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Full Length Research Paper

Immunologic effects of *Moringa oleifera* methanolic leaf extract in chickens infected with Newcastle disease virus (kudu 113) strain

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This study was aimed at evaluating the immune boosting potential of crude methanolic extract of *Moringa oleifera* in chickens experimentally challenged with Newcatle disease (ND) virus. One hundred and twenty forty two (42) day old chicks were randomly divided into four equal groups. Groups I and II were given daily oral treatment of methanolic extract of *M. oleifera* at 200 mg/kg body weight until day 56 of age. Groups II and III were vaccinated with the La Sota strain of ND vaccine. Group I was not vaccinated while group IV was left as untreated/unvaccinated control. All the groups were challenged with the velogenic strain of ND virus (VNDV) on day 56. Following challenge, the birds were assessed for cellular and humoral immune responses. Data on cellular and humoral immune responses were analysed using the statistical package for social sciences (SPSS). Increases in total and differential cell numbers and haemagglutination inhibition (HI) titre in the extract-treated groups did not correlate with total protection against ND. *M. oleifera* extract increased ND HI titre and the total and differential leukocyte counts in the treated and unvaccinated group I birds much more than those of treated and vaccinated group II birds, hence it could be recommended as a prophylactic treatment against ND in non vaccinated birds.

Key words: Velogenic Newcastle disease, chickens, *Moringa oleifera*, immunity.

INTRODUCTION

Newcastle disease (ND) is a serious threat to aviculturists and poultry industry worldwide. ND belongs to OIE listed diseases and is characterized as "a transmissible disease that has the potential for very rapid spread irrespective of national borders; a disease of serious socio-economic or public health consequence, and of major importance in the international trade of animals and animal products (OIE 2005; Facon et al., 2005)". Thus outbreaks of velogenic ND are characterized by high mortality, condemnation of other infected flocks, trade restrictions associated with quarantine and surveillance of affected areas within individual states where outbreaks have been detected (Talebi, 2006; Yongola et al., 2006). ND is caused by ND virus (NDV) which is an Avian Paramyxovirus type 1 (APMV-1) that belongs to the family Paramyxoviridae and genus *Avulavirus* (Aldous and Alexander, 2001; Alexander, 2003). The only effective means of preventing NDV is through vaccination commonly given by the oral, ocular and intranasal routes (Wambura, 2009). *Moringa oleifera* is the most widely

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cultivated species of the family Moringaceae. It is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. This rapidly growing tree (also known as the horseradish tree, drumstick tree, benzolive tree, or Ben oil tree), was utilized by the ancient Romans, Greeks and Egyptians. It is now widely cultivated and has become naturalized in many locations in the tropics. It is a perennial softwood tree, producing low quality, but which for centuries has been advocated for traditional medicinal and industrial uses (Anwar et al., 2007). All parts of the *M. oleifera* tree are edible and have long been consumed by humans. *M. oleifera* is said to be a natural anthelmintic, mild antibiotic, detoxifier and an outstanding immune builder and is used in many countries to treat malnutrition and malaria (Khesorn, 2009). It is also regarded by water purification experts as one of the best hopes for reducing the incidence of waterborne diseases. Moreover, there has been recently an increased interest in the utilization of *M. oleifera*, as a protein source for livestock (Sarwatt et al., 2002; Kakengi et al., 2007). It is therefore a multipurpose tree of significant economic importance with industrial and medicinal uses (Umar, 1998; Anwar et al., 2007). However, there is paucity of information on the use of the leaves as an immunomodulator, especially in reducing the mortality rate in chickens infected by NDV, and also as an adjuvant to vaccination. In this project, the effects of the leaf extract of *M. oleifera* on antibody responses to ND were evaluated. This study was therefore designed to investigate the effects of crude methanolic extract of *M. oleifera* in chickens experimentally challenged with velogenic Newcastle disease virus.

MATERIALS AND METHODS

Plant material

The green leaves of *M. oleifera* were collected during the months of March, and April at Ibagwa-Aka, Nsukka, Enugu State, Nigeria. The plant was identified at the Bioresources Development and Conservation Programme, Nsukka. Extraction of the dried leaves was performed by soaking in absolute methanol (98%) for 24 h at room temperature (28°C). The resulting extract was concentrated *in vacuo* and subsequently air dried in a shade. The extract was solubilized in 5% Tween 80. Phytochemical analyses of the extraxts were performed using standard methods (Evans, 2002).

Experimental animals

One hundred and twenty day-old White Harco cockerels procured from Zartec Ltd Ibadan, South west Nigeria were used for the study. The chicks were not vaccinated against any disease. The birds were housed in an isolated pen at the Poultry Disease Research Unit of the Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka. They were fed with commercial poultry feed *ad-libitum* and provided with drinking water.

Experimental procedure

The birds were randomly divided into four equal groups of 30 chicks each per group at 42 days of age. Group IV was isolated from the other groups. Groups I and II were treated orally with 200 mg/kg body weight of the extract daily for two weeks while other groups were not treated. Groups II and III chicks were vaccinated with La Sota® vaccine. Two weeks post treatment; birds in all the groups were inoculated intramuscularly with 0.2 ml challenge dose of VNDV strain (Kudu 113) with titre $10^{9.5}$ EID_{50} per milliliter of the inoculum. On days 42, 49, 56, 63, 70 and 77 of age, blood samples were collected from each group for serology and haematology. Sera from the blood samples were stored at -20°C until used.

Haemagglutination (HA) and heamagglutination inhibition (HI) tests

Two milliter of blood was collected from each of three adult birds in a test tube containing EDTA as anticoagulant. The blood was washed in phosphate buffered saline (PBS) and centrifuged at 3000 rpm for 5 min. This was repeated until a clear supernatant was obtained. The packed red blood cells (RBC) were re-suspended in a measured volume of PBS solution to make 0.5% RBC suspension (Beard, 1989).

The antigen titre for running the HI test was determined by standard HA technique using La Sota ND vaccine as antigen (Alexander, 2003). The reciprocal of the highest dilution of the La Sota ND antigen causing 100% agglutination of an equal volume of standardized RBCs was taken as the HA titre of the antigen. The HI titres were determined, also by the method of Beard (1989). The HI titers were reciprocal of the highest dilutions of the sera at which 100% RBC HI occurred. The geometric mean titre (GMT) was calculated using the Tube Number Method and Table (Villegas and Purchase, 1989).

Haematology

An assessment of the cellular response was made by determining the total and differential counts of white blood cells (WBC). Total white cell counts were obtained standard methods using improved Neubeur heamocytometer while the differential counts were done in stained thin blood smears (Coles, 1986).

Statistical analyses

The antibody titres were transformed to base 2 logarithms while the total and differential WBC counts were subjected to Analysis of Variance (ANOVA) using the Statistical Package for Social Sciences (SPSS). Significant means were separated using the Duncan's New Multiple Range Test and tests were considered significant at a probability of $P < 0.05$ (Duncan, 1955).

RESULTS

Serology

The titres on week 1 of the experiment indicated low levels of antibody in the range of 1.3 to 2.8 (Figure 1). The GMT post treatment and vaccination indicated obvious differences between the vaccinated Groups II (294.1) and III (274.4) and the unvaccinated Groups I (73.3) and IV (nil). Also the GMT of the treated and vaccinated Group II and untreated and vaccinated Group III showed steady increase until the end of the experiment

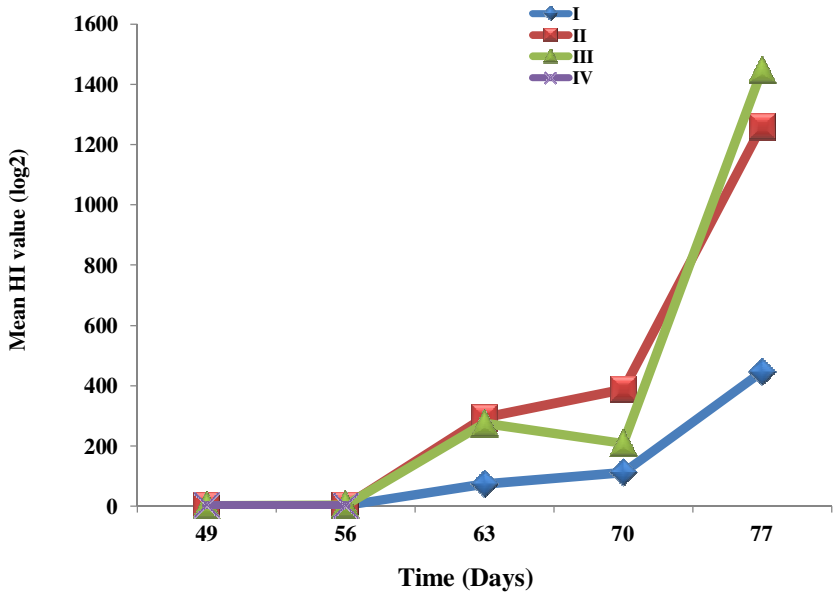

Figure 1. The HI titre for Newcastle disease antigen of the different groups of the birds treated with *M. oleifera* and or NDV vaccination. Group I = treated, unvaccinated, challenged; Group II = treated, vaccinated, challenged; Group III = untreated, vaccinated, challenged; Group IV = untreated, unvaccinated, challenged.

Table 1. The mean leukocyte counts of the different groups of the birdstreated with *M. oleifera* and or NDV vaccination.

Age (Weeks)	Mean leukocyte counts (10^3/µl) ± S.D.			
	Group I	Group II	Group III	Group IV
0 (Vaccination and commencement of treatment with the extract 0	17.18±0.22^{a}	15.76±0.28^{bc}	15.16±0.48^{c}	17.42±0.36^{a}
1	42.68±0.27^{a}	32.14±1.02^{b}	25.36±1.17^{c}	22.38±2.09^{d}
2 challenge and end of treatment	58.82±2.93^{a}	30.46±1.37^{b}	43.94±1.44^{c}	28.22±0.89^{b}
3	66.30±2.04^{a}	48.20±0.90^{b}	232.90±8.68^{c}	-
4	57.18±1.32^{a}	44.32±230.40^{b}	36.58±1.48^{c}	-
5	33.16±0.72^{a}	33.48±1.53^{a}	39.90±1.44^{b}	-

^{a,b,c}Different alphabetical superscripts in a row indicate significant differences between the means: $p < 0.05$. Group I = treated, unvaccinated, challenged; Group II = treated, vaccinated, challenged; Group III = untreated, vaccinated, challenged; Group IV = untreated, unvaccinated, challenged.

on week 5.

Total leukocyte counts (TLC)

On week 1 and 2 of the experiment there were significant ($P < 0.05$) differences in the mean TLC across the groups (Table 1). The mean TLC of the treated and unvaccinated Group I was significantly ($P < 0.05$) higher than the mean TLC of the treated and vaccinated Group II on weeks 1, 2, 3 and 4 while the mean TLC of the treated and vaccinated group II was significantly ($P < 0.05$) lower than the values of the untreated but vaccinated Group III throughout the study. On week 1 and 2 of the experiment, the mean TLC of the untreated and vaccinated Group III was significantly ($P < 0.05$) higher than those of the untreated and unvaccinated Group IV (Table 1).

Differential leukocyte counts (DLC)

The mean absolute lymphocyte counts (ALC) of the treated and unvaccinated Group I was significantly ($P<0.05$), higher than those of treated and vaccinated Group II and untreated and vaccinated Group III, on weeks 1, 2, 3 and 4; and higher than those of untreated and unvaccinated Group IV on weeks 1 and 2. The ALC of the treated and vaccinated Group II was also

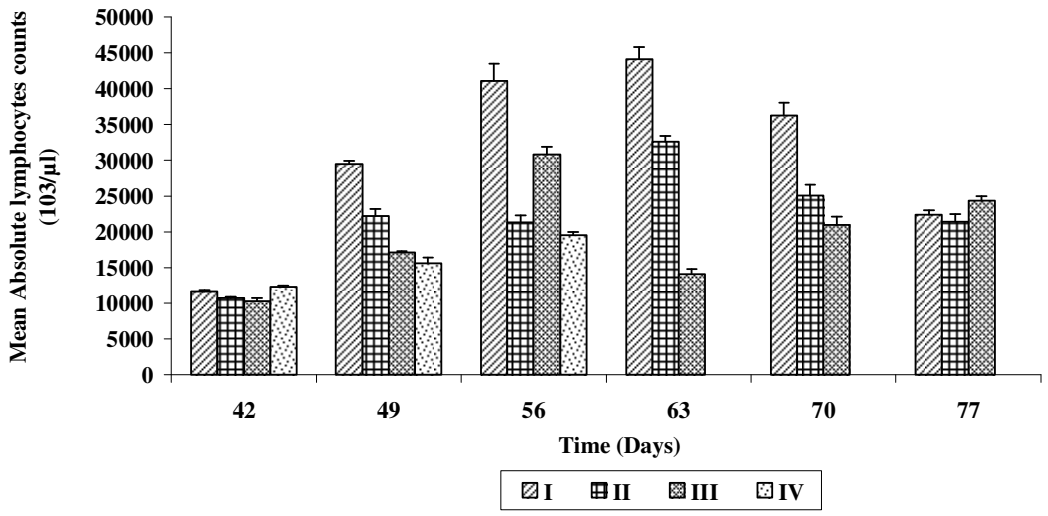

Figure 2. The absolute lymphocytes counts of the different groups of the birds treated with *M. oleifera* and or NDV vaccination. Group I = treated, unvaccinated, challenged; Group II = treated, vaccinated, challenged; Group III = untreated, vaccinated, challenged; Group IV = untreated, unvaccinated, challenged.

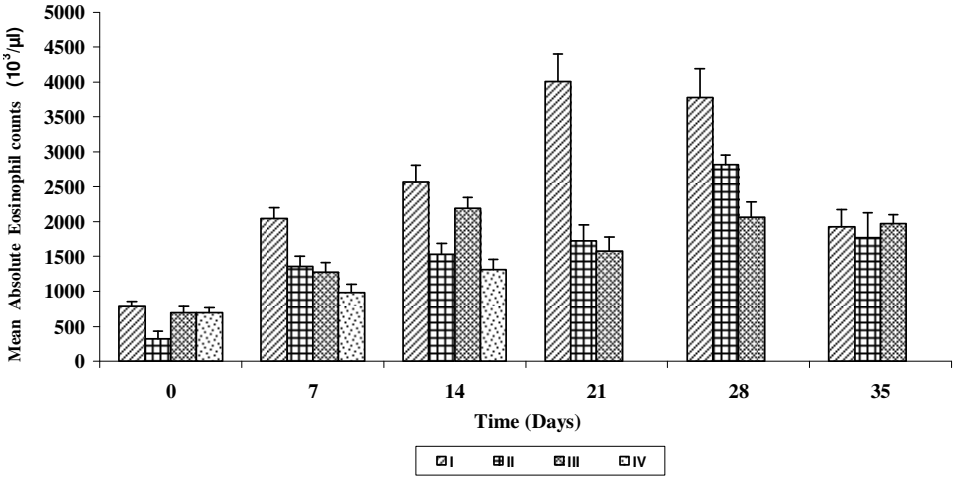

Figure 3. Absolute eosinophils counts of the different groups of the birds treated with *M. oleifera* and or NDV vaccination. Group I = treated, unvaccinated, challenged; Group II = treated, vaccinated, challenged; Group III = untreated, vaccinated, challenged; Group IV = untreated, unvaccinated, challenged.

significantly (P< 0.05) higher than the untreated and vaccinated Group III on weeks 2, 3, and 4 (Figure 2). On weeks 1, 3 and 4, the mean absolute eosinophils count AEC in treated and unvaccinated Group I was significantly (P< 0.05) higher than those of the Groups I to IV while the AEC of the treat and vaccinated Group II was significantly (P< 0.05) higher than those of untreated and vaccinated group III on weeks 2 and 4 (Figure 3). On weeks 1, 2, and 3, the mean absolute heterophils count (AHC) of the treated and unvaccinated Group I was significantly (P < 0.05) higher than those of treated and vaccinated Group II and untreated and vaccinated Group III (Figure 4). On week 3 of the study, the treated and unvaccinated group I had significantly (P< 0.05) higher mean absolute basophil counts (ABC) per chick than treated and vaccinated group II and untreated and vaccinated Group III (Figure 5).

DISCUSSION

The sera collected immediately before vaccination of chicks had very low maternal antibody titres, suggesting that the birds had not been exposed to NDV, and that the

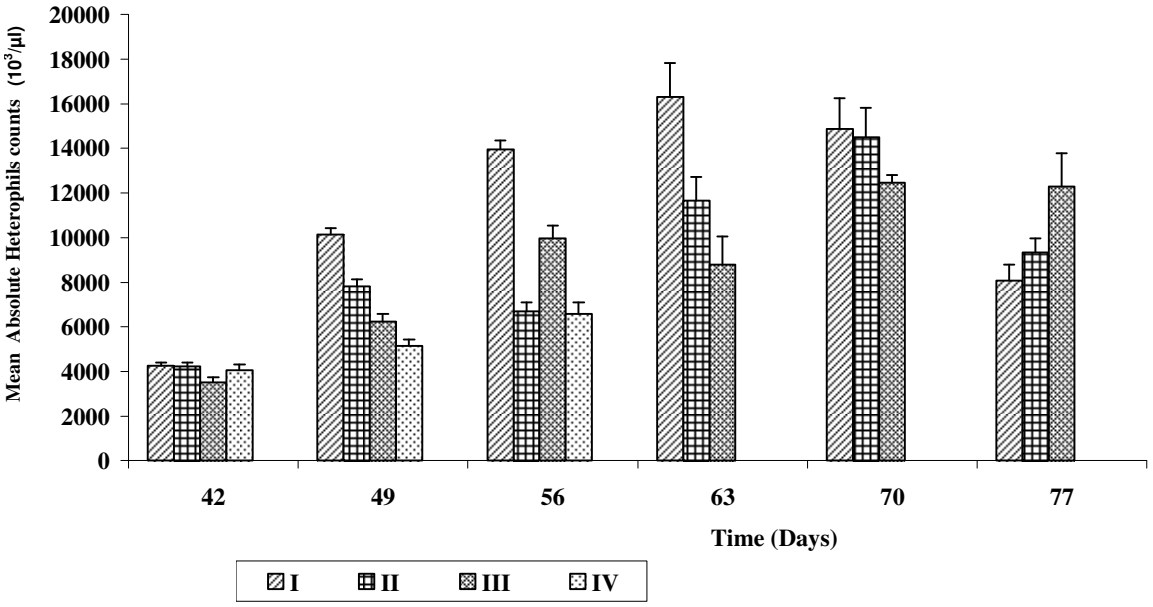

Figure 4. Absolute Heterophils counts of the different groups of the birds treated with *M. oleifera* and or NDV vaccination. Group I = treated, unvaccinated, challenged; Group II = treated, vaccinated, challenged; Group III = untreated, vaccinated, challenged; Group IV = untreated, unvaccinated, challenged.

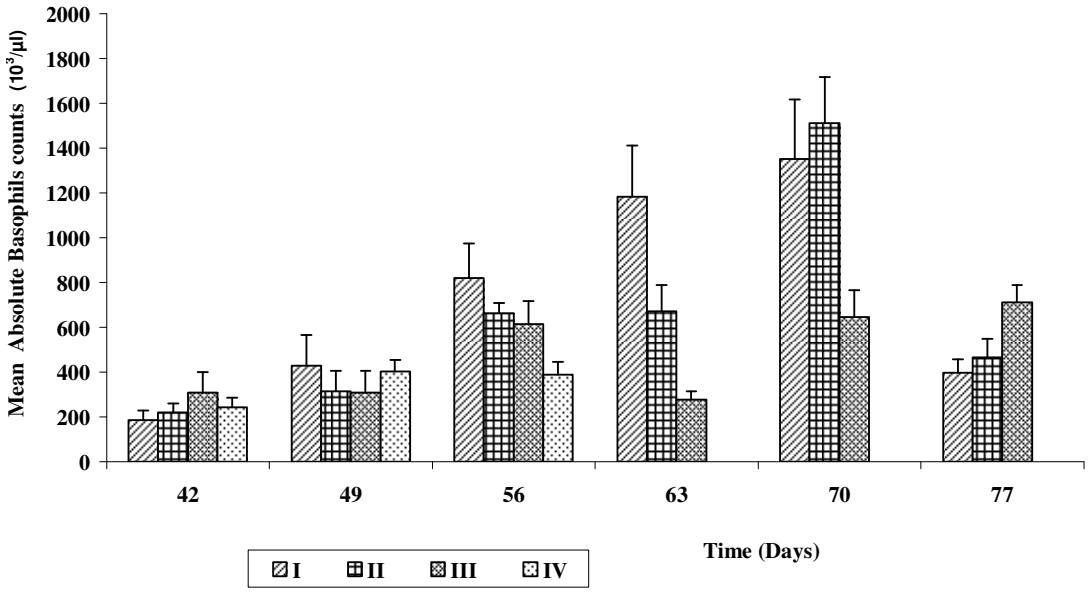

Figure 5. Graph showing the absolute basophils counts of the different groups of the birds treated with *M. oleifera* and or NDV vaccination. Group I = treated, unvaccinated, challenged; Group II = treated, vaccinated, challenged; Group III = untreated, vaccinated, challenged; Group IV = untreated, unvaccinated, challenged.

maternally derived antibodies to NDV acquired from their parents had waned at 42 days of age. This progressive decrease in maternally derived antibody titers was in agreement with earlier observations of (Alexander, 2003; Facon et al., 2005), who reported that chicks from vaccinated parent stocks have high levels of maternally derived antibodies at day old which continued to decline in its protection level within 15 to 20 days after hatching. Following vaccination with La Sota vaccine and challenge with VNDV strain Kudu 113, the HI titre was significantly

high in all the vaccinated and challenged Groups II and III than in the unvaccinated and challenged Groups I and IV. This is similar to what was reported that following challenge with VNDV, the HI titre is usually high (Illango and Olaho-Mukani, 2005; Kakengi et al., 2007). The presence of this high NDV HI antibody is necessary to provide long-term protection against ND (Ritchie et al., 1994; Sa' idu, 2006). Throughout the period of experimentation, there was progressive increase in the HI values of groups II and III, while there was equally an appreciable increase in the HI value of the *M. oleifera* treated group I chicks. It is important to note that on 56 weeks post challenge, the antibody titre of Group III fell below the HI value obtained on day 63 of age. This may be due to the continuous challenge by the NDV that was being secreted by the birds within their confined environment (Alexander, 2003). All the chicks in Group II in this study did not responded to the vaccination in the same way. Individual variation in the production of HI antibody could have occurred following vaccination. This variation most often is due to the presence of variable passive immunity in chicks or to the varying degree of susceptibility of immune mechanism to antigen as was also suggested by Hunduma et al. (2010). This might be due to genetical incapability of some birds to produce any reaction to NDV antigen (Alexander, 2003; Herholz et al., 2006). Other possible reasons included impaired immune-competence due to immunosuppressive agents in the feed or due to immunosuppressive diseases such as Infectious bursal disease, etc (Herholz et al., 2006).

Throughout the study, the mean TLC in Group I was significantly ($P < 0.05$) higher than the mean TLC in Group IV. The leucocytosis may be attributed to increased production of leukocytes in the haematopoietic tissues (Yongola et al., 2006; Ravindraa et al., 2009). The primary consequence of low leukocyte count in stressed chickens is suppression of the immune system and increased susceptibility to disease (Wambura, 2009). The AHC started increasing on day 49 and reached the peak level on day 63 in Group I and day 70 in Groups II and III. The value in Group I was significantly ($P < 0.05$) higher than that of Group IV and this might have resulted from the fact that heterophils exhibit high level of apoptosis when infected by NDV (Ravindraaa et al., 2009). The level of the heterophils usually indicates the severity of the initial immune response; therefore their high values in Group I showed that *M. oleifera* possibly protected them from apoptosis (Fahey, 2005).

The mean ALC increased from day 49 to day 63 in Groups I and II, but subsequently decreased. This is in agreement with the report that the increase in lymphocytes might be physiologic, reactive, or proliferative in disease conditions (Wambura, 2009). Birds that normally have high numbers of circulating lymphocytes in the initial response to infective pathogens might develop leucopenia due to lymphopenia (Ritchie et al., 1994). The low values of mean ALC in Groups III and IV is in agreement with the report that NDV has the ability to cause agglutination and lyses of the lymphocytes of affected birds thereby reducing the number of circulating lymphocytes (Bennett et al., 2003; Khesorn, 2009).

Conclusively, *M. oleifera* extract increased ND HI titre and the total and differential leukocyte counts in the treated and unvaccinated Group I birds much more than those of treated and vaccinated Group II, hence, it could be recommended as an immune-booster treatment against ND in non vaccinated birds.

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