSSION

3.1 | Growth Performance

Dietary treatments significantly affected the productive and slaughter performances of growing rabbits (Table 1). Live weight (LW) was consistently different among groups at the end of the trial. Particularly, PE and VE fed animals had higher LW and ADG compared to the other groups (Figure 2).

Table 1: Effect of supplementation of *Aloe vera* leaf extract and Vitamin E in diet on weight gain and feed intake in Rabbits

Treatments	S	S+VE	S+PE	S+PE+VE
Weight gain (g)	201.6±3.20^{b}	266.0±2.13^{a}	271.3±2.10^{a}	383.3±2.23^{a}
Feed intake (g/Rabbit)	1138±5.52^{b}	1283±05.70a	1275±7.05^{a}	1383±3.35^{b}
Feed: gain ratio	4.94±0.36^{a}	4.87±0.30	4.84±0.42^{b}	4.34±0.42a

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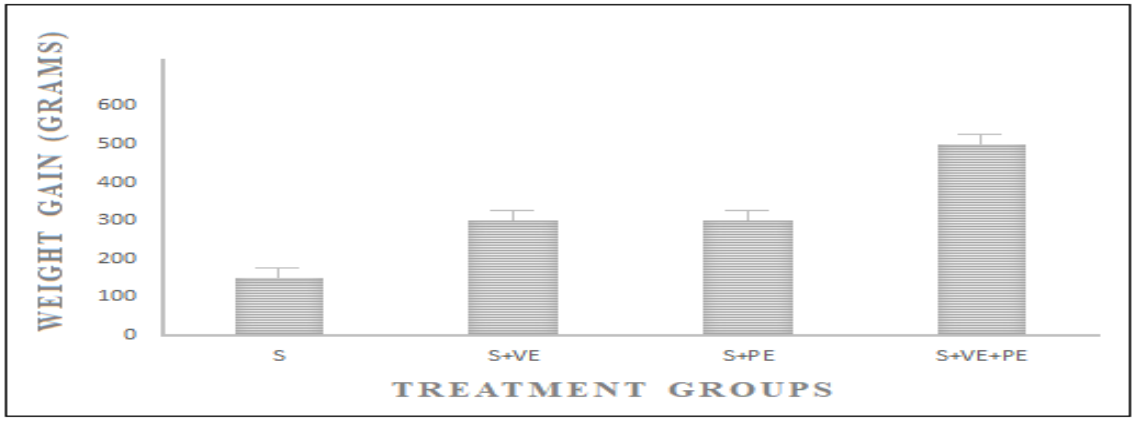

Figure 2: Effect of supplementation of *Aloe vera* leaf extract and Vitamin E in diet on weight gain in Rabbits

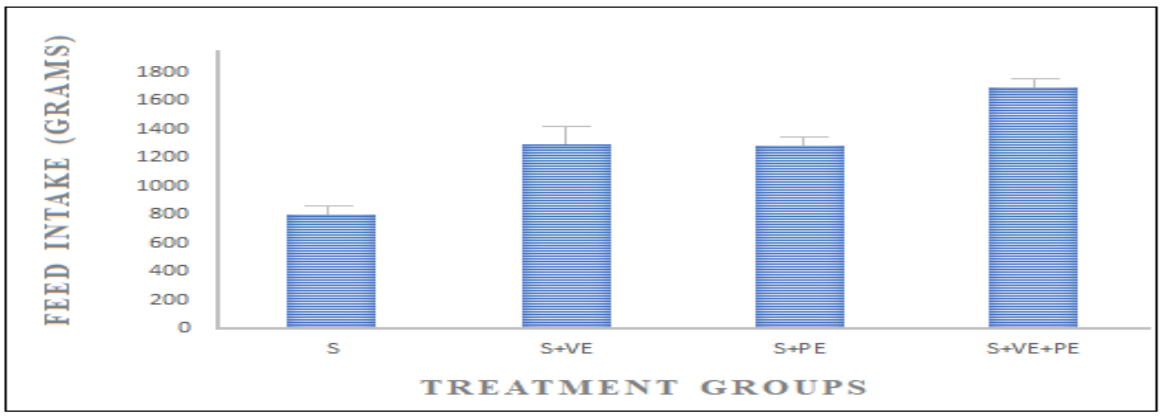

Figure 3: Effect of supplementation of *Aloe vera* leaf extract and Vitamin E in diet on feed intake in Rabbits

3.2 | Meat Quality

The influence of PE and VE addition to the diet on the meat quality of rabbits[8]. Supplementation of 300 mg/kg PE and VE significantly increased the redness of the muscle compared to other groups ($P < 0.05$). Additionally, supplementation with 100, 300, and 400 mg/kg PE and VE resulted in decreased water loss rates compared to the control group ($P < 0.05$). Moreover, PE and VE supplementation led to a reduction in shear force compared to the control group ($P < 0.05$), with no significant dosage effect observed ($P > 0.05$) [9]. However, PE and VE had no discernible effect on the pH, lightness, and yellowness of the muscle ($P > 0.05$).

3.3 | Immunological Parameters Levels of immunoglobulins

The levels of immunoglobulins IgM, IgA, and IgG, measured in milligrams per deciliter (mg/dl), with different experimental conditions denoted as S, S+VE, S+PE, and S+PE+VE, along with their standard errors (±S. E) [10].

3.4 | IgM (mg/dl)

Under the control condition S, the IgM level measures 18.96 mg/dl, which slightly increases to 20.59 mg/dl under condition S+VE, further rising to 22.40 mg/dl under condition S+PE, and peaking at 23.68 mg/dl under condition S+PE+VE, with a standard error of ±0.511.

3.5 | IgA (mg/dl)

The IgA levels exhibit relative consistency across all conditions, ranging from 5.47 to 6.66 mg/dl, with a slight increase observed under condition S+PE compared to the baseline condition S [11]

3.6 | IgG (mg/dl)

Under condition S, the IgG level measures 23.39 mg/dl, significantly increasing to 101.89 mg/dl under condition S+VE, further rising to 129.98 mg/dl under condition S+PE, and reaching 245.93 mg/dl under condition S+PE+VE. These measurements come with a standard error of ±3.376.

Table 2: Immunological assay of rabbits as affected by addition of Aloe vera leaf extract (PE) and Immuno-booster Vitamin E (VE).

Item	S	S+VE	S+PE	S+PE+VE	±S. E
IgM (mg/dl)	18.96[b]	20.59[b]	22.40[a]	23.68[a]	0.511
IgA (mg/dl)	5.47	5.59	6.66	5.73	0.101
IgG (mg/dl)	23.39[d]	101.89[c]	129.98[b]	245.93[a]	3.376

[a, b, c and d]Means in the same row with different superscripts are significantly different at (P≤0.05).SE = Standard error of means. IgM: Immunoglobulin's M, IgA: Immunoglobulin's A, IgG: Immunoglobulin's G.

The results suggest that the presence of both PE (Aloe vera plant extract) and VE (Vitamin E) individually and in combination leads to a significant increase in IgM levels compared to the Control condition S. This indicates that these treatments may be stimulating or enhancing the production of IgM antibodies[12]. IgA levels show less variation across conditions, suggesting that the treatments tested do not have a significant impact on IgA levels compared to the control. IgG levels show a remarkable increase with the introduction of immune-booster Vitamin E (S+VE), which further increases with the addition of Aloe vera plant extract (S+PE) and reaches a peak under the combined effect of PE and VE (S+PE+VE). This suggests that both VE and PE may have a synergistic effect on enhancing IgG production.Overall, these results indicate that the interventions tested have varying impacts on the levels of different immunoglobulins, with IgM and IgG showing more pronounced responses compared to IgA [13]

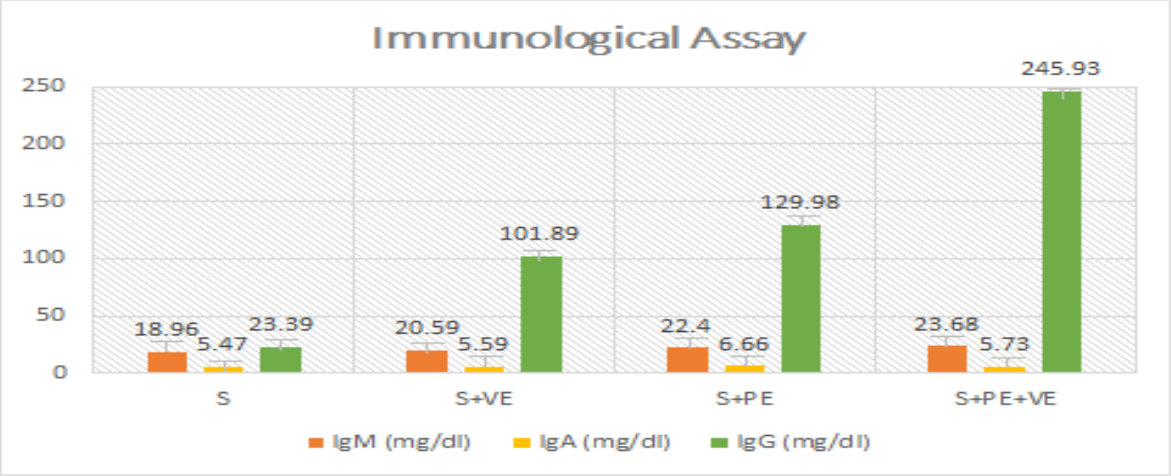

Figure 4: Immunological assay showing effect of supplementation of Aloe vera plant extract (PE) & Vitamin E as an immune-booster

3.7 | Effect of Plant Extract and Vitamin E on Cytokine induction IL-6 induction

Changes IL-6 levels in blood were investigated in rabbits exposed to Aloe vera plant leaf extract and Vitamin E. The control group exhibited a statistically significant lower IL-6 level compared to the three groups treated with Aloe vera Plant Extract and Vitamin E. Furthermore, all combinations of treatments led to a significant decrease in IL-6 levels compared to the group receiving both Aloe vera Plant Extract and Vitamin E, with more pronounced changes observed in the former[14]. Upon analyzing individual extracts, a decrease in IL-6 levels was observed in all cases compared to the group receiving both plant extract and Vitamin E. These findings are shown in Figure 4.

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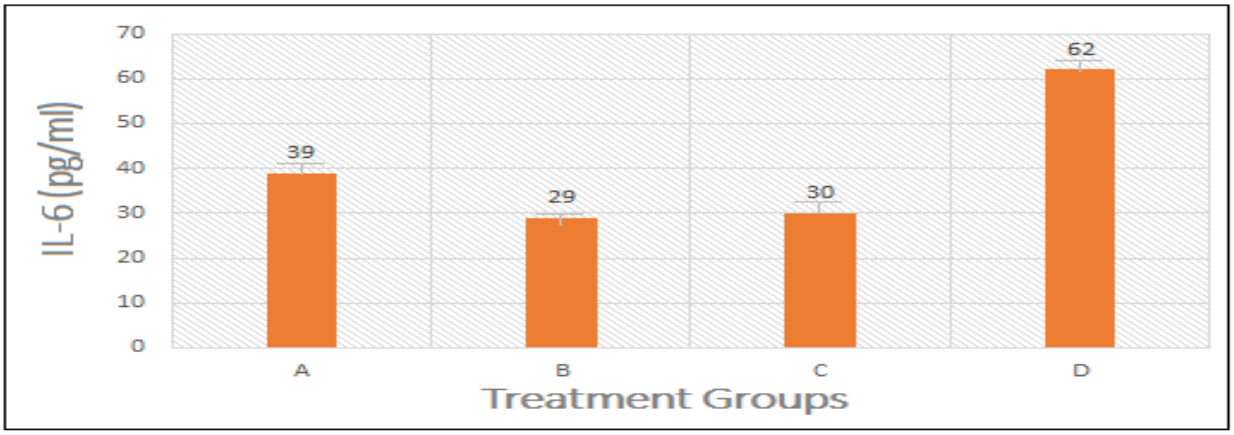

Figure 5: Serum IL-6 in Rabbits treated with Aloe vera plant extract and Vitamin E individually and combinations (A represents controlled group, B treated with vitamin E, C treated with Aloe Vera leaf extract and D treated with both).

3.8 | TNF-α induction

The rabbits in the combined group (PE and VE) exhibited a high level of TNF-α as compared to the control as well as other groups. Interestingly, the oral provision of Plant leaf extract and Vitamin E have shown high increase in Tumor necrosis factor alpha (TNF-α) levels in all groups as shown in the Figure 5 below.

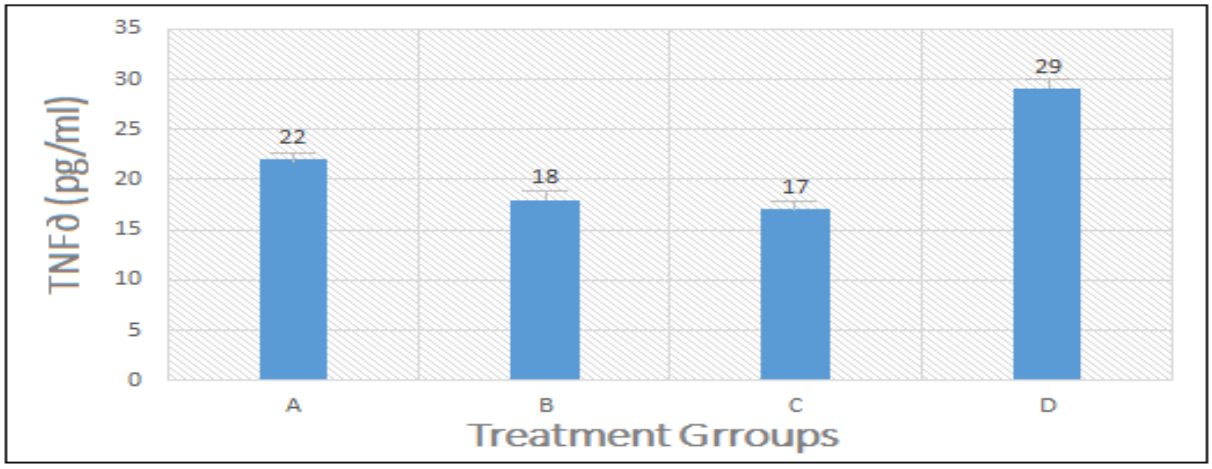

Figure 6. Serum TNF-α in Rabbits treated with plant extract and Vitamin E (A represents controlled group, B treated with vitamin E, C treated with Aloe Vera leaf extract and D treated with both).

3.9 | Histopathological Studies Duodenum

In the experimental group receiving a combination of Standard diet, Aloe vera Plant leaf extract, and Vitamin-E (S+PE+VE), significant changes were observed in the duodenum upon examination[15]. The tunica mucosa displayed densely packed intestinal villi with pronounced hypertrophy, extensive subdivisions and reunions. The mucosal epithelium showed a simple columnar epithelium, indicating active hyperplasia with a noticeable enhancement in goblet cell count and function of secretion. In the lamina propria and submucosa, there was notable lymphocytic aggregation developing lymphocytic nodes of limbs. Brunner's glands in the submucosa showed 2 types of secretory units: primarily upper layer of alveoli with large, lightly stained cytoplasmic alveoli, and less frequently, small serous acinar units with granular eosinophilic cytoplasm[16].

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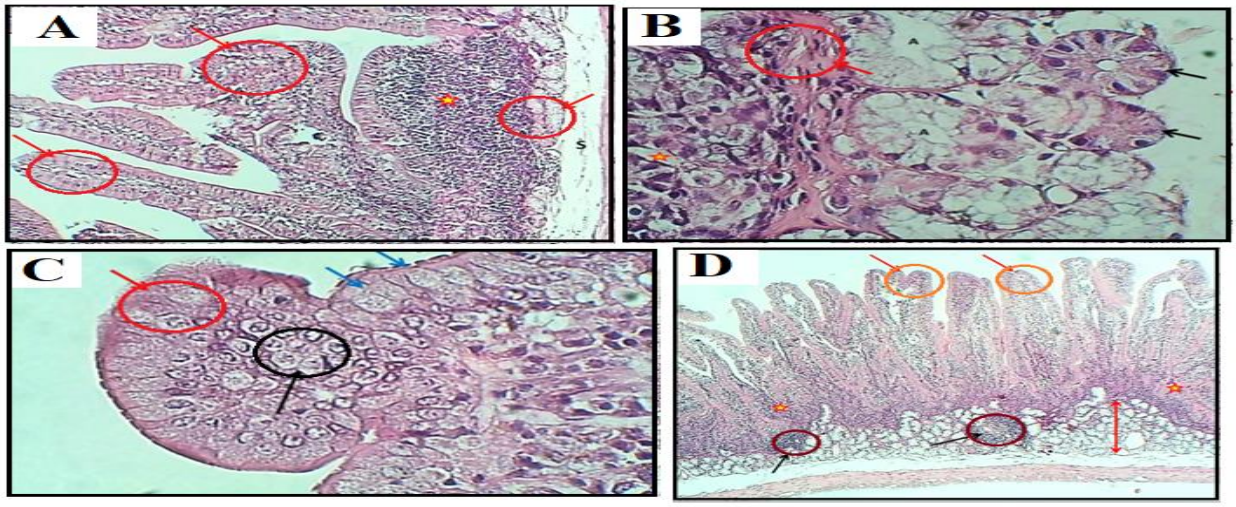

Figure 7. exhibits the histological section of the duodenum within the treatment group (S+PE+VE), highlighting the muscularis mucosa (indicated by a red arrows and circles), crypts of epithelium (marked with an star), mucous alveolar units (labeled as A), and serous acini of Brunner's glands (denoted by black arrows).

3.10 | Jejunum

In the treatment group IV (S+PE+VE), the jejunum exhibited distinct features, with the tunica mucosa displaying a significant augmentation of intestinal plica circularis, accompanied by the projection of pyramidal-shaped intestinal mucosa. Additionally, the submucosal connective tissue revealed 6-8 villi [16.] Moreover, the epithelial mucosa, characterized by a pseudostratified columnar epithelium, was enveloped by a dense brush border of microvilli[1].

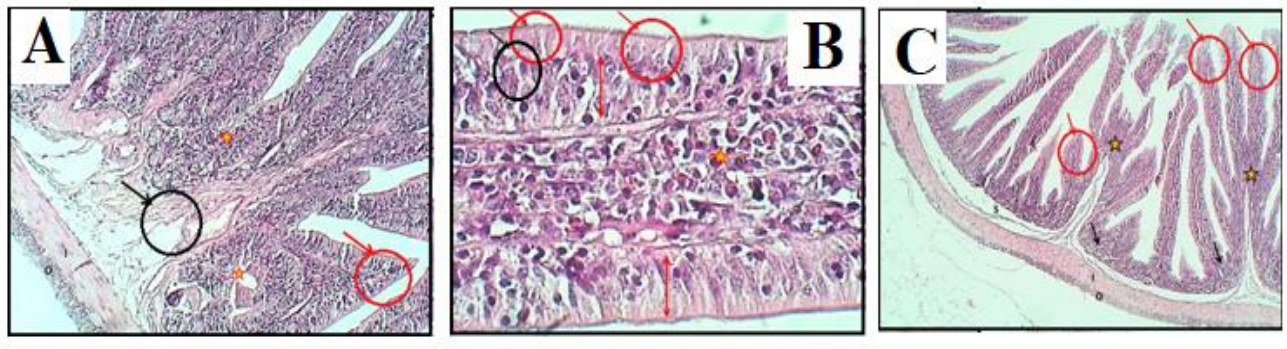

Figure 8: illustrates the histological section of the jejunum within the treatment group (S+PE+VE), indicating a noticeable augmentation in plica circularis (highlighted by asterisks) and the thickening of villi (indicated by red arrows). Additionally, it shows sparse epithelial crypts (marked by black arrows), a thin submucosa (asterisks), with thick inner smooth muscle fibers of the tunica muscularis (labeled as I) and a thin outer layer (labeled as O).

The central region of each villus, consisting of the lamina propria, exhibited notable thickening accompanied by a dense layer of loose connective tissue. Historical measurements indicated a substantial rise in the count of goblet cells and other parameters in the jejunum of the treatment group (S+PE+VE) in comparison to animals in the control group (Deshmukh et al., 2008).

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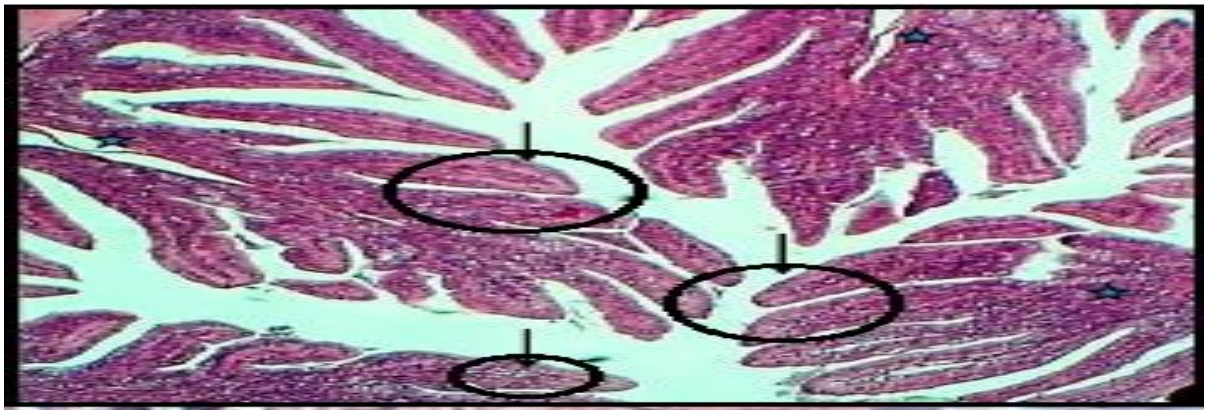

Figure 9: illustrates the histological section of the ileum within the treatment group (S+PE+VE), displaying a substantial amount of mucosal plica circularis (marked by asterisks) and numerous villi (indicated by arrows).

3.11 | Ileum

In experimental animals treated with the extract, there were no notable distinctions between the findings of the ileum and jejunum. This likeness is demonstrated by the notable increase in intestinal plica circularis, which appears as 8-9 short villi projecting into the mucosal and submucosal layers (Figures 9 and 10). The mucosal epithelium of the villi is comprised of pseudostratified columnar epithelium adorned with microvilli forming a brush border, the center of the villi is made by a thick lamina propria. Previous observations revealed a significant rise in villus length, crypt depth, and overall parameters observed in the ileum of the treated animals[19].

3.12 | Wound Healing Studies Skin Irritation Test

A prospective investigation on skin irritation was performed. No inflammation was observed in skin, such as inflammation, swelling, or any adverse reactions, were detected after applying the Standard ointment containing Aloe vera leaf extract and immuno-booster Vitamin E. The PE + VE formulation demonstrated non-irritating characteristics, indicating its suitability for topical use

3.13 | In Vivo Studies

In vivo experiments were performed to evaluate the relative effectiveness of Aloe vera extract and the immune-boosting properties of Vitamin E in recovery of wound. Male rabbits were utilized to examine their impact on its recovery. Group I have rabbits treated solely with standard skin ointment cream (Polyfax), serving as negative controls. Groups II, III, and IV included rabbits treated with Vitamin E, Aloe vera extract, and a combination of both Vitamin E and Aloe vera extract, respectively. Figures 9 and 10 illustrate the percentage reductions in wound areas for Groups I to IV

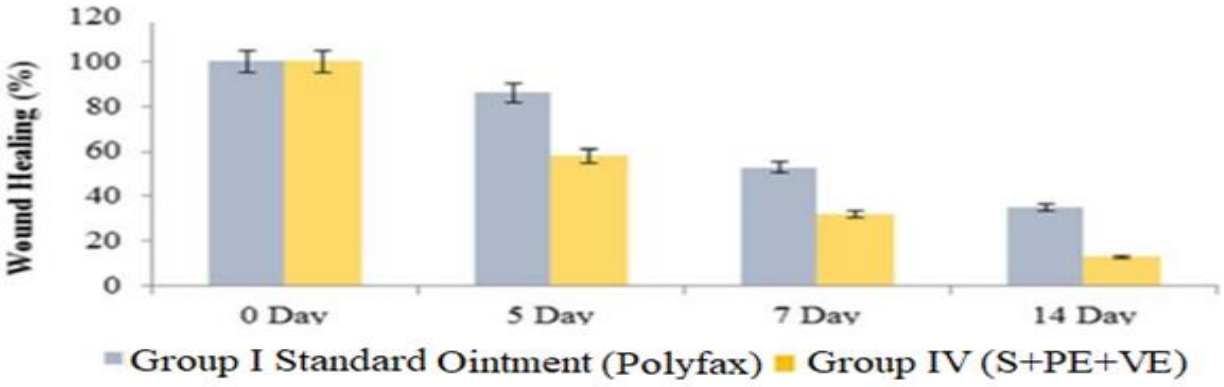

Figure 10: *In vivo* wound healing activity of *Aloe vera* extract with Vitamin E

Upon comparing the wound healing activity between Group I (negative control) and Group IV, which was treated with Aloe vera plant extract and Vitamin E, significant enhancements were noted in Group IV throughout a 14-day period. Initially, during the first 24–48 hours, all four groups showed same features such as inflammation. However, starting from the third day, Group IV showcased notable reductions in these symptoms, indicating accelerated healing [20] By the end of the initial week, Group IV showed signs of the healing process beginning, with either a scab forming or a thin layer of tissue over the wound. Throughout the subsequent week, Group IV continued to progress in wound recovery, with the scab becoming more substantial. Conversely, Group I (the negative control) experienced persistent inflammation, resulting in a slower healing process and no observable scab formation. By the end of the two-week period, Group IV displayed complete wound healing, as the scabs naturally detached and the skin returned to a normal appearance. In contrast, Group I (negative control) still had open wounds displaying visible signs of inflammation.

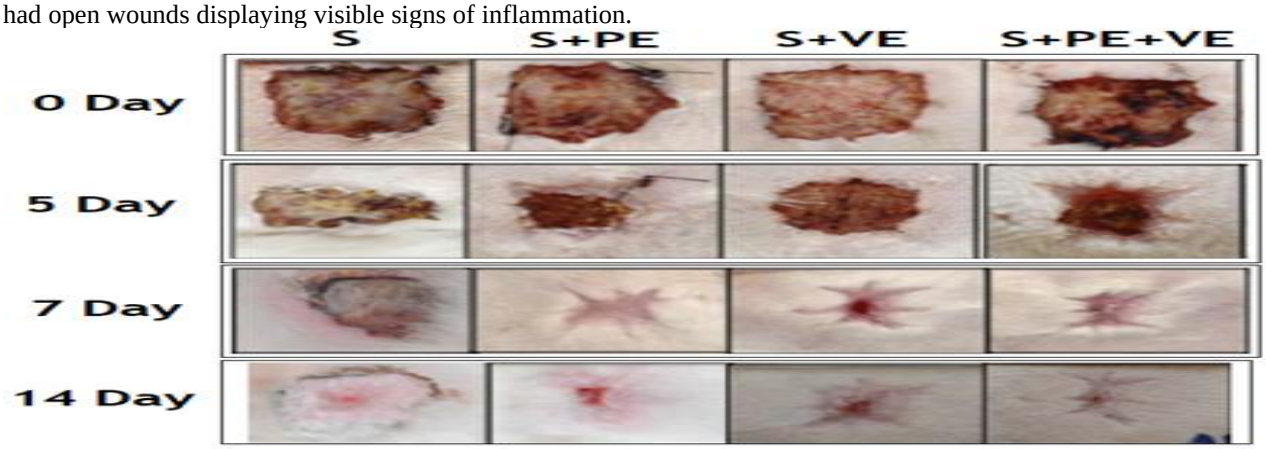

Figure 11: Photographic record of wound healing for each rabbit in the control and treatment groups.

Our investigation revealed that dietary inclusion of PE and VE significantly enhances feed intake. This aligns with findings by[20, 21, 22], and others support that PE and VE improve feed intake through mechanisms such as taste alteration, appetite stimulation, and enhanced digestive enzyme secretion. Weight gain was also improved with Aloe vera extract, consistent with prior research VE supplementation increased villus height and nutrient absorption, contributing to weight gain, likely due to VE's gut microflora maintenance and acemannan polysaccharide's immunostimulatory effects.

4 | DISCUSSION AND CONCLUSION

Our study found that PE and VE supplementation led to significant increases in IgM and IgG levels, but not IgA, indicating specific immune system enhancements. Histopathological studies showed that a combination of Aloe vera extract and VE significantly altered the intestinal mucosa, enhancing absorptive and secretory functions. In wound healing studies, a formulation containing Aloe vera extract and VE showed no adverse reactions and significantly improved wound recovery in rabbits, demonstrating potential for therapeutic use. Our findings suggest that PE and VE supplementation can enhance feed intake, weight gain, immune response, and intestinal health in animals. Further research is needed to understand the mechanisms and optimize supplementation strategies for maximum benefit.

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