



Immunomodulatory effect of Stinging nettle (*Urtica dioica*) and Aloe vera (*Aloe barbadensis*) in broiler chickens

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ABSTRACT

A study was conducted to identify the immunomodulatory effect of Stinging nettle (*Urtica dioica*) and Aloe vera (*Aloe barbadensis*) in broiler chicken. A total of 240 birds were divided into 4 treatment groups with 4 replications in each group, each replication containing 15 birds. Treatments were comprised of no Nettle and Aloe vera (T₀); Nettle 5% (T₁), Aloe vera @5 ml/lit drinking water (T₂) and Nettle 5% and Aloe vera @5 ml/lit drinking water (T₃). The study was conducted under a randomized design. The immunomodulating effect was evaluated by measuring antibody titer against NDV vaccine; and by estimating the albumin, globulin and total protein upto 40 days of age. The effect on liver was estimated by measuring liver enzymes alanine aminotransferase (ALT). The hypoglycemic effect was estimated by measuring glucose level in the serum, and morphological changes in the spleen and bursa were estimated by measuring the ratio score of the respective organs to the body weight. Throughout the experiment, the value for HI titer, albumin, globulin, total protein, spleen weight ratio score, bursa weight ratio score, liver function test and blood glucose of all treatment group were non significant ($p > 0.05$) except for albumin on 10th day, globulin on 0th day, total protein on 0th day and ratio score of spleen were found to be significant ($p \leq 0.05$) at 30 days in all treatment compared to control group. Histopathology of spleen and bursa appeared normal in all treatment and control groups. Result thus suggests that nettle and Aloe vera have non-significant immunomodulatory effects.

1. Introduction

Intensive poultry production with fast growing strains and high stocking densities are usually susceptible to infectious agents due to various reasons, one of the reasons being reduced immune potential (Vander, 1983), and immunosuppression, a recurring economic problem in commercial poultry flocks, may be caused by a number of factors such as stress, nutrition and infectious agents.

Plants and their extracts have immense potential for the management and treatment of a variety of diseases and conditions. Phyto-medicines may not only be cheap and affordable but also can be purportedly safe with no or minimal side reactions (Jagadeeswaran, 2007). Among them nettle and Aloe vera have an immunomodulatory properties. The stinging hairs contain acetylcholine, histamine, serotonin, choline and small amount of leukotrienes (ESCOP, 2003). The root of the plant contains scopoletin, sterols, fatty acids, polysaccharides and isolectins. Several of nettle lectin chemicals having antiviral action (Golshan, Toghiani, & Gholamreza, 2015). Other chemicals like

flavonoids in the leaves and lectin in the root have been documented with interesting immune stimulant actions in the treatment of systemic lupus. These compounds are responsible for the typical skin reactions (Golshan et al., 2015).

Increased lymphocyte proliferation was demonstrated for isolated nettle root fioica polysaccharides as well as for aqueous lyophilized extracts, and a methanolic and an ethanolic nettle root extract (Wagner, Willer, & Kreher, 1989). Likewise, pure *Urtica dioica* agglutinin (UDA) increased lymphocyte proliferations was demonstrated for isolated nettle root polysaccharides as well as for aqueous lyophilized extracts, and a methanolic and an ethanolic nettle root extract (Wagner et al., 1989). Likewise, pure *Urtica dioica* agglutinin (UDA) affected lymphocyte proliferation in a dose-dependent manner. UDA is a complex mixture of at least 11 isolectins that differ with respect to their amino acid composition. The active components of aloe include anthraquinones, chromones, polysaccharides and enzymes. The anthraquinones and chromones are responsible for the anti-cancer activity, anti-inflammatory and evacuating. The other active principles of

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Aloevera include hydroxyanthrone, aloemodin-anthrone 10-C-glucoside and chrones (Yadav, 2009). The bitter yellow latex containing anthraquinones and glycosides has been reported from the middle layers of leaf.

Likewise, pure *Urtica dioica* agglutinin (UDA) affected lymphocyte proliferation in a dose-dependent manner. The gene for nettle lectins encodes lectin (chitin-binding domain) and a chitinase domain (Lerner & Raikhel, 1992).

Aloevera contains anthraquinones and chromone in the inner gel which possesses strong anti-inflammatory effects as shown in murine macrophages. A recent report of a clinical study evaluated the therapeutic effect of *Aloe vera* gel wherein 2% oral gel is not only effective in decreasing the pain score and wound size in recurrent aphthous stomatitis patients but also decreasing the aphthous wound healing period (Maharjan & Nampoothiri, 2015). It affects cytokine production (Reynolds, 1999) and found to have an action in promoting maturation of dendritic cells (Lee et al., 2001) both of which may explain possible effects on the immune system. Aloe juice has virucidal capacity against herpes simplex, varicella zoster and influenza virus (Sydiskis, 1991).

2. Materials and Methods

2.1. Experimental trial

The experimental study included rearing of broilers chicken and was conducted at Rampur livestock farm, Chitwan, Nepal. Laboratory examination was conducted at Paklihawa Campus, IAAS, Pathology lab; National Avian Laboratory, Bharatpur, Chitwan, Nepal and Narayani Samudaik Hospital, Bharatpur, Chitwan, Nepal.

2.2. Experimental broiler chickens

A total of two hundred and forty- day old Cobb- 500 broiler chicken of the same breeder flock and same hatching lot vaccinated against Marek's disease were procured from a commercial hatchery and commercial mash feeds were acquired from a commercial feed mill. The nettle leaf powder was from Asan, Kathmandu, Nepal and the commercial juice of the *Aloe vera* was from a reputed company Patanjali Yogapitha, Haridwar, India.

2.3. Experimental design

Two hundred and forty day old Cobb - 500 broilers chicken from commercial hatchery were divided into four (4) treatment groups each consisting of sixty birds. Initially, birds were kept for one week in experimental house. Each group was replicated four times with each replication consisting of 15 birds. Experimental groups were given different treatments which are mentioned in Table 1 below. The birds were allocated randomly to the different groups. The chickens in each of the four groups were given ND vaccine. Primary ND vaccination was performed on 6th day with F₁ strain intra-ocularly, on 21st day with Lasota strain and on 35th day again with Lasota strain. Four birds were selected randomly and sacrificed and samples (blood, bursa and spleen) were collected from day old chickens before treatments. After that, one bird from each replication was selected randomly, weighed and was sacrificed at 10th, 20th, 30th and 40th days; and their blood, bursa and

Table 1
Nomenclature and distribution of four treatments groups.

T ₀	Basal diet with no nettle and Aloe vera
T ₁	Basal diet with Nettle 5% in feed
T ₂	Basal diet with Aloe vera @ 5 ml/ lit i.e. (0.5%) drinking water per day
T ₃	Basal diet with Nettle (5%) in feed + Aloe vera @ 5 ml/ lit i.e. (0.5%) drinking water per day

spleen were collected. The serum was immediately separated from blood and stored at -20 °C. The bursa and spleen were weighed separately and preserved in 10% neutral buffer formalin for histopathological examination.

2.4. General management

The birds were reared on a deep litter system and maintained with all recommended husbandry norms in relation to light, feed and water. All the groups were reared on commercial feeds with fulfillment of all its requirements according to the increasing life time and days of life. They were fed with broiler starter feed (B₁) from zero day to 3rd weeks, broiler grower feed (B₂) after 3rd week upto 5th weeks and broiler finisher (B₃) from 5th week onwards. Frequent changes of water with proper arrangement of space and light were managed for all the birds (Table 2).

2.5. Laboratory examination

2.5.1. Determination of NCDV antibody titer

The antibodies titer against ND was determined by Haemagglutination Inhibition (HI) test using NCDV (F1) antigen by beta procedure utilizing 4HA units of virus and 1% suspension of fresh chicken RBC making serial two fold dilution of serum in antigen.

2.5.2. Biochemical estimation

In the biochemical test, total serum protein (Albumin and Globulin) was determined by BCG method, glucose by GOD/POD method and liver enzyme (ALT) by Reitman & Frankel's method as per crest bio-systems test kit with the help of a spectrophotometer.

2.5.3. Histopathological examination

Preserved tissues were processed by standard techniques of dehydration in ascending grade of alcohols and cleared in xylene. The tissues were then embedded in paraffin wax and sectioned into 5 mm thick tissue, then stained with hematoxyline and eosin. The histopathological examination and photographs were taken with Olympus (D2S) photographic camera as CX41 microscope (Olympus)

2.5.4. Calculation of bursa-spleen: body weight ratio

The body weights of birds were taken before they had been sacrificed with the help of electronic balance then after necropsy weighing of bursa and spleen was done with the help of sensitive electronic balance weighing for 0.001–20 g. The bursa was detached from the cloacal area and from the rectum of intestines with every care not to detach any part or the content there in. The spleen was collected by detaching from mesentery. Any extra tissue attached thereof was removed. The organs to body weight ratio were calculated as -

$$\text{Bursa: body weight ratio} = \frac{\text{Bursa weight (g)}}{\text{Bodyweight(g)}}$$

$$\text{Spleen: body weight ratio} = \frac{\text{Spleen weight (g)}}{\text{Bodyweight(g)}}$$

2.6. Recording of morbidity and mortality in treatment and control groups

The records of morbidity and mortality of broiler chicken were determined.

2.7. Bio-statistics and data analysis in excel

Prior to analysis, all data were recorded in Excel. For analysis of antibody response to ND vaccine, data were expressed as the reciprocals of end point dilution in logarithms to the base 2. The mean \pm SD antibody titer was obtained. The mean antibody titer of different groups at

Table 2
Various activities within the samples collection.

Time (days)	Activities			
	T ₃	T ₂	T ₁	T ₀
	Nettle (5%) in feed + Aloe vera 5 ml/lit	Aloe vera 5 ml/ lit	Nettle (5%) in feed	Control
0	Samples collected before experimentation (Blood, bursa and spleen)	Samples collected before experimentation (Blood, bursa and spleen)	Samples collected before experimentation (Blood, bursa and spleen)	Samples collected before experimentation (Blood, bursa and spleen)
10	Blood, bursa and spleen	Blood, bursa and spleen	Blood, bursa and spleen	Blood, bursa and spleen
20	Blood, bursa and spleen	Blood, bursa and spleen	Blood, bursa and spleen	Blood, bursa and spleen
30	Blood, bursa and spleen	Blood, bursa and spleen	Blood, bursa and spleen	Blood, bursa and spleen
40	Blood, bursa and spleen	Blood, bursa and spleen	Blood, bursa and spleen	Blood, bursa and spleen

different days of treatment was determined using analysis of variance (ANOVA) at $P \leq 0.05$. Significance difference between groups were then analyzed by Tukey test at $P < 0.05$ if there is significance difference in ANOVA.

The bursa body weight ratio and spleen body weight ratio were calculated and their mean and SD were calculated. Biochemical parameters and their mean and SD were calculated. The analyses were done with the help of software Open - epi and SPSS (16).

3. Results

3.1. Effect on antibody titre against Newcastle disease

Geometric means of HI titre against ND antigen in the serum of experimental broilers has been presented in Table 3.

The highest titre was found 32.57 ± 3.32 in T₀ on the 20th day followed by 29.23 ± 4.28 in T₃ on same day and the lowest titre was found 9.30 ± 3.32 in T₀ on 10th day. In control group, the highest titre was found 32.57 ± 3.32 on 20th day falling down to 17.60 ± 8.13 on 30th day and antibody titre was increased onwards. In T₁ group, the highest titre was found 22.59 ± 1.91 on 30th day and the lowest titre was found 12.62 ± 1.91 on the 10th day.

In the T₂ group, titre was found almost similar on 30th and 40th days which were the highest titres but the lowest titre was found 12.62 ± 5.75 on 10th day. Similarly, in T₃ group, the highest titre was observed on 20th day decreasing down upto 30th day and from 30th day while increasing upto 25.91 ± 5.75 from 30th days onwards. The antibody titre against ND showed non significant ($p > 0.05$) differences among the treatment groups as compared to the control group throughout the observation period. So, it can be regarded that the nettle and Aloe vera had no effect on antibody titre against ND.

3.2. Effect of stinging nettle and Aloe vera on albumin levels

Table 4 below presents changes in the Albumin levels (g/dl) in the serum of broiler chickens under experimentation at different levels of Stinging nettle and Aloe vera treatment.

Table 3

Effect of stinging nettle and Aloe vera levels on log₂ HI titres (Mean \pm SD) against ND at different days of age of broilers.

Treatments	Days (Mean \pm SD)			
	10	20	30	40
T ₀ : Basal diet with no Nettle and Aloe vera	11.79 \pm 4.18	32.57 \pm 3.32	17.60 \pm 8.13	27.57 \pm 0
T ₁ : Basal diet with Nettle 5% in feed	12.62 \pm 1.91	18.43 \pm 8.73	22.59 \pm 1.91	20.93 \pm 8.13
T ₂ : Basal diet with Aloe vera @ 5 ml / lit i.e. (0.5%) drinking water/day	12.62 \pm 5.75	23.42 \pm 10.63	26.74 \pm 4.98	26.74 \pm 1.66
T ₃ : Basal diet with Nettle (5%) in feed + Aloe vera @ 5 ml/lit i.e. (0.5%) drinking water/day	9.30 \pm 3.32	29.23 \pm 4.28	23.42 \pm 8.73	25.91 \pm 5.75
p-value	0.62 ^{ns}	0.08 ^{ns}	0.30 ^{ns}	0.29 ^{ns}

Means in column with different superscripts are significantly different. ^ssignificant at 5% ($p < 0.05$); ^{ns}non significantly different.

The highest level of serum albumin was found 2.04 ± 0.57 in T₁ group on 40th days i.e. basal diet with nettle 5% in feed followed by 1.84 ± 0.10 in T₃ group on 40th days i.e. basal diet with nettle (5%) in feed + Aloe vera @5 ml/ It drinking water and the lowest value was 0.6 ± 0 in T₀ group on 0 day i.e. basal diet with no nettle and Aloe vera.

The values of the control group are below the treatment group during 0–10 days and it increased 1.54 ± 0.32 on 20th day then it increased and was found 1.68 ± 0.24 on 40th day. In T₁ group, the highest value was found 2.04 ± 0.57 on 40th day and the lowest value was found 0.69 ± 0.53 on 10th day. Similarly, in T₂ group, the highest albumin level was found 1.48 ± 0.14 on 40th day whereas lowest value was found 1.11 ± 1.12 on 0th day. Similarly, in T₃ group, lowest value was observed 1.02 ± 0.12 on 0th day and it increased upto 20th day and the highest value was observed on 40th day. The result of albumin levels showed non significant ($p > 0.05$) differences among the treatment groups as compared to the control group throughout the observation period except on 10 days which showed significant ($p < 0.05$).

3.3. Effect of stinging nettle and Aloe vera on globulin levels

The Table 5 below presents the changes in serum globulin levels (g/dl) of broiler chickens under experimentation at different levels of stinging nettle and Aloe vera treatment.

The highest serum globulin level was found 5.4 ± 1.41 in T₀ group on 0 day i.e. basal diet with no nettle and Aloe vera followed by 3.6 ± 1.27 in T₁ group on 0 day i.e. basal diet with nettle (5%) in feed while the lowest value was found 1.83 ± 1.02 in T₂ group on 10th day i.e. basal Diet with Aloe vera @ 5 ml /lit drinking water/day.

In the control group, the highest value of globulin was observed 5.4 ± 1.41 on 0 day but it decreased on 10th day and further very slightly increased upto 30th days. In T₁ group, the highest level of globulin was found 3.6 ± 1.27 on 0 day and the lowest value was found 2.18 ± 0.46 on 40th day. In T₂ group, the highest globulin level was found 2.9 ± 1.3 on 0 day and the lowest value was observed 1.83 ± 1.02 on 10th day whereas on 20th, 30th and 40th day its value

Table 4
Effect of stinging nettle and Aloe vera feeding on albumin levels (Mean \pm SD) in serum at different days of broilers.

Treatments	Days (Mean \pm SD)				
	0	10	20	30	40
T ₀	0.6 \pm 0	0.63 \pm 0.13	1.54 \pm 0.32	1.54 \pm 0.17	1.68 \pm 0.24
T ₁	0.9 \pm 0.56	0.69 \pm 0.53	1.47 \pm 0.24	1.45 \pm 0.16	2.04 \pm 0.57
T ₂	1.11 \pm 1.12	1.31 \pm 0.26	1.41 \pm 0.23	1.33 \pm 0.17	1.48 \pm 0.14
T ₃	1.02 \pm 0.12	1.30 \pm 0.21	1.34 \pm 0.18	1.25 \pm 0.82	1.84 \pm 0.10
p-value	0.68 ^{ns}	0.01 ^s	0.72 ^{ns}	0.79 ^{ns}	0.14 ^{ns}

Means in column with different superscripts are significantly different. ^ssignificant at 5% ($p < 0.05$); ^{ns}non significantly different.

was almost similar. Similarly, in T₃ group, these values were almost similar. The result of globulin levels showed non significant differences except on 0 days which showed significant ($p \leq 0.05$) throughout the observation period among the treatment groups as compared to the control group.

3.4. Effect of stinging nettle and Aloe vera on total protein levels

The Table 6 below presents changes in the serum total protein levels (g/dl) of broiler chickens under experimentation at different levels of Stinging nettle and Aloe vera treatment.

In the control group, the highest value of total protein was observed 6 \pm 1.41 on 0 day but it decreased to 3.22 \pm 0.53 on 10th day and got increased in 20th and 30th days. In T₁ group, the highest level of total protein was found 4.5 \pm 0.70 on 0 day and the lowest value was found 3.82 \pm 0.51 on 10th day. In T₂ group, the highest level of total protein was found 4.2 \pm 0.18 on 40th day while the lowest value was observed 3.15 \pm 1.17 on 10th day but on 0th, 20th and 30th day the values were almost similar. Similarly in T₃ group, values were almost similar in 0th and 10th day and it increased from 20th day but almost similar value was observed on 20th, 30th and 40th days. The result of total protein levels showed non significant differences except on 0 days which showed significant ($p < 0.05$) among the treatment groups as compared to the control group throughout the observation period.

3.5. Effect on immune organ to body weight ratio

Relative weight (g) of the spleen and bursa are depicted in Tables 7 and 8 with mean and standard deviation of the group.

The scores was the absolute numbers obtained by the division of weight of spleen into body weight converted in the same unit (g), described in methodology, with mean have been obtained from the statistical analysis.

The highest score (Table 7) was found in T₂ group on 40th day followed by T₃ group on 10th day and the lowest value was obtained in T₀ group on 30th day. Score value of spleen was highest on 10th days in all control and treatment groups then it decreased on 20th and 30th day and again from 30th day onward it slightly increases except for T₃ group. The ratio score of spleen were found to be significant ($p < 0.5$) at 30 days in all treatment and control group but non significant were noted on 0th, 10th, 20th and 40th day respectively.

Table 5
Effect of stinging nettle and Aloe vera feeding on serum globulin levels (Mean \pm SD) on different days of broilers.

Treatments	Days (Mean \pm SD)				
	0	10	20	30	40
T ₀	5.4 \pm 1.41	2.59 \pm 0.48	2.53 \pm 0.25	2.62 \pm 0.45	2.29 \pm 0.38
T ₁	3.6 \pm 1.27	3.13 \pm 0.64	2.43 \pm 0.08	2.77 \pm 0.20	2.18 \pm 0.46
T ₂	2.9 \pm 1.3	1.83 \pm 1.02	2.59 \pm 0.42	2.69 \pm 0.33	2.71 \pm 0.13
T ₃	2.38 \pm 1.63	2.34 \pm 1.00	2.75 \pm 0.09	2.74 \pm 0.62	2.35 \pm 0.30
p-value	0.05 ^s	0.22 ^{ns}	0.36 ^{ns}	0.96 ^{ns}	0.19 ^{ns}

Means in column with different superscripts are significantly different. ^ssignificant at 5% ($p < 0.05$); ^{ns}non significantly different.

The highest score (Table 8) was found 2.20 \pm 0.60 in T₀ group on 20th day followed by 2.15 \pm 0.46 in T₁ group on same day and the lowest value was obtained 0.17 \pm 0.01 in T₂ group on 30th days.

In control group of ratio score of bursa, mean value was 1.14 \pm 0.58 on 0th day then it increased in 10th and 20th days and from 30th day onward value started to decline. Similarly, in T₁, T₂ and T₃ groups mean value was 1.33 \pm 0.06, 1.156 \pm 0.283 and 1.15 \pm 0.39 respectively on 0th day then it increased on 10th and 20th days and started to decline. Ratio score of bursa was non significant.

3.6. Effect on liver function tests

The Table 9 below presents changes in the serum ALT levels (IU/L) of broiler chickens under experimentation at different levels of Stinging nettle and Aloe vera treatment.

The highest level of ALT was found 124.5 \pm 41.93 in T₁ group on 20th day i.e. basal diet with nettle 5% in feed followed by 119 \pm 1.73 in T₀ group on 10th day i.e. basal diet with no nettle and Aloe vera and the lowest value was 58.25 \pm 42.16 in T₂ group on 40th day i.e. basal diet with Aloe vera @ 5 ml /lit drinking water/day. The biochemical assessment of liver enzyme revealed Alanine Aminotransferase (ALT) which was non significant at level of ($p > 0.05$), thus indicating treatment to be not effective for the liver function.

3.7. Effect on blood glucose

The table shows the effect of blood glucose levels (mg/dl) in the serum of broiler chickens with non significant differences throughout the observation period. The highest level of blood glucose was found in T1 group on 30th days and the lowest value was observed in T1 group on 0th day. The result on the blood glucose levels (Table 10) showed non significant differences among the treatment groups as compared to the control group throughout the observation period so it can be regarded as the nettle and Aloe vera is having no effect on blood glucose level.

3.8. Histopathology of bursa and spleen

The bursa and spleen appeared normal in all group. In bursa cortex and medulla were well filled with lymphoid cells and were clearly distinguishable (Fig. 1). There was scant interfollicular space and the

Table 6
Effect of stinging nettle and Aloe vera on total protein levels (Mean \pm SD) in serum at different days of broilers.

Treatment	Days (Mean \pm SD)				
	0	10	20	30	40
T ₀	6 \pm 1.41	3.22 \pm 0.53	4.07 \pm 0.09	4.17 \pm 0.35	3.97 \pm 0.35
T ₁	4.5 \pm 0.70	3.82 \pm 0.51	3.9 \pm 0.21	4.22 \pm 0.05	4.22 \pm 0.17
T ₂	4 \pm 0.17	3.15 \pm 1.17	4 \pm 0.23	4.02 \pm 0.35	4.2 \pm 0.18
T ₃	3.4 \pm 1.50	3 \pm 1.46	4.1 \pm 0.14	4.25 \pm 0.17	4.2 \pm 0.23
p-value	0.03 ^s	0.67 ^{ns}	0.45 ^{ns}	0.63 ^{ns}	0.45 ^{ns}

Means in column with different superscripts are significantly different. ^ssignificant at 5% ($p < 0.05$); ^{ns}non significantly different.

bursal epithelium was very regular and few cells thick (Fig. 2). Dark staining cortex was filled mainly with many small lymphocytes and the paler medulla with fewer cells of various sizes (Fig. 1). There was no any difference in lesion score in any treatment groups.

3.9. Morbidity and mortality during experiment

The mortality occurred in control group, one bird died on 10th and the other one on 20th day of age after sample collection from control group. Clinical signs were not observed prior to mortality as well as morbidity was not seen in any of the group. Dead birds do not show any post mortem lesions.

4. Discussion

The research was designed to evaluate the immunomodulatory effect of Stinging nettle (*Urtica dioica*) and Aloe vera (*Aloe barbadensis*). There are many parameters through which we can measure immunity. HI titres, globulin levels are the indicators of the humoral immunity (Chauhan, 1999); spleen, and bursa weight to body weight ratio as the broad indicators of nonspecific immunity (Chauhan & Singh, 2001). The hemagglutination inhibition (HI) antibody titre is most commonly used serological test and exclusive measure for determining immunological status of chicken against viral infections of family paramyxoviridae, mainly Newcastle Disease Virus (Giambrone, 1981; Spanoghe, Peeters, Cotlear, Devos, & Viaene, 1977). All of these parameters are important and thus have been studied in this research and discussed hereby. The research also comprised of histopathological study of bursa and spleen as well as effect of Stinging nettle and Aloe vera on blood glucose levels.

Throughout the experiment, the value for HI titres, albumin, globulin, total protein, spleen body weight ratio score, bursa body weight ratio score, liver function test (ALT) and blood glucose of all treatment group were non significant ($p > 0.05$) except for albumin on 10th day ($p < 0.05$), globulin on 0 day ($p \leq 0.05$), total protein on 0 day ($p < 0.05$) and spleen body weight ratio score on 30 days ($p < 0.5$) were found to be significant differences among the treatment group as compared to control group.

The result of antibody titre against ND showed non significant ($p > 0.05$) differences among the treatment groups as compared to the control group throughout the observation period. So, it can be regarded

as the nettle and Aloe vera having no effect on antibody titre against ND. According to Golshan (2015) who has done research on Evaluation of Nettle (*Urtica dioica*) and Ginger (*Zingiber officinale*) powder on Serum Antioxidants and Immune responses of Broiler Chicks. He found no significant difference in antibody titer against Newcastle and Influenza viruses ($P > 0.05$). Therefore, nettle or ginger cannot be used as an immunomodulator but can improve serum antioxidant capacity of broiler chicks. Similarly, Nasiri, Nobakht, and Safamehr (2011) applied different levels of Nettle (*Urtica dioica*) on various parameters including immunity parameters of broilers and reported experimental diets did not have any significant effects on immunity parameters of broilers which is similar to the present study on nettle. Fallah (2015) done research on Effects of supplementing Aloe vera gel and garlic powder on blood biochemical parameters in immune response of broilers stated that there was no significance difference between the treatment and control groups in antibody titres against ND which support the present study of Nettle and Aloe vera.

The result of albumin levels (Table 8) showed non significant ($p > 0.05$) differences among the treatment groups as compared to the control group throughout the observation period except on 10 days which showed significant ($p < 0.05$). Total protein of birds were not influenced by different levels of nettle when total of 300 (Ross-308) broilers were randomly allocated into one of the following dietary treatments i.e. control, basal diet; basal diet with 0.5% nettle; basal diet with 1% nettle; basal diet with 2% nettle (Safamehr, Mirahmadi, & Nobakht, 2012). According to Singh, Koley, Chandrakar, and Pagrut (2013) who has done research on Effects of Aloe vera on dressing percentage and haemato-biochemical parameters of broiler chickens showed that no significant result was obtained in the levels of albumin amongst all the groups which match the current research of Aloe vera.

Similarly, the result of globulin levels showed non significant except on 0 day which show significant ($p \leq 0.05$) differences among the treatment groups as compared to the control group throughout the observation period so it can be regarded as the nettle and Aloe vera is having no effect on globulin level. Value was highest on 0th day in all treatment and control group but on 10th, 20th, 30th and 40th days value was almost similar. Total protein of birds were not influenced by different levels of nettle when total of 300 (Ross-308) broilers were randomly allocated into one of the following dietary treatments i.e. control, basal diet; basal diet with 0.5% nettle; basal diet with 1%

Table 7
Effect of stinging nettle and Aloe vera feeding on spleen weight ratio score (Mean \pm SD) at different days of age of broilers.

Treatment	Days (Mean \pm SD)				
	0	10	20	30	40
T ₀	0.32 \pm 0.12	0.51 \pm 0.19	0.48 \pm 0.12	0.05 \pm 0.01	0.11 \pm 0.03
T ₁	0.60 \pm 0.18	0.75 \pm 0.31	0.51 \pm 0.06	0.08 \pm 0.01	0.09 \pm 0.03
T ₂	0.61 \pm 0.09	0.79 \pm 0.18	0.64 \pm 0.03	0.06 \pm 0.00	0.10 \pm 0.00
T ₃	0.76 \pm 0.51	0.91 \pm 0.42	0.61 \pm 0.15	0.08 \pm 0.02	0.07 \pm 0.01
p-value	0.22 ^{ns}	0.33 ^{ns}	0.13 ^{ns}	0.02 ^s	0.21 ^{ns}

Means in column with different superscripts are significantly different. ^ssignificant at 5% ($p < 0.05$); ^{ns}non significantly different.

Table 8Effect of stinging nettle and Aloe vera feeding on bursa weight ratio score (Mean \pm SD) at different days of age of broilers.

Treatment	Days (Mean \pm SD)				
	0	10	20	30	40
T ₀	1.14 \pm 0.58	1.99 \pm 0.07	2.20 \pm 0.60	0.23 \pm 0.04	0.16 \pm 0.02
T ₁	1.33 \pm 0.06	1.76 \pm 0.49	2.15 \pm 0.46	0.21 \pm 0.08	0.17 \pm 0.03
T ₂	1.15 \pm 0.28	1.93 \pm 0.16	2.11 \pm 0.29	0.17 \pm 0.01	0.20 \pm 0.03
T ₃	1.15 \pm 0.39	1.69 \pm 0.26	1.62 \pm 0.42	0.25 \pm 0.03	0.19 \pm 0.03
p-value	0.87 ^{ns}	0.45 ^{ns}	0.29 ^{ns}	0.18 ^{ns}	0.25 ^{ns}

Means in column with different superscripts are significantly different. ^ssignificant at 5% ($p < 0.05$); ^{ns}non significantly different.

nettles; basal diet with 2% nettle (Safamehr et al., 2012). According to Singh et al. (2013) who had done research on Effects of Aloe vera on dressing percentage and haemato-biochemical parameters of broiler chickens showed no significant result was obtained in the levels of globulin amongst all the groups which match the current research of Aloe vera. Similarly, according to (Yadav, 2009) who has done research on Effect of Aloe vera on immunomodulation, liver function, blood glucose and performance of broiler chickens showed significant difference which do not match the current research it may be due to sample size, difference in doses of Aloe vera.

The result of total protein levels (Table 6) showed non significant differences except on 0 day which show significant ($p < 0.05$) among the treatment groups as compared to the control group throughout the observation period. So, it can be regarded as the nettle and Aloe vera is having no effect on total protein level. Total protein of birds were not influenced by different levels of nettle when total of 300 (Ross-308) broilers were randomly allocated into one of the following dietary treatments i.e. control, basal diet; basal diet with 0.5% nettle; basal diet with 1% nettle; basal diet with 2% nettle (Safamehr et al., 2012). According to Singh et al. (2013) who had done research on Effects of Aloe vera on dressing percentage and haemato-biochemical parameters of broiler chickens showed no significant result was obtained in the levels of total protein, albumin, globulin, A/G ratio amongst all the groups which match the current research.

The spleen body weight ratios were found to be significant ($p < 0.5$) at 30 days in all treatment compared to control group but non significant were noted on 0th, 10th, 20th and 40th day respectively. Similarly, non significance was shown by ratio score of bursa body weight. The result shows that ratio score of spleen in control group was below than treatment groups. Similarly, score value is highest at 10th days in all control and treatment groups then it decline in 20th and 30th day and again from 30th day onward it slightly increases except T₃ and the result shows that ratio score of bursa in which control group was 1.142 \pm 0.584 on 0th day then it increases in 10th and 20th days and from 30th days onward value start to decline. Similarly in T₁, T₂ and T₃ groups mean value was 1.333 \pm 0.065, 1.156 \pm 0.283 and 1.150 \pm 0.393 at 0th day then it start to increases in 10th and 20th days again start to decline. There are many reports of either no effect on the weight of lymphoid organ (Augustine & Thomas, 1979) or an increased weight of lymphoid organs (Venkatratnam, Reddy, & Hafeez, 1985) due to coccidial infection/ vaccination.

Table 9Effect of stinging nettle and Aloe vera feeding on ALT levels (Mean \pm SD) in serum at different days of broilers.

Treatment	Days (Mean \pm SD)				
	0	10	20	30	40
T ₀	110 \pm	119 \pm 1.73	117.5 \pm 37.74	81.5 \pm 41.93	109 \pm 4.24
T ₁	106 \pm	110 \pm 14.14	124.5 \pm 41.93	107.33 \pm 24.02	62.66 \pm 24.44
T ₂	100 \pm	73.75 \pm 32.23	90 \pm 42	70.5 \pm 46.85	58.25 \pm 42.16
T ₃	110 \pm	102.66 \pm 47.93	101.33 \pm 42.01	77.75 \pm 38.87	88 \pm 33.94
p-value	NA	0.21 ^{ns}	0.64 ^{ns}	0.58 ^{ns}	0.10 ^{ns}

Means in column with different superscripts are significantly different. ^ssignificant at 5% ($p < 0.05$); ^{ns}non significantly different.

Table 10
Effect of Stinging nettle and Aloe vera feeding on blood glucose levels (Mean \pm SD) in serum at different days of broilers.

Treatment	Days (mean \pm SD)				
	0	10	20	30	40
T ₀	NA	46.24 \pm 86.61	137.31 \pm 31.28	144.59 \pm 39.31	98.37 \pm 51.65
T ₁	13.81 \pm 19.53	32.39 \pm 81.01	82.00 \pm 82.08	153.00 \pm 33.04	115.08 \pm 27.84
T ₂	47.18 \pm 73.72	88.06 \pm 101.73	114.94 \pm 70.65	133.98 \pm 70.65	101.22 \pm 30.81
T ₃	NA	11.14 \pm 102.34 ^{ss}	83.22 \pm 72.48	127.45 \pm 37.82	83.74 \pm 51.15
p-value	NA	NA	0.60 ^{ns}	0.87 ^{ns}	0.77 ^{ns}

Means in column with different superscripts are significantly different. ^ssignificant at 5% ($p < 0.05$); ^{ns}non significantly different.

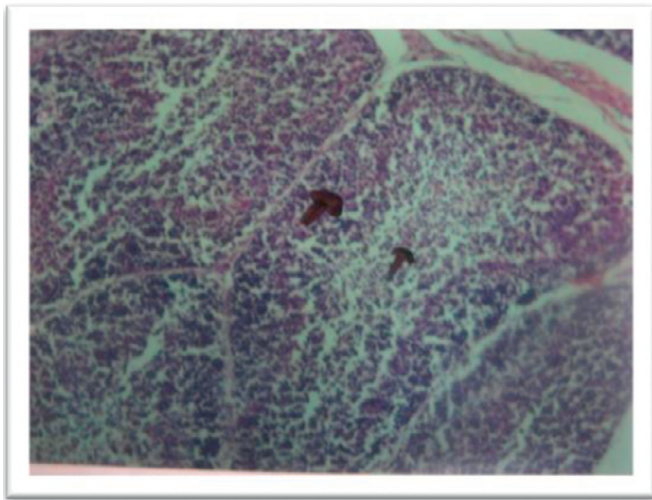


Fig. 1. Cortex (▬) and medulla (→) well filled with lymphoid cells and clearly distinguishable (H & E), 100X.

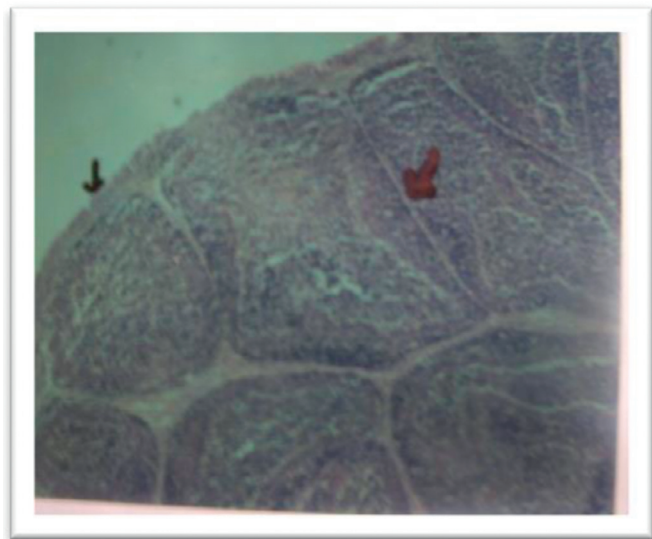


Fig. 2. Scant interfollicular space (▬) the bursal epithelium is regular and a few cells thick (→) and, (H&E) 40 X.

species, with difference in the parts of the plant used as well as the model of diabetes chosen; the present study are well supported by Okyar, Can, Akev, Baktir, and Sutupinar (2000). He conducted research on three main groups of rats, namely, non-diabetic (ND), type I (IDDM) and type II (NIDDM) diabetic rats were. Aloe vera leaf pulp and gel extracts do not lowered blood sugar level of ND rats but aloe vera leaf pulp extract showed hypoglycemic activity on IDDM and NIDDM rats. Also, reports have been published from human medicine. The claim oral

administration of Aloe vera might be useful adjunct for lowering blood glucose in diabetic patients and for reducing blood lipid levels in patients with hyperlipidemia. The use and effect of Aloe vera on Aloe vera treatment of wounds in diabetic rats has been reported to be due to hypoglycemic effects of aloe gel and it is indicated that may be due to its role influence on inflammation, fibroplasia, collagen synthesis and maturation, and wound contraction.

Assessed by histopathology, the bursa and spleen appeared normal in all group. In bursa cortex and medulla were well filled with lymphoid cells and were regularly distinguishable. There was scant interfollicular space and the bursal epithelium was very regular and few cells thick. Dark staining cortex was filled mainly with many small lymphocytes and the paler medulla with fewer cells of various sizes. The undifferentiated epithelial cells were cuboidal with an acidophilic cytoplasm clearly separated the medulla from cortex. There was no any difference in lesion score in any treatment groups.

Mortality occurred in the control group, one bird died on 10th and other on 20th days of age after sample collection from control group.

5. Conclusion

The result of our studies showed that birds feed with stinging nettle and Aloe vera have no detected immune response for both generalized cellular and humoral immunity which act against any opportunistic pathogens as well as specific immunity against New castle disease virus. It can be concluded that stinging nettle and Aloe vera having no toxic effect on the spleen and bursa which is also seen by no measured effects on liver enzymes. This study was done to assess the effect of stinging nettle and Aloe vera on liver of normal birds but further experiment should be done to determine whether these two plants have a protective effect on liver damaged by other infections or toxins.

Ethical approval

Approval for this research was granted from Nepal Veterinary Council (NVC) which is national statutory body of Nepal.

Conflict of interest

The authors have no conflict of interest regarding this work.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.vas.2018.07.002.

References

- Augustine, P. C., & Thomas, O. P. (1979). E. meleagrititis in young turkeys: Effect on weight, blood and organ parameters. *Avian Disease*, 23, 854–862.
- Bottenberg, M. M. (2007). Oral Aloe vera- induced hepatitis. *The Annals of Pharmacotherapy*, 41(10), 1740–1743.
- Chauhan, R. S., & Singh, G. S. (2001). Immunomodulation: An overview. *Journal of Immunology and Immunopathology*, 3(2), 1–15.
- Chauhan, R. S. (1999). *Illustrated veterinary pathology*. Luknow, U.P: International Book Distributing Company.
- ESCOP. (2003). *Urticae folium/ herbal*. (2nd ed.), 521–527.
- Fallah, R. (2015). Effect of adding aloe vera gel and garlic powder on performance and liver functions of broiler chickens. *Global Journal of Animal Scientific Research*, 3(2), 491–496.
- Giambone, J. J. (1981). An overview: Immunity against newcastle infections. *Poultry Science*, 2, 102.
- Golshan, M. R., Toghyani, M., & Gholamreza, G. (2015). Evaluation of nettle (*urtica dioica*) and ginger (*zingiber officinale*) powder on serum antioxidants and immune responses of broiler chicks. *Der Pharmacia Lettre*, 7(7), 411–415.
- Jagadeeswaran, A. (2007). *Exploration of growth promoting and immunomodulating potentials of indigenous drugs in broiler chicken immunized against Newcastle viral disease (Ph.D. thesis)* Chennai, India: Tamil Nadu Veterinary and Animal Sciences University.
- Keshavarz, M., Rezaeipour, V., & Asadzadeh, S. (2014). Growth performance blood metabolites, antioxidant stability and carcass characteristics of broiler chickens fed diets containing nettle (*Urtica dioica*. L) powder or essential oil. *International Journal of Advance Biological Biomedical Research*, 2(9), 2553–2561.
- Lee, J. K., Lee, M. K., Yun, Y. P., Kim, Y., Kim, J. S., & Lee, C. K. (2001). Acemannan purified from Aloe vera induces phenotypic and functional maturation of immature dendritic cells. *International Immunopharmacology*, 1, 1275–1284.
- Lerner, D. R., & Raikhel, N. V. (1992). The gene for stinging nettle lectin (*Urtica dioica*) agglutinin encodes both a lectin and a chitinase. *Journal of Biology Chemistry*, 267, 11085.
- Maharjan, H. R., & Nampoothiri, L. (2015). Evaluation of biological properties and clinical effectiveness of *Aloe vera*: A systematic review. *Journal of Traditional Complementary Medicine*, 5(1), 21–26.
- Nasiri, S., Nobakht, A., & Safamehr, A. R. (2011). *5th national conference on new ideas in agriculture, 16-17 February* (pp. 1–5).
- Okyar, A., Can, A., Akev, N., Baktir, G., & Sutupinar, N. (2000). *Effect of aloe vera leaves on blood glucose level in type i and type ii diabetic rat models* Istanbul, Turkey: Department of Biochemistry, Istanbul University Report.
- Rechinger, K. H. (1963). *Flora iranica: flora des iranischen hochlandes und der umrahmenden gebirge* (1st Ed.). Verlagsanstalt, Graz, Austria: Akademische Druck University.
- Safamehr, A., Mirahmadi, M., & Nobakht, A. (2012). Effect of nettle (*Urtica dioica*) medicinal plant on growth performance, immune responses, and serum biochemical parameters of broiler chickens. *International Research Journal of Applied and Basic Sciences*, 3(4), 721–728.
- Singh, J., Koley, K. M., Chandrakar, K., & Pagrut, N. S. (2013). Effects of aloe vera on dressing percentage and haemato-biochemical parameters of broiler chickens. *Veterinary World*, 6(10), 803–806.
- Spanoghe, L., Peeters, J. E., Cotlear, J. C., Devos, A. H., & Viaene, N. (1977). Infectious bursal disease infections. *Avian Pathology*, 6, 101.
- Sydiskis, R. J. (1991). Inactivation of enveloped viruses by anthraquinones extracted from plants. *Antimicrobial Agents and Chemotherapy*, 35(12), 2463–2466.
- Vander, Z. A. J. (1983). Breeding for immune responsiveness and disease resistance. *World's Poultry Science Journal*, 39, 118–131.
- Venkatratnam, A., Reddy, K. R., & Hafeez, M. (1985). Caecal coccidiosis experimental transmission through cloaca and pathogenesis. *Cheiron*, 14, 95–97.
- Wagner, H., Willer, F., & Kreher, B. (1989). Biologically active compounds from the aqueous extract of *Urtica dioica*. *Plant Medicine*, 55, 452–454.
- Yadav, D. K. (2009). *Effect of aloe vera on immunomodulation, liver function, blood glucose and performance of broiler chickens* (Unpublished M.V.Sc thesis). Rampur, Nepal: Institute of Agriculture and Animal Science.