Immunomodulatory effects of levamisole hydrochloride and *Nigella sativa* against infectious bursal disease (IBD) in chicks

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Accepted 31 May, 2016

**ABSTRACT**

Infectious bursal disease (IBD, Gumboro) is a viral disease of poultry well known all over the world. Failure of IBD vaccine after vaccination as well as the immunosuppressant effect represented a major risk for poultry producers. The effects of two immunomodulatory agents namely levamisole hydrochloride and *Nigella sativa* ground seeds were *in vivo* assessed in broiler and layer chicks through two different experiments. Each experiment has 100 chicks that was divided into four subgroups each with 25 chicks and treated as follows: Group I received *N. sativa* ground seeds in the recommended dose as 1.5 g/L of drinking water; Group II received a mixture of Levamisole hydrochloride *N. sativa* ground seeds (1.5 g/L) each; Group (III) received Levamisole hydrochloride and Group (IV) serve as controls. Broiler chicks showed significant effect of treatments during the 4th week (P < 0.01) and 5th week (< 0.05). *N. sativa* reported a significantly high mean of titer (6146.78). Body weight of broiler chicks of *N. sativa* treatment obtained a significantly higher mean of body weight. For layer chicks, the result of both levamisole and *N. sativa* during the 2nd week. had a significant higher mean titer (7033.56 and 7746.89, respectively). It was observed that the total leukocyte count of all groups were higher after treatment than before (46.63 and average of 52.77, respectively). The biochemical measurements for layer chicks showed that *N. sativa* group had a higher level of serum globulin. Histopathological examinations after challenging the chicks revealed a partial shedding of the epithelial layers, inflammatory cells in connective tissue and depletion of lymphocytes in lymphoid follicles. In conclusion, levamisole hydrochloride and *N. sativa* have an immunomodulatory effect through improvement of the immune system hence supporting the birds against pathogens that causing immune suppression such as IBD.

**Keywords:** *Nigella sativa*, levamisole hydrochloride, immune system, Infectious bursal disease.

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**INTRODUCTION**

Infectious bursal disease (IBD, Gumboro) is a highly contagious viral disease of young chickens. The age of great susceptibility is between 3 and 6 weeks. The recognition of the specific disease entity affecting the bursa of fabricius of chicken was reported by Cosgrove in 1962, in Gumboro, Delaware, USA. The disease is often referred to as Gumboro because it was first diagnosed in this city (Winterfield and Hitchner, 1962). The first outbreak of IBD in the Sudan was observed in Elobied (North Kordofan State) in 1981 with pronounced mortality that toll up to 36% (Shaubib et al., 1982). Since then the disease has been reported in many parts of the Sudan and became a serious problem facing the poultry industries (Hajer et al., 1987). Gaffer et al. (1987) found the disease in Kassala, and in Nyala 1986 with a mortality up to 50%. Thereafter it became endemic whenever massive poultry production is practiced in the country. Shaubib et al. (1982), Salman et al. (1983), Mahasin (1998) and El Hassen et al. (1998) observed some seasonal cases in Northern Sudan. The target
organ of IBDV is the bursa of fabricius when B lymphocytes mature in avian species and the severity of the disease is directly related to the number of susceptible cells present. Initially infectious bursal disease virus (IBDV) was classified as Picorna virus (Lunger and Madux, 1972), then as a Reovirus (Pelex and Mandelli, 1968; Koster et al., 1972). Lastly the IBDV was regarded as a member of Birnaviridae family in the avibirnavirus genus. The virus is non enveloped, icosahedral symmetry with a diameter of 55 to 65 nm. All the strains of this virus share a group antigen that can be detected with enzyme-linked immunosorbent assay test (ELISA) and can be serotyped into serotypes I and II with the neutralization test (Lukert and Saif, 1991).

The economic impact of IBD is influenced by the strain of the virus, susceptibility of flocks under current primary and secondary environmental, mange mental factors, flock livability, weight gain, conversion and reproductive efficiency (Shane et al., 1994). In addition to high mortality rate and the immunosuppressant effects of IBD, it also affects the growth rate, feed conversion rate in broiler flocks. Eradication in affected countries thus seems unrealistic and prevention of IBD necessitates both hygienic measures and medical prophylaxis. Indeed, no vaccine can solve the problem if major sanitary precautions are not taken. These include ‘all-in/all-out’ farming methods, cleaning and disinfection of premises and observance of a ‘down time’ period. The virus is heterogenic and this heterogeneity results in frequent outbreaks in the flied even in vaccinated flocks (Hassan et al., 1998).

The control of the disease in Sudan now depends on vaccination. Despite the regular use of these vaccines and the different vaccination practices, the disease still prevails. The resulting immunosuppressant after infection has influence on the feed conversion, growth rate and egg production. Hence, they increase the susceptibility of the birds to other diseases (Shane et al, 1994). The effect of this immunosuppressant can be adjusted by certain compounds that enhance the immune system of birds (Renoux and Renoux, 1971). These immunomodulators are Levamisole hydrochloride and Nigella sativa. Levamisole hydrochloride has an antiviral role and was used to improve the immune response of birds under stress (Renoux and Renoux, 1971). In the current proposal, levamisole was used to enhance the chicken to be protected against Gumboro. This is because Levamisole is known to restore cell mediated immune response in peripheral T-lymphocytes and stimulate phagocytosis by monocytes (Mikata and Donland, 2003-2006). N. sativa has been used for medical purposes for centuries; an active ingredient thymoqinone acts as an immune booster in HIV, it has an immune enhancing effect of human T-cell production (Stern, 2000). Soliman et al. (1999) studied the synergistic effect of feeding black seed and garlic on broilers performance and immunity. These results showed that using 0.3% black seed in broiler diet improved the development of immunity.

MATERIALS AND METHODS

Experimental chicks

Experiment 1

One hundred broilers chicks at one day old of Cobb breed were purchased from Fakieh Company in Bisha town, Saudi Arabia. The chicks were divided into four subgroups of 25 chicks each. For the system of housing, floor was used and separated into four partitions.

Experiment 2

One hundred layer chicks at one day old of White High sex breed were purchased from Coral Company in Sudan. The chicks were divided into four subgroups of 25 chicks each. For the system of housing, four cages were used, each measured 2 m × 1 m. The cages were cleaned and disinfected before the introduction of the chicks.

In both experiments each cage was supplied with one feeder and one drinker. The temperature, light and ventilation were adjusted to optimum according to age. The chicks were fed and supplied with water ad libitum.

Experimental design

For both experiments the groups were treated as follows:

Group I: 25 chicks received N. sativa ground seeds in the recommended dose as 1.5 g/L of drinking water from 1 to 35 days of age.

Group II: 25 chicks received a mixture of Levamisole hydrochloride and N. sativa ground seeds in the same dose daily starting the 12th of age and continued up to the 16th day.

Group III: 25 chicks received Levamisole hydrochloride at the 12th and 16th day.

Group IV: 25 chicks served as controls.

Sera collection

The chicks were bled via heart puncture (easy and safer than wing and jugular veins) on the 7th, 14th, 21th, 28th and the 35th day; while for the broiler chicks the bleeding started at two weeks post administration of the treatment. Then the blood samples were left at room temperature for 1 to 2 h and sera were collected in sterile container, placed at 4°C till tested by the ELISA kit. The IBD ELISA kit measured the amount of antibody to IBD in the serum of chicks. Microtitre plates had been pre-coated with inactivated IBD antigen. Chicken serum sample was diluted and added to microtiter well where any anti IBD antibodies present will bind and form antigen – antibody complex. Non-specific antibodies and other serum proteins are then washed away. Anti-chicken IgG labelled with the enzyme alkaline phosphatase is then added to the wells and binds to any chicken anti-IBD antibodies bound to the antigen. After another wash to remove unreacted conjugate, substrate is added in the form of pNPP chromogen. A yellow color is developed if anti-IBD antibody is present and the intensity is directly related to the amount of anti-IBD antibody present in the sample.

Challenging

After 21 and 30 days of the experiments, layer treated chicks were challenged to test were any immunity to two types of challenging viruses were used, the live local field isolate and a live attenuated
challenged CEVAC IBDV was against hyper virulent form of Infectious Bursal Disease in poultry was also been used in the challenge.

Pilot test

Preliminary pilot test was performed in 27 chicks at 21 days old and were divided into two subgroups each of 10 birds while control consisted of 7 chicks. Group 1 each of 10 birds received 0.1 ml of 1/10 concentration of the local homogenate bursa intra nasally. Group 2 was challenged with 1/100 of local IBD virus, Group 3 was left uninoculated. Observations were conducted for 9 days post inoculation for any changes. Secondly after 30 days of the experiment the rest layer treated chicks were challenged with a live attenuated challenged CEVAC IBDV. Observations were conducted for 9 days post inoculation for any changes.

All chicks were subjected to the following: The level of antibody to IBDV in chicken serum, recording, mortality, clinical signs, postmortem finding and percentage bursa weight to spleen weight before and after infection. Histopathological finding after challenging whole blood with EDTA for TLC before and after infection for differential leucocytes cell. Blood serum for biochemical test (total protein).

Statistical analysis

Statistical analysis was carried out by using SPSS version11.5 software (the main result the level of antibody to IBDV in chicken serum). Of one way analysis of variance, Tukey’s multiple range test (HSD). Duncan’s New Multiple Range Test (DNMRT) is a multiple comparison procedure developed by David B. Duncan in 1955. Duncan’s MRT belongs to the general class of multiple comparison procedures that used the student zed range statistic qr to compare sets of means, was used to test significance between different treatments at P < 0.01 and P < 0.05.

RESULTS

Antibody titers of broiler chicks were significantly affected by the immunomodulatory supplementation only during the 1st week (P<0.01) and 5th week (P<0.05), whereas during both the 2nd and 3rd week no significant effect on antibody level was detected. On the 4th week N. sativa (L.H) reported a significantly higher mean of titer (6146.78) compared to all other treatments (control, levamisole (L.H) and mixture (L.H. and N.S) and no significant differences was detected between treatment (Table 1).

As for the layer chicks, level of antibodies titer was significantly affected by the immunomodulatory supplementation during the 2nd week (P < 0.05) but not during the rest period (1st, 3rd, 4th and 5th week). Both levamisole hydrochloride and N. sativa during 2nd week had significantly higher mean of titer (7033.56 and 7746.89) than mixture, whereas these treatments (L.H, N.S. and mixture) showed no significant difference with control.

Morbidity and mortality among challenged layer chicks

Morbidity after challenging the layer chicks was significantly (p < 0.05) influenced by treatment. G3 exhibited the least morbidity and G2 exhibited the higher morbidity (Table 2). The mortality rates were significantly (p < 0.05) influenced by treatment. G3 showed no mortality however it was not different (p > 0.05) from G1. 80% of the challenged chicks of G2 died (Table 2).

Differential leucocyte cell

For differential leucocyte cell (DLC), it was observed that the total leucocyte count of all groups were higher after the treatments than before (an average of 52.77 and 46.63, respectively) (Table 4).

Effect of treatments on layers biochemical measurements

Treatments significantly (P < 0.01) affected the level of protein for the infected layer chicks, whereas the condition and the interaction did not significantly affect this parameter (Table 4).

Clinical sign after challenge

There was no clinical signs and mortality seen in pilot test (Table 3). The study showed that after challenge with the CEVC, challenged layer exhibited anorexia, depression, profuse and diarrhea sign and at 33 days old anorexia and depression still existed among the stock (Figure 1). At 35 days old, sign of diarrhea and lost appetite was observed, while by the next day chicks started to eat. This clinical finding usually occurs in the chicken. The clinical disease has a sudden onset. Clinical signs of disease include dehydration, trembling, ruffled feathers, vent pecking, and depression. Affected chickens experience transient and depression.

Postmortem finding

In postmortem changes in the study looks at third day post infection, thigh linear hemorrhage, swollen, edematous, hemorrhagic B.F. and for 2 day after BF hemorrhagic later become pale then after 5th day P.I. there is rudimentary bursa this agrees with Gray and Richard (1995) (Figure 2). Initially, the BF is swollen (inflamed), appears edematous and hyperemic, and has a gelatious yellowish transudate covering the serosal membrane. Hemorrhage and area of necrosis may be present in more severe cases. Five day after infection, the BF diminishes in the size rapidly (atrophies).

Meanwhile, treated chicks that received N. sativa reported 1/0.3 percentage bursa at 33 days and 0.6/0.7 and 0.4/0.5 at 34 and 35 days old, respectively, whereas treated chicks that received mixture (L.A and N.S) at 33, 34, and 35 days old reported 0.9/0.3, 1.0/0.8 and 1.0/0.8
Table 1. Level of antibodies titer for broiler and layers chicks as affected by immunomodulatory (L.H and N.S).

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Titer of broiler chicks</th>
<th>Titer of layer chicks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2^nd wk.</td>
<td>3^rd wk.</td>
</tr>
<tr>
<td>Group 4 (control)</td>
<td>489.44 a</td>
<td>196.89 a</td>
</tr>
<tr>
<td>Group 3 (LH)</td>
<td>392.44 a</td>
<td>135.44 a</td>
</tr>
<tr>
<td>Group 1 (N.S)</td>
<td>893.56 a</td>
<td>212.67 a</td>
</tr>
<tr>
<td>Group 2 (N.S + L.H) mixture</td>
<td>721.33 a</td>
<td>184.44 a</td>
</tr>
<tr>
<td>S.E ±</td>
<td>177.31</td>
<td>33.06</td>
</tr>
</tbody>
</table>

*Means within same column which having similar letters are not significantly different at 0.05 level of probability according to Duncan’s New Multiple Range Test (DNMRT).

Table 2. Overall morbidity and mortality of the challenged layer chicks.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>No. of chicks treated</th>
<th>Morbidity rate (%)</th>
<th>Mortality rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (N.S)</td>
<td>25</td>
<td>13 (52.0) a</td>
<td>2 (8.00) a b</td>
</tr>
<tr>
<td>Group 2 (N.S + L.H)</td>
<td>25</td>
<td>20 (80.0) b</td>
<td>6 (24.0) a</td>
</tr>
<tr>
<td>Group 3 (LH)</td>
<td>25</td>
<td>5 (20.0) c</td>
<td>0 (0.00) b</td>
</tr>
<tr>
<td>Group 4 control</td>
<td>25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>38 (38.0)</td>
<td>8 (8.00)</td>
</tr>
</tbody>
</table>

*The chicks were challenged with hot vaccine on day 30 of the treatment. NS means Nigella sativa and LH means levamisole hydrochloride. *Values with different superscripts in the same column differ at p < 0.05.

Table 3. Pilot test for evaluated signs and mortality (group 1 and group 2).

<table>
<thead>
<tr>
<th>Day P. 1</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical signs</td>
<td>No signs seen</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>Mortality</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

No clinical signs and mortality seen.

percentage bursa, respectively. On the other hand, percentage bursa was 1.2/0.6, 0.7/0.9 and 0.7/0.8 at 33, 34 and 35 days old for treated chicks received levamisole was 0.8/-, 1.0/- and 0.5/- for the same period of control.

The study therefore showed the immune organs bursa/spleen weight. The percentage of N.S treated chicks and levamisole treated chicks were 1/0.3 and 1.2/0.6 g respectively which were the highest in comparison with the lowest recorded in mixture and control that are 0.9/0.3 and 0.8/0 g, respectively at 33 days old. They regressed the following day which means that bursa was rudimented. Black seeds and levamisole powder groups showed an elevated percentage of the organs B/S in comparison to other group. This indicates that N. sativa and levamisole had an immunostimulating effect against IBD infection.
Table 4. Mean differential leucocytes cell (D.L.C) infected layers as affected by treatments.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total leukocytes</td>
<td>Control</td>
<td>46.63 ± 2.3</td>
<td>47.26 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>Nigella</td>
<td>50.31 ± 4.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Levamisole</td>
<td>64.03 ± 2.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mixture</td>
<td>49.05 ± 3.4</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>Control</td>
<td>37.84 ± 1.7</td>
<td>37.02 ± 2.1b</td>
</tr>
<tr>
<td></td>
<td>Nigella</td>
<td>41.45 ± 2.6b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Levamisole</td>
<td>52.33 ± 2.3a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mixture</td>
<td>38.33 ± 2.7b</td>
<td></td>
</tr>
<tr>
<td>Neutrophils</td>
<td>Control</td>
<td>59.66 ± 2.2</td>
<td>42.65 ± 3.1b</td>
</tr>
<tr>
<td></td>
<td>Nigella</td>
<td>57.75 ± 2.26b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Levamisole</td>
<td>61.72 ± 2.7a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mixture</td>
<td>43.58 ± 3.98b</td>
<td></td>
</tr>
<tr>
<td>Eosinophils</td>
<td>Control</td>
<td>0.91 ± 0.1</td>
<td>1.0 ± 0.11b</td>
</tr>
<tr>
<td></td>
<td>Nigella</td>
<td>1.37 ± 0.3ab</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Levamisole</td>
<td>2.23 ± 0.49a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mixture</td>
<td>1.01 ± 0.2b</td>
<td></td>
</tr>
<tr>
<td>Monocytes</td>
<td>Control</td>
<td>1.08 ± 0.2</td>
<td>0.81 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Nigella</td>
<td>1.08 ± 0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Levamisole</td>
<td>1.58 ± 0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mixture</td>
<td>0.91 ± 0.2</td>
<td></td>
</tr>
</tbody>
</table>

ab means within same column with different letters differ (P < 0.05).

Table 5. Mean square values of the treatments on biochemical test of layers.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f</th>
<th>Globulin</th>
<th>Albumin</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments (T)</td>
<td>3</td>
<td>0.400 **</td>
<td>0.019 *s</td>
<td>0.399 **</td>
</tr>
<tr>
<td>Condition (C)</td>
<td>1</td>
<td>0.027 *s</td>
<td>0.054 *s</td>
<td>0.101 *ns</td>
</tr>
<tr>
<td>TXC (interaction)</td>
<td>3</td>
<td>0.012 *s</td>
<td>0.026 *s</td>
<td>0.004 *ns</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>0.053</td>
<td>0.024</td>
<td>0.039</td>
</tr>
<tr>
<td>C.V (%)</td>
<td>6.81</td>
<td>4.64</td>
<td>2.95</td>
<td></td>
</tr>
</tbody>
</table>

Histopathological finding

The histopathological examination of Hematoxylin and Eosin stained sections of bursa and spleen from the different group treatments. Group one (N. sativa) shows sloughing of the epithelial layer, hemorrhage, congestion and inflammatory cells and depletion of lymphocytes, thickening of arteries, lymphocyte replaced by inflammatory cells and degeneration, depletion of lymphocytes (Figure 3). Group two (N. sativa and levamisole) shows cysts formed and inflammatory cells in connective tissues also degeneration, necrosis, congestion and hemorrhage. Group three (levamisole) sloughing of the epithelial layers of bursal villi also and inflammatory cells in connective tissue lymphocytes and depletion of lymphocyte in lymphoid follicle. Group four shows normal pseudostratified columnar epithelium, follicle, cortex, medulla and septum. The bursa shows inflammation over 80% of the distal areas. The lumen is filled with blood and shedding of mucosa. Edema and necrosis are seen in mucosa and submucosal areas. There are increased numbers of inflammatory cells (eosinophils, lymphocytes, monocytes and plasma cells). Most of the mucosa is destroyed. Regeneration of the epithelium and lumen is complete by the 10th day in light infection.
Figure 1. Layer chicks received Nigella at 33 days old. The clinical sign is depression.

Figure 2. Swollen, hemorrhagic of bursa of fabricius in layer chicks at 34 days old.

Figure 3. Section in the bursa of layer chicks challenged with IBDV Showing sloughing of the epithelial layer (red arrow), hemorrhage, congestion and inflammatory cells (back arrow) (H&E X200).
DISCUSSION

Infectious bursal disease (IBD) is of high economic effect due to the high mortality rate and the immunosuppressant effects which reduce the growth rate, feed conversion efficiency in broiler flocks. For the control of the disease vaccines are usually used, despite that the disease still prevails in Sudan. The immunosuppressant could be modulated by certain compounds that enhance the immune system of birds (Renoux and Renoux, 1971). Some of these immunomodulators are Levamisole hydrochloride and *N. sativa*. This study investigated the possibility of immunomodulatory or immunotherapeutic effects of Levamisole hydrochloride and/or *N. sativa* against IBDV.

Two experiments were designed to investigate this; one of them was done in Saudi Arabia and the other in Sudan. In the first investigation, chicks without IBD infection and had only maternal immunity. Decline of immunity was noticed in the third week then it started to rise till five weeks of age. This may be due to enhancement of both humoral and cellular immune cells.

The results of the present experiment antibody titers of broiler chicks are in agreement with increase titer of antibody that carried out by Saima et al. (2014) in Pakistan using broiler chicks where titer increased from control which gave 470 in comparison to other treated groups where 873 and 1030 were reported also. These findings are in agreement with the findings of Soliman et al. (1999) who reported the significance of black seed on immune response of broiler. Similarly, Toghyaniat et al. (2010) and Khan et al. (2012) reported that adding black seed in the diet significantly improved the immune response of chicks (p < 0.05) but increased the weight of lymphoid organs, also in agreement with the findings of Osman and Barody (1999). Also, it supports the findings of Salem and Hussain (2000). Swamy and Tan (2000) found *N. sativa* oil to possess antiviral properties and immune potentiating activities.

The second investigation was carried out in Sudan by infecting layer chicks and finding out the level of antibody titers of challenged layer chicks. Also results of the present experiment were in agreement with the findings of Soppi et al. (1978). The present findings suggested that different concentrations of levamisole may affect different populations of lymphocytes because later during the experiment the mutagenic responses decreased and reached the starting level of day 3. The effect is probably mediated by the activation of the T-cell function and effects only antibody responses to thymus dependent antigen, these finding confirm the observation regarding the ability of levamisole to modulate immune-response.

Body weight of challenged layers reported a significantly higher mean of this character. Those results disagreed with those obtained by Abbas and Ahamed (2010) who reported that birds fed with diet supplemented with 1% or 2% black cumin showed significantly (p ≤ 0.05) low body weight. This also disagrees with those obtained by Soppi et al. (1978) who reported that levamisole had no effect on body weight. Investigation of whole blood with EDTA for total leukocyte count (TLC) before and after infection and for differential leukocyte cell (DLC) was carried out. Total and differential leukocyte counts are important indicators in investigating the immunomodulatory effects of the compounds (Doul et al., 1986). The investigations may provide information concerning the functional status of the immunocompetent cells to assess the status of the immune response of the chicks during the infection period. In the present study, it was observed that the total leukocyte count with the addition of levamisole hydrochloride and *N. sativa* group were higher than the control and mixture group. Alodan and Mashaly (1999) suggested that the leukocyte was reduced during the infection. The result showed that the infection suppressed the immune response and this indicated that the increase post infection increase the leukocyte count during infection may be attributed to the renewal in bursa or more addition of levamisole hydrochloride and *N. sativa* as reported by (Tengerdy, 1989; Scheideler and Fonning, 1996; Sünner and Flachowsky, 2001).

The levamisole hydrochloride and *N. sativa* in development of immune response in birds as stated by some investigator increased functional and proliferation of lymphocytes, neutrophil, eosinophil against the oxidative impairment in the cells. On the other hand, investigation of the effect of treatments on layers measurements of globulin content (g/dl), indicates that globulin of layer chicks was significantly affected by treatments (P<0.01) while infection between treatments did not significantly affected this character. *N. sativa* can be used as natural growth promoter in poultry diet due to its pharmacological properties and wide margin of safety; in addition to its positive effects on broilers, layers performance and health. These results are in agreement with those by Abbas and Ahamed (2010). In the study, significant effect on the control group and *N. sativa* treated group was observed by the serum globulin level of layer chicks. However, highest level of serum globulin was observed in control chicks. *N. sativa* had a higher level of serum globulin. These results disagreed with those obtained by Bhardwaj et al. (2011, 2012) who observed increased serum globulin level in levamisole treated chicks and decreased level in unvaccinated control birds. On other biochemical measurement albumin content (g/dl), statistical analysis revealed that neither treatments and their interaction significantly affected the level of the serum albumin among studied treatments were closely related to each other for control the level of albumin before infection was slightly higher as compare after infection condition but without significant. Protein content (g/dl) treatments significantly affected the level of protein for the infected layer chicks (P < 0.01), whereas the condition and the interaction did not
significantly affect this parameter by both control and *N. sativa* treatments, which showed no significant difference between them.

Protein level in the body is directly linked to the liver function and consumption of protein. The serum protein concentration in indicator of hydration showed a decline in total serum protein in infectious bursa disease (IBD) infected chicks. This might be attributed to inflammatory exudation of serum albumin into the bursa of fabricius. There was a reported change in total serum protein level after IBD infection. This study aimed to explain the effect of immunomodulatory and innate and resist the infection induced by virus.

The result of the study agrees with Karnataka et al. (1993), Chakraborty and Chatterjee (1998) and Kumari et al. (2011a, b, 2012a, b).

Mortality of challenged layer was higher (24%) for chicks treated with mixture (L.H and N.S), followed by chicks treated with N.S (8%), whereas those treated with L.H and control reported no mortality for all studied period.

Levamisole was able to enhance cellular immune response in these groups resulting in faster response. This study therefore agrees with Oladele et al. (2012) Levamisole hydrochloride (Lev. HCl) has been acclaimed to boost immune response particularly in immunocompromised state. Its routine use as an immunomodulatory in poultry production is yet to be well embraced, thus its effects on cellular immunity and flock performance of commercial broilers were evaluated. Mortality rates were 4.17 and 29.17% in 1A and 1C, respectively.

The study therefore agrees with Salem and Hussain (2000) and Al-Ankari (2005). The cumulative values of mortality rate showed that feeding of powdered back cumin (*N. sativa*) seeds to growing broilers has been show to lower mortality rate from 2 to 1%; the black cumin seed has been reported to improved immunity, and stimulate bone marrow and immune cells.

In the present study, the percentage bursa weight to spleen weight for layer chicks was studied. This study agrees with Kamal and Kassab (2009). Regarding the BW/BW the highest ratio (P = 0.05) recorded in BS group (0.22), this is due to its immunogenic effect as mentioned earlier, while in GP group the ratio was the lowest (P = 0.05; 0.13) when comparing the results of pathological indexes with the organ weights.

Histopathological examination of the BF identified the occurrence of IBD because very typical microscopic alterations are present after infection. Lymphocyte necrosis is the most common histopathological lesion and is accompanied by edema, hyperemia, accumulation of heterophilis and cystic cavities replaces lymphocytes in follicles and later there is some regeneration of lymphocytes. The intensity of microscopic alterations in the BF may also be quantified to evaluate the level of immune protection or immune modulation of the infection

The study therefore shows that the histopathological examination of bursa and spleen sections from the different groups revealed alteration of the epithelium, lymphocytes replaced by inflammatory cellular degeneration, necrosis among the different groups. Group 1 showed severe infection showing sloughing of the epithelial layer, hemorrhage and congestion and inflammatory cells, and lymphocytes replaced by inflammatory cells, degeneration and depletion of lymphocyte. Also Group 1 spleen showed depletion of lymphocytes and thickening of arties. In Group 2, bursa showed cysts formed and inflammatory cells in connective tissue, cells filled by inflammatory cells and debris, degeneration, necrosis, congestion and hemorrhage (Figure 4). In Group 3, bursa showed partial shedding of the epithelial layers, inflammatory cells in connective tissue and depletion of lymphocytes in lymphoid follicle (Figure 5). In Group 4, bursa histologically not only resembles a lymph gland with lymph follicles, but also contains pseudostratified columnar epithelial cells (Calhoun, 1933). In both growth and histological structure the bursa resembles the thymus and has been nicknamed the “cloacal thymus”.

This study therefore agrees with Mudhar et al. (2012) who mentioned the histopathological lesion scores of the vaccinated birds of different groups. The distribution of the observed lesions is variable within the different ages post-vaccination. They were characterized by atrophy and lymphocytes depletion. In the more severe lesions, follicular lymphoid necrosis, an inter-follicular interstitial fibrosis and degeneration of the coating epithelium, were notes. The severity of lesions was caused by intermediate-plus vaccine. Bursal lesion scores were surprisingly high in most sections but moderate one was recorded at 21st day. Depending on the virulence of the live attenuated viruses, some vaccine strains can cause bursal damage (Mazariegos et al., 1990) and lead to immunosuppression in the vaccinated birds (Edward et al., 1982; Reece et al., 1982). The highest bursal lesion scores with cyst formation (Tsukamoto et al., 1995), lymphocytic depletion with inflammation (Mazariegos et al., 1990), acute necrosis (Rautenschlein et al., 2001), follicular atrophy (Franciosini and Coletti, 2001), extensive bursal damage with follicular repopulation (Rautenschlein et al., 2001) and increased interstitial connective tissue proliferation (Franciosini and Coletti, 2001) were produced by intermediate-plus vaccine and less with intermediate type. The histopathological features and remarkably high score of bursal lesions in this study would evaluate the virus as undoubtedly pathogenic virus vaccine. However, the pathogenicity of virus vaccines are yet to be determined in a separate experiment and further experiment to evaluate it can be conducted.

It should be mentioned that challenging with the isolated field strain failed to develop any signs or histopathological changes. This could be an answer to
the effect of the used treatments and a prove that the treatments protected the chicks from infection. However more work is needed to establish a solid base for this hypothesis.

It can be concluded that levamisole hydrochloride and N. sativa were able to enhance cellular immune response in the treated chicks resulting in faster response and reported no mortality for all studied period. Also, this indicates an immunostimulanting effect against IBD infection.

ACKNOWLEDGEMENTS

The authors wish to thank Sudan Academy of Sciences and Animal Resources Research Corporation for their financial support and advice, also would like to My deepest thank is also goes to Dr. Abudl bagi Ahmad.
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Abdul bagi and Azmi Elhag for helping in the statistical analysis of this work.


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