Research Article

Amelioration of pathological effects of Newcastle disease by *Aloe vera*

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Citation

Raza Ali Shahid, Shahzad Akbar Khan, Zafar Iqbal Chaudhary and Muhammed Ali Abdullah Shah. Amelioration of pathological effects of Newcastle disease by *Aloe vera*. Pure and Applied Biology. Vol. 9, Issue 1, pp290-302. <http://dx.doi.org/10.19045/bspab.2020.90034>

Received: 16/04/2019

Revised: 15/09/2019

Accepted: 18/10/2019

Online First: 23/10/2019

Abstract

The aim of study was to observe the ameliorative effects of *Aloe vera* on Newcastle disease (ND) in broilers. Commercial broiler chicks (Hubbard) were divided into different groups i.e. negative control group, non-vaccinated *Aloe vera* group, vaccinated *Aloe vera* group and vaccinated non-treated group and challenged with velogenic ND virus. Blood samples were collected at different days post inoculation for sero-conversion and hematology. Moreover, lymphoid organs were collected for weight and histological analysis. Antibody titer was highest in vaccinated *Aloe vera* group followed by vaccinated non-treated group (A) and non-vaccinated *Aloe vera* group. Lymphoid organ's weight was significantly less ($p < 0.05$) in vaccinated *Aloe vera* treated group followed by *Aloe vera* treated group and non-treated group (A). Histopathology revealed congestion, depletion of lymphocytes, dysplasia of thymic lobules, thinning of cortex, focal necrosis, disappearance of lymph follicles and interfollicular edema like lesions within lymphoid organ of the groups challenged with Newcastle disease virus. However, there was significant difference ($p < 0.05$) between *Aloe vera* treated and non-treated group (A) as treated groups showed less pathological changes as compared to non-treated groups (A). Hematology showed significant difference ($p < 0.05$) within differential leukocyte count (DLC) of the chickens in all groups. Feed consumption of non-treated group (A) was higher than treated groups but the weight gain was more in *Aloe vera* treated groups. After infection the feed intake of non-treated group was lowered than treatment group due to disease stress. The body weight gain of 2% *Aloe vera* supplemented broiler was significantly ($p < 0.05$) higher than without *Aloe vera* treated group. It was concluded that *Aloe vera* contains many biological active components which plays a significant role in ameliorating all kinds of pathological effects produced by New Castle Disease Virus in broilers.

Keywords: *Aloe vera*; Broilers; ELISA; Haemagglutination inhibition test; Histopathology; New castle disease

Introduction

Poultry industry is one of the biggest industries of the world but still it is facing many challenges for its future development. One of the most important challenges is less

resources to face outbreaks of poultry diseases, which kills thousands of birds. In Pakistan, poultry industry ranks as second largest industry. Newcastle disease virus (NDV), which belongs to the genus,

Avulavirus, within the family, Paramyxoviridae [1] can cause 100 % mortality depending upon the pathogenicity of virus. ND is highly infective and wide spreading disease as a survey revealed that Newcastle disease is most common in Pakistan [2]. Thus it causes serious economic losses to poultry industry. NDV has a very wide host range, affecting at least 241 avian species [3]. The most virulent strains cause systemic lesions of lymphoid tissues, necrosis and severe lymphoid depletion. Less virulent strains do not cause as much necrosis but may predispose to secondary infections with other pathogens. In previous literature histopathological changes have been described in the tissues of immune system of chicken, after infection with different strains of NDV. The lesions described were severe lymphoid depletion due to necrosis and apoptosis in the spleen, bursa of Fabricius and thymus [4].

Aloe vera (*Aloe Barbadensis* Miller) is a perennial succulent belonging to the Liliacea family [5]. *Aloe vera*, a cactus-like plant has been used for traditional medical purposes for thousands of years. *Aloe* leaves can be separated into two basic products: the latex, a bitter yellow liquid beneath the epidermis of the leaf and the gel; a colorless and tasteless substance in the inner part of the leaf. Both of them have many biologically active components, mainly anthraquinones and polysaccharides (the most active is acemannan), which may act alone or in synergy [6]. According to [7], *Aloe vera* contains 200 compounds among which glycoproteins, anthraquinones, saccharides, vitamins, enzymes and especially, low molecular weight substances are biological active components.

The current project was designed to observe the effects of *Aloe vera* for amelioration of pathological effects on the lymphoid organs of ND infected birds and to evaluate the response of *Aloe vera* on antibody titre of broilers confronted with ND experimental

infection along with growth promotion.

Materials and methods

A total of 120 day old chicks were divided into 4 groups (n=30 birds in each) A, B, C & D. Group A acted as negative Control. Group B was challenged with NDV at day 21st as well as supplemented with *Aloe vera* without vaccination against ND named as non-vaccinated *Aloe vera* group. Group C was challenged with NDV at day 21st along with vaccination against ND and supplemented with *Aloe vera* named as vaccinated *Aloe vera* group. Group D was challenged with NDV, without supplementation of *Aloe vera* but vaccinated against ND. On day 1st, 7th, 20th, 24th, 26th & 28th, blood samples were collected from four birds of each group for determination of antibody titer against NDV.

Aqueous extraction of *Aloe vera* gel

Whole fresh leaves of *Aloe vera* separated from locally available plant, identified by botanist of University of Veterinary And Animal Sciences, Lahore. Whole leaves of *Aloe vera* were cut, using a pocket knife. Latex of the leaf was removed and gel was collected into a jar up to 1000 ml. This gel was homogenized with the help of homogenizer. Then the mixture was filtered and put into flask within rotary vacuum evaporator. The temperature of evaporator was set at 65°C and rotation of flask was performed for two days for complete removal of water so that only active biological components may remain within the mixture. After two days rotation, the mixture was placed in incubator at 37°C to get the powder form of extract. About 100mg of aloe Vera powder was given to treated groups orally from 1st day of age.

Collection of serum samples

Blood was collected aseptically without EDTA from the wing vein of three randomly selected birds from each group at day 1st, 7th, 20th, 24th, 26th and 28th, to check the antibody titers against ND with the help of 25 gauge needles (Approximately 2ml). Then

syringes will be kept in tilt position at ambient temperature for 2 hours. The plunger of the syringe will be removed after separation of the serum and the serum will be poured into the sterile microfuge tubes by manual pouring or using a glass Pasteur pipette. The sera samples will be labeled and stored at -20°C till further use.

Performance of different tests

Sera samples were used to determine the antibody titer (humoral immunity) against NDV as done for Haemagglutination inhibition test [8]. Haemagglutination inhibition test was performed in order to know haemagglutinating inhibition antibody whereas ELISA was performed in order to check the levels of antibodies.

All the birds except control were vaccinated against ND at 6th day subcutaneously and were challenged at 21st day with NDV.

Indirect ELISA was performed to check the antibody response from the birds. To detect the antibodies directed against NDV a commercial ELISA kit, IDEXX (IDEXX Corporation, Westbrook-ME-USA) was used.

The weight of lymphoid organs was also calculated post slaughter as done by [9].

Blood was collected from wing vein of three birds per group at 20th, 24th, 26th and 28th days of age for Hematology. Syringe containing heparin was used to avoid blood clot formation. Blood samples were prepared on slides, stained by Giemsa methods as studied by [10].

1 ml blood was collected from the wing vein aseptically for the calculation of TLC and DLC.

Calculation of FCR

FCR was calculated on weekly basis throughout the experiment. Feed intake per group was recorded every week individually. The weight of all chicks was determined on every first day of experiment and then on weekly basis up to 28 days of age, in order to calculate the mean body weight gain. Data recorded regarding feed

intake and body weight gain were used to calculate feed conversion ratio.

Histopathology of lymphoid organs

After inoculation of virus, three birds were randomly selected from each group and postmortem examination was performed on 24th, 26th and 28th day, respectively. (Dead birds were examined in the same manner). Lymphoid organs (bursa, thymus, spleen, and caecal tonsils along with trachea and lungs) were isolated and processed for gross pathological lesions. The weight and volume of the lymphoid organs (thymus, bursa, and spleen) was also determined [11]. Trachea, bursa, spleen and lungs were further used for histopathology. These organs were preserved in 10% buffered formalin solution, dehydrated, embedded in paraffin wax, sectioned and stained with hematoxylin and eosin (H & E) as described by (Elizabeth et al. 2007). The samples were brought to the Histopathology Laboratory, University of Veterinary and Animal Science Lahore.

Statistical analysis

Data was statistically analyzed by one-way ANOVA. Data were considered significantly different when *P* values were less than 0.05.

Results and discussion

Estimation of antibody response against new castle disease (ND) through HI test

The estimation of antibody response against ND was determined by Haemagglutination inhibition test as described by [8]. According to the values Antibody titers showed significant difference as *P* values are less than 0.05. The results declared that control group has no ND as it was control group. Vaccinated *Aloe vera* group has highest antibody titer post challenge of virus as it was vaccinated along with *Aloe vera* treatment followed by vaccinated non-treated group due to vaccination. Non-vaccinated *Aloe vera* group also possessed good titer against ND as it was treated with

Aloe vera but the titer was low as compared to vaccinated groups as shown in table 1.

From the above mentioned results it was declared that there was no significant difference between all groups at 1st day due to equal presence of maternal antibodies against ND. At day 7 the antibody titer showed non-significant difference between control group and non-vaccinated *Aloe vera* group i.e. ($P \leq 0.842$) due to no administration of ND vaccine at 6th day. Similarly vaccinated *Aloe vera* group and vaccinated non-treated group showed non-significant results ($P \leq 0.967$) as both groups were vaccinated against ND at same days. But the difference between vaccinated and non-vaccinated groups was significant ($P \leq 0.00$). At 20th day control group and non-vaccinated *Aloe vera* group showed no antibody titer due to lack of vaccination and non-persistence of maternal antibodies. So the result was non-significant i.e. ($P \leq 0.10$). Vaccinated *Aloe vera* group and vaccinated non-treated group have non-significant difference ($P \leq 0.24$) between them as both were vaccinated against ND at 6th and 17th day respectively, whereas the result between vaccinated and non-vaccinated groups was significant ($P \leq 0.00$). At 24th day control group and non-vaccinated *Aloe vera* group showed significant difference i.e. ($P \leq 0.15$) because non-vaccinated *Aloe vera* group was challenged with ND virus at day 21st. Vaccinated *Aloe vera* group and vaccinated non-treated group also showed non-significant results i.e. ($P \leq 0.85$). At 26th day all groups showed significant difference i.e. ($P \leq 0.00$) except vaccinated *Aloe vera* group and vaccinated non-treated group which showed non-significant difference i.e. ($P \leq 0.12$). At 28th day all groups showed significant difference i.e. ($P \leq 0.00$).

ELISA test for antibodies detection

To confirm the antibody titer through Haemagglutination inhibition test, ELISA was performed at same days before and after inoculation of virus.

The Log₁₀ titer for ND ELISA is shown in table 2.

According to these values Antibody titer showed significant difference between treated and non-treated groups as P values is less than 0.05

Weight of lymphoid organs

At 24th day the weight of bursa showed non-significant changes between non-vaccinated *Aloe vera* group and vaccinated *Aloe vera* group i.e. ($P \leq 0.17$). Non-vaccinated *Aloe vera* group and vaccinated non-treated group also showed non-significant result as i.e. ($P \leq 0.99$) while vaccinated *Aloe vera* group and vaccinated non-treated group showed significant difference i.e. ($P \leq 0.01$). Control group also possessed significant difference from all other groups i.e. ($P \leq 0.00$).

Weight of thymus at 24th day showed non-significant changes between control group and vaccinated *Aloe vera* group i.e. ($P \leq 0.167$). Non-vaccinated *Aloe vera* group and vaccinated *Aloe vera* group also showed non-significant result as i.e. ($P \leq 0.330$) while non-vaccinated *Aloe vera* group, vaccinated *Aloe vera* group and vaccinated non-treated group showed significant difference i.e. ($P \leq 0.00$). Control group also possessed non-significant difference from all other groups i.e. ($P \leq 0.06$). Weight of spleen at 24th day showed significant difference between all groups i.e. ($P \leq 0.00$). At 26th day, weight of bursa thymus and spleen showed significant difference i.e. ($P \leq 0.00$) between all groups.

At 28th day, weight of bursa, thymus and spleen showed significant difference i.e. ($P \leq 0.00$) between all groups except non-vaccinated *Aloe vera* group and vaccinated *Aloe vera* group in which spleen showed non-significant difference i.e. ($P \leq 0.971$).

Hematology

The total leukocyte count (TLC) reflected marked difference between the different groups of chickens. But, the differential

leukocyte count (DLC) of the chickens reflected highly significant difference within groups. The P value for TLC between all groups was less than 0.05. P value within all groups was 0.00 except within non-vaccinated *Aloe vera* group and vaccinated non-treated group where P value was 0.038 (less than 0.05). The P value for lymphocytes percentage within all groups was 0.00 i.e. significant (less than 0.05) except within non-vaccinated *Aloe vera* group and vaccinated *Aloe vera* group where P value was 0.775 i.e. non-significant (greater than 0.05). The P value for monocytes percentage within all groups was 0.00 i.e. significant (less than 0.05) except within non-vaccinated *Aloe vera* group and vaccinated *Aloe vera* group where P value was 0.998 i.e. non-significant (greater than 0.05). The P value for Eosinophil percentage within all groups was 0.00 i.e. significant (less than 0.05) except within control group and non-vaccinated *Aloe vera* group where P value was 1.00 i.e. non-significant (greater than 0.05).

The P value for heterophil percentage within all groups was 0.00 i.e. significant (less than 0.05). The P value for basophil percentage within all groups was 0.00 i.e. significant (less than 0.05) except within control group and non-vaccinated *Aloe vera* group where P value was 1.00 i.e. non-significant (greater than 0.05). The values are shown in table 3.

Feed conversion ratio

Feed conversion ratio was measured on weekly basis from day 1st till the end of the trial. Total feed intake of the birds within each group was obtained along with total weight gain of chicks within each group.

According to these results *Aloe vera* treated groups showed significant difference i.e. ($P \leq 0.00$) then non-treated groups.

According to formula the FCR calculated at day 7th for negative control group was 1.13 ± 0.50^a , for non-vaccinated *Aloe vera* group = 1.12 ± 0.59^a , for vaccinated *Aloe vera* group = 1.11 ± 0.62^a , and for vaccinated non-treated group = 1.13 ± 0.50^a . There was no significant difference at day 7th. At day 14th FCR calculated for control group was 1.29 ± 0.68^a , for only *Aloe vera* treated group = 1.25 ± 0.45^b , for vaccinated *Aloe vera* group = 1.21 ± 0.51^b , and for vaccinated non-treated group = 1.34 ± 0.019^c . According to these values non-vaccinated *Aloe vera* group and vaccinated *Aloe vera* group showed significantly less FCR then control group and vaccinated non-treated group. At day 21st FCR calculated for control group was 1.39 ± 0.48^a , for only *Aloe vera* treated group = 1.31 ± 0.50^b , for vaccinated *Aloe vera* group = 1.31 ± 0.61^b , and for vaccinated non-treated group = 1.42 ± 0.44^c . According to these results non-vaccinated *Aloe vera* group and vaccinated *Aloe vera* group showed significantly less FCR then control group and vaccinated non-treated group. At day 28th FCR calculated for control group = 1.51 ± 0.57^a , for non-vaccinated *Aloe vera* group = 1.44 ± 0.68^b , for vaccinated *Aloe vera* group = 1.42 ± 0.44^b , and for vaccinated non-treated group = 1.61 ± 0.028^c . According to these results non-vaccinated *Aloe vera* group and vaccinated *Aloe vera* group showed significantly less FCR then control and vaccinated non-treated group.

Table 1. Estimation of antibody response against new castle disease through HI test

Days	Groups			
	A	B	C	D
1	2.30±0.23 ^a	2.30±0.23 ^a	2.30±0.23 ^a	2.30±0.23 ^a
7	1.0±0.0 ^a	1.14±0.29 ^a	2.22±0.28 ^b	2.14±0.29 ^b
20	0.00±0.00 ^a	0.00±0.00 ^a	2.75±0.11 ^b	2.63±0.11 ^b
24	0.00±0.00 ^a	0.89±0.65 ^b	3.04 ±0.08 ^c	2.84 ±0.19 ^c
26	0.00±0.00 ^a	2.14±0.29 ^b	3.17 ±0.00 ^c	2.90±0.10 ^c
28	0.00±0.00 ^a	2.22 ±0.28 ^b	3.28±0.07 ^c	3.00±0.07 ^d

Superscripts having different letters among groups differ significantly (P<0.05)

Table 2. Pre-challenge and post challenge antibody titers by HI test

Days	Groups			
	A	B	C	D
1	2.95±0.03 ^a	2.95±0.03 ^a	2.95±0.03 ^a	2.94±0.90 ^a
7	1.22±0.90 ^a	1.27±0.03 ^b	1.34±0.01 ^c	1.30±0.01 ^d
20	0.09±0.15 ^a	0.61±0.70 ^b	3.77±0.01 ^c	3.69±0.01 ^d
24	0.09±0.01 ^a	1.01±0.01 ^b	4.11±0.01 ^c	3.99±0.01 ^d
26	0.09±0.01 ^a	1.18±0.01 ^b	4.16±0.01 ^c	4.00±1.06 ^d
28	0.09±0.01 ^a	1.70±0.03 ^b	4.24±0.02 ^c	4.10±0.01 ^d

Superscripts having different letters among groups differ significantly (P<0.05)

Table 3. Pre-challenge and post challenge antibody titers by ELISA test

Groups	TLC WBCs = 10 ³ /Mm ³	Lymphocytes	Monocytes	Eosinophils	Heterophils	Basophils
A.	22.30±0.03 ^a	56.29±0.04 ^a	2.00±0.03 ^a	2.50±0.06 ^a	37.10±0.03 ^a	1.2±0.03 ^a
B.	22.50±0.05 ^b	57.00±0.03 ^b	2.10±0.02 ^b	2.50±0.06 ^a	39.4±0.03 ^b	1.7±0.04 ^b
C.	22.40±0.04 ^c	57.66±0.32 ^b	2.10±0.04 ^b	2.60±0.04 ^b	39.7 ±0.03 ^b	1.4±0.03 ^b
D.	22.60±0.05 ^d	59.70±0.03 ^c	2.30±0.04 ^b	2.90±0.03 ^c	37.30±0.03 ^c	1.7±0.03 ^b

Superscripts having different letters among groups differ significantly (P<0.05)

Histopathological examination of lymphoid organs

Histopathology was performed to check the microscopic results of lymphoid organs to detect the changes post inoculation of virus. Post infection results showed depletion of splenic lymphocytes, disappearance of boundaries between red and white pulp due to bleeding and congestion, reduction of lymph follicles in vaccinated non-treated group as shown in figure 1as compared to vaccinated *Aloe vera* group as shown in figure 2. More severe congestion, depletion of lymphocytes population in

medulla of thymus, dysplasia of thymic lobes and focal necrosis were observed in thymus of vaccinated non-treated group as shown in figure 3as compared to vaccinate *Aloe vera* group as shown in figure 4. Cellular atrophy, swollen tonsils revealed hemorrhages, congestion and necrosis in the lymphoid tissue and sloughing off of the necrotic tissues neutrophils and rupture of capillaries were observed in cecal tonsil. Thymus of vaccinated *Aloe vera* group showed few multifocal necrotic centers and mild degree of congestion. The bursa of vaccinated non-treated group showed inter-

follicular edema, slight depletion of lymphocytes and mild cellular infiltration as compared to vaccinated *Aloe vera* group and negative control group as shown in figures 5 and 6. Cellular hypertrophy, increase lymphocytes population and presence of lymphoblast were observed in lymphoid organs of vaccinated non-treated group. The values for Histopathological scoring are shown in table 4.

According to these results the histopathology of lung, trachea and thymus declared significant results between control and all other groups. At 24th day non-significant difference was observed in non-vaccinated *Aloe vera* group with vaccinated *Aloe vera* group i.e. ($p \leq 0.135$) and vaccinated non-treated group i.e. ($p \leq 0.645$). Vaccinated *Aloe vera* group and non-treated group showed significant difference i.e. ($p \leq 0.001$). Spleen and bursa showed non-significant difference.

Significant difference was observed between all groups at 26th day ($p \leq 0.010$). At 28th day all groups showed non-significant difference although the severity of lesions was less in vaccinated *Aloe vera* group as compared to non-vaccinated *Aloe vera* group and non-treated group.

Similarly the histopathology of spleen and bursa declared non-significant results at 24th day while significant difference was observed between vaccinated *Aloe vera* group with non-vaccinated *Aloe vera* group and non-treated group at 26th and 28th day i.e. ($p \leq 0.001$) but non-significant changes were observed between non-vaccinated *Aloe vera* group and non-treated group.

Discussion

Broilers immune system is easily unbalanced under modern rearing conditions leading to high productive losses, increased morbidity and mortality. Thus, intense efforts put together to minimize these losses. The present research was formulated to determine the efficacy of *Aloe vera* to ameliorate the pathological effects of

New castle disease in broilers along with growth promotion and overall health status of birds. The antiviral drugs are not frequently used in poultry sector due to their high costs and toxicity. Exploration of medicinal plants against viral diseases is the only way to overcome these problems in poultry sector. Such studies were also conducted by [12-14].

Aloe vera is an herbal plant and it has a significant effect as an anti-bacterial as well as anti-viral. It possesses significant effects to decrease the viral load, the results agreed with [15].

Aloe vera as a member of the Liliaceae family of plants has been used topically for wounds due to its extra ordinary effects in healing properties [16]. Two herbal preparations are derived from *Aloe* plant; *Aloe* gel and *Aloe* latex. *Aloe* gel is the clear gel produced by parenchymal cells located in the central region of the leaf. The gel is composed mainly of water (99%) and 25% of the dry weight of the gel are monosaccharide and polysaccharides. The most prominent monosaccharide in *Aloe* gel is mannose-6-phosphate, and the most common polysaccharides are called glucomannans as studied by [17]. There are some other studies about antiviral properties of *Aloe vera* especially about the antiviral activity of its anthraquinones as described by [18, 19]. In another study this property is attributed to the mannose-6-phosphate which is also present in *Aloe vera* gel [20].

The hepato protective ability of *Aloe vera* was studied time and again and it was found that isolated phytosterols, namely cycloartanol and lophenol have the ability to induce the down regulation of fatty acid synthesis and a tendency for up regulation of fatty acid oxidation in the liver, which favors the reduction in intra-abdominal fat and improvement of hyperlipidemia. Furthermore, it decreases the ratio of sterol regulatory element-binding transcription factor i.e. peroxisome proliferator activated

receptor (PPAR)- α along with some metabolic syndrome-related disorders and liver steatosis as studied by [21].

In this study the cumulative geometric mean titer of group A (3.8), B (4.8), C (6.8), and group D (3.5) before infection while CGMT values after infection i.e. A (2.3), B (8.2), C (8.9), and group D (3.2) showed that 2 percent oral administration of *Aloe vera* gel extract improved the antibody titer against NDV in broilers as studied by [22]. It was due to the Immuno modulatory activities of *Aloe vera* [23]. *Aloe vera* increases the efficiency of lymphoid organs. *Aloe vera* gel has strong Immuno modulatory activity wherein it down regulates lipopolysaccharide-induced inflammatory cytokine production and expression of NLRP3 (NACHT, LRR, and PYD domain-containing protein 3) inflamma some in macrophages. Administration of *Aloe vera* has been universally demonstrated as marked increase in phagocytic and proliferative activity of the reticulo endothelial system as studied by [24].

Aloe vera directly inhibits the cyclooxygenase pathway and reduces prostaglandin E2 production, which plays an important role in inflammation as studied by [25].

Weight of lymphoid organs i.e. thymus, spleen, and bursa of Fabricius was improved in broiler in which 2% aqueous extract of *Aloe vera* was supplemented orally as compared to without *Aloe vera* diet. This was due to the Immuno-modulatory activities of *Aloe vera* in the diet at moderate level increase the activation of immune system as *Aloe* contains anthraquinones and chromone in the inner gel, which possess strong anti-inflammatory effects as shown in murine macrophages as studied by [26].

Weight gain of all the birds and feed intake including *Aloe vera* treated and without *Aloe vera* group increased in regular ascending order with advancement of age. *Aloe vera* supplemented broiler intake less feed as compared to without *Aloe vera* and they gained more body weight. This was due to the laxative property of *Aloe vera* gel which is very useful for the digestive system. This is the fact that *Aloe vera* improves the absorption of poly-unsaturated fatty acids from the intestines which increase the metabolic energy and because of the dietary fat composition, it makes it possible to increase diet digestibility and to stimulate growth. These results agreed with the observations of same study by [27].

In *Aloe vera* treated groups, low mortality was observed when challenged with Newcastle disease virus as compared to non-treated groups. This was due to the increase in antibody titer against ND virus and the effects of biologically active components present in *Aloe vera* gel which have antiviral activity as heparin, acemannan, anthraquinones and polysaccharides as studied by [28] who compared acemannan with azdothymidine and acyclovir in vitro. He studied the immunomodulatory and antiviral properties of acemannan and found that acemannan supposed to modify the viral glycoproteins. Acemannan have a synergistic effect against enveloped viruses by inhibiting the virus replication.

From our study we concluded that that Histopathological lesion were more severe in non-treated groups, challenged with NDV variant as compared to treatment groups. It was also concluded that that production of Histopathological lesions in spleen bursa and thymus were more severe in group D as compared to other treatments group.

Figure 1. Spleen of Group D, a= splenic arteries are intact b= less no of red cells and more interfollicular spaces

Figure 2. Spleen of group C showing normal a= red pulp, b= white pulp

Figure 3. Thymus of group D showing multiple areas of congestion and increased sinusoidal spaces. (10 X)

Figure 4. Thymus of group C showing normal (10X) a= cortex, b= medulla

Figure 5. Bursa of group B (arrow show normal) 10X

Figure 6. Group A Bursa showing normal (10 X) a=Lamina propria, b=medulla, c=cortex, d= pseudo stratified epithelium

Table 4. Hematological analysis of all groups

Lungs	Groups			
	A	B	C	D
24	1.0±0.00 ^a	3.25±0.95 ^{bc}	2.25±0.5 ^b	3.75±0.50 ^c
26	1.0±0.00 ^a	2.75±0.50 ^c	2.0±0.0 ^b	3.75±0.0 ^d
28	1.0±0.00 ^a	3.25±0.95 ^b	2.75±0.50 ^b	3.75±0.50 ^b
Trachea	Groups			
	A	B	C	D
24	1.0±0.00 ^a	3.25±0.95 ^{bc}	2.25±0.5 ^b	3.75±0.50 ^c

26	1.0±0.00 ^a	2.75±0.50 ^c	2.0±0.0 ^b	3.75±0.0 ^d
28	1.0±0.00 ^a	3.25±0.95 ^b	2.75±0.50 ^b	3.75±0.50 ^b
Bursa	Groups			
	A	B	C	D
24	1.0±0.00 ^a	2.5±0.57 ^{ab}	2.0±0.81 ^{ab}	2.25±0.95 ^b
26	1.0±0.00 ^a	3.0±0.81 ^b	2.5±0.57 ^b	3.5±0.5 ^b
28	1.0±0.00 ^a	3.0±0.81 ^b	2.75±0.50 ^b	3.5±0.57 ^b
Thymus	Groups			
	A	B	C	D
24	1.0±0.00 ^a	3.25±0.95 ^{bc}	2.25±0.5 ^b	3.75±0.50 ^c
26	1.0±0.00 ^a	2.75±0.50 ^c	2.0±0.0 ^b	3.75±0.0 ^d
28	1.0±0.00 ^a	3.0±0.81 ^b	2.75±0.50 ^b	3.5±0.57 ^b
Spleen	Groups			
	A	B	C	D
24	1.0±0.00 ^a	2.5±0.57 ^b	2.0±0.81 ^{ab}	2.25±0.95 ^{ab}
26	1.0±0.00 ^a	2.75±0.50 ^c	2.0±0.0 ^b	3.75±0.0 ^d
28	1.0±0.00 ^a	3.75±0.95 ^c	3.0±0.50 ^b	4.0±0.0 ^c

Superscripts having different letters among groups differ significantly (P<0.05)

1=Normal; 2=Mild changes; 3=Moderate changes; 4=Severe changes

Conclusion

At the end it was concluded that *Aloe vera* contains many biological active components which plays a significant role in ameliorating all kinds of pathological effects produced by New Castle Disease Virus in broilers.

Authors' contributions

Designed the research project: Z Iqbal & S Akbar, Performed the experiments: RA Shah, Provides financial support: Z Iqbal, SA Khan & AA Shah, Analysis and interpretation of data: Z Iqbal, SA Khan & AA Shah.

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