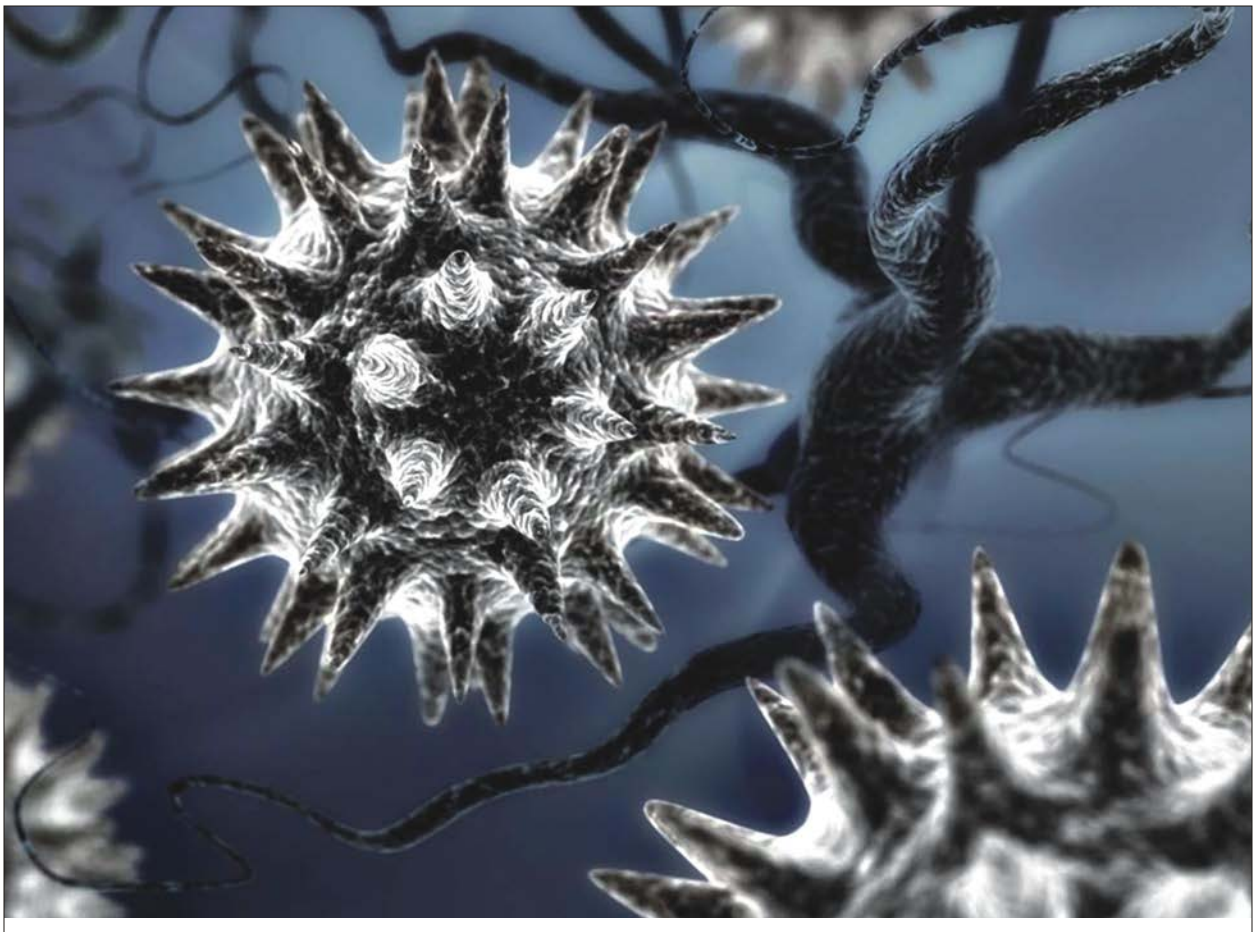

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Curcuma longa for Protecting Chicks Against Newcastle Disease Virus Infection and Immunosuppressive Effect of Marek's Disease Viral Vaccine

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ABSTRACT

A total of 300 one day old Hubbard chicks were divided into 6 groups (G1-G6: 50 chicks/each) The G1 (control neither vaccinated nor treated), G2 vaccinated with NDV, G3 (vaccinated with MDV Rispen strain), G4 (vaccinated with MDV and NDV), Group 5 vaccinated with MDV vaccine and treated with *Curcuma longa* and G 6 vaccinated with MDV, NDV and treated with *Curcuma longa*. Chicks vaccinated with NDV vaccine received Hitciner B-1 strain at 7th day of age then boosted with LaSota strain at 21st day of age in drinking water, while groups vaccinated with MDV vaccine (0.2 mL/chick) at one day of age by S/C injection. Serum samples at 10, 14, 17, 21, 28, 35 and 42 of age for HI test against NDV. Heparinized blood samples at 10, 14, 17, 21, days of age for phagocytic activity of macrophages. All groups were challenged with vvNDV for detecting the protection percent. From this study, it could be concluded that MDV vaccine has an immunosuppressive effect on chicks and this could be antagonized by immunostimulant as *Curcuma longa*. The surprising immunostimulatory effect of curcuma is in the induction of protection level 80% in treated but not NDV vaccinated group which equivalent to that group vaccinated with NDV vaccine only and not treated. From the obtained results we recommend the use of *Curcuma longa* powder in poultry rations for enhancing the immune response against either field infection or vaccination.

Key words: Marek's disease, new castle disease, immune, curcuma

INTRODUCTION

MDV is known since long time as immunosuppressive agent and this virus immunosuppression interfere with the immune response against microbial agents infection and vaccines and the degree of immunosuppression be associated with the severity of the disease (Purchase *et al*., 1968; Sharma, 1987; Rivas and Fabricant, 1988; Heidari *et al*., 2010). Field as well as vaccinal virus strains of MD has gross changes in both bursa of Fabricious and thymus glands of chickens with drastic reduction in packed cell volume and hematopoiesis which results in immunosuppression (Jakowski *et al*., 1969; Sharma, 1978; Purchase and Sharma, 1974; Jakowski *et al*., 1970). Curcumin (diferuloylmethane) is an orange-yellow component of turmeric (*Curcuma longa*), a spice often found in curry powder. Traditionally known for its an anti-inflammatory effects, curcumin has been shown in the last two decades to be a potent immuno-modulatory agent that can modulate the activation of T cells, B cells, macrophages, neutrophils, natural killer cells and dendritic cells.

Curcumin can also down regulate the expression of various pro-inflammatory cytokines including TNF, IL-1, IL-2, IL-6, IL-8, IL-12 and chemokines, most likely through inactivation of the transcription factor NF-kappaB. Interestingly, however, curcumin at low doses can also enhance antibody responses. This suggests that curcumin's reported beneficial effects in arthritis, allergy, asthma, atherosclerosis, heart disease, Alzheimer's disease, diabetes and cancer might be due in part to its ability to modulate the immune system. Together, these findings warrant further consideration of curcumin as a therapy for immune disorders (Bright, 2007; Jagetia and Aggarwal, 2007; Sikora *et al*., 2010). Modern science has revealed that curcumin mediates its effects by modulation of several important molecular targets, including transcription factors (e.g., NF-kappaB, AP-1, Egr-1, beta- catenin and PPAR-gamma), enzymes (e.g., COX2, 5-LOX, iNOS and heme oxygenase-1), cell cycle proteins (e.g., cyclin D1 and p21), cytokines (e.g., TNF, IL-1, IL-6 and chemokines), receptors (e.g., EGFR and HER2) and cell surface adhesion molecules. Because it can modulate the expression of these targets, curcumin is now being used to treat cancer, arthritis, diabetes, Crohn's disease, cardiovascular diseases, osteoporosis, Alzheimer's disease, psoriasis and other pathologies (Shishodia *et al*., 2005).

The laboratory studies have identified a number of different molecules involved in inflammation that are inhibited by curcumin including phospholipase, lipo-oxygenase, cyclooxygenase 2, leukotrienes, thromboxane, prostaglandins, nitric oxide, collagenase, elastase, hyaluronidase, monocyte chemoattractant protein-1 (MCP-1), interferon- inducible protein, tumor necrosis factor (TNF) and interleukin-12 (IL-12). Curcumin has been demonstrated to be safe in six human trials (human trials using 1125-2500 mg of curcumin per day and up to 8000 mg of curcumin per day for 3 months found no toxicity from Curcumin) (Chainani-Wu, 2003). Curcumin significantly reduced Coxsackievirus RNA expression, protein synthesis and virus titer and protected cells from virus-induced cytopathic effect and apoptosis (Si *et al*., 2007).

Mazumber *et al*. (1995) demonstrated that curcumin has an antiviral activity, being a HIV-1 integrase inhibitor (IC_{50} = 40 μM) and suggested that curcumin analogs may be developed as anti-AIDS drugs. Data showed that curcumin inhibited the replication of HIV-1 integrase protein. Eigner and Scholz (1999) reported that curcumin was claimed for anti- HIV-1 and HIV-2 activities in a recent patent application.

The aim of the present study is to clarify the non-specific immuno- stimulatory effect of *Curcuma longa* against NDV infection and immunosuppressive effect of MDV.

MATERIALS AND METHODS

Chicks materials: A total of 500 one day old Hubbard chicks were fed balanced ration and reared in good hygienic measures and divided into 10 groups (G1-G10 50 chicks/each). The G1 (control neither vaccinated nor treated), G2 vaccinated with NDV, G3 (vaccinated with MDV Rispen strain), G4 (vaccinated with MDV and NDV), Group 5 vaccinated with MDV vaccine and treated with *Curcuma longa* and G 6 vaccinated with MDV, NDV and treated with *Curcuma longa*. Chicks vaccinated with NDV vaccine received Hitciner B-1 strain at 7th day of age then boosted with LaSota strain at 21st day of age in drinking water (Both vaccines purchased from Serum and vaccine research Institute, Abbassia, Egypt), while groups vaccinated with MDV(TAD Marek Vac Forte) vaccine received only one dose (0.2 mL/chick) at one day of age by S/C injection. Serum samples of all chicks were collected at 10,14, 17, 21, 28, 35 and 42 of age for determining the antibodies against NDV using HI test. Heparinized blood samples were collected at 10, 14, 17, 21 days of age for detecting the phagocytic activity of macrophages. All groups were challenged

with vvNDV (Serum and vaccine research and production, Abbassia, Egypt) for detecting the protection percent.

***Curcuma longa*:** *Curcuma longa* was purchased as a powder from popular supper market for buying aromatic and medicinal herbal plants and used as feed additives in a percentage of 1%.

Blood samples: Two groups of blood samples were collected, from each chick by wing vein puncture, in sterile plastic centrifuge tube with heparin (20 IU mL^{-1}) for macrophage cells separation (10, 14, 17, 21 days of age for detecting the phagocytic activity of macrophages) or without heparin for serum separation (10, 14, 17, 21, 28, 35 and 42 of age for determining the antibodies against NDV using HI test).

Roswell park memorial institute (RPMI-1640) medium: RPMI-1640 medium was purchased from GibcoBRI Cat No. 51800-019, Lot No, 3072701, used in phagocytic activity assay.

Ficoll hypaque: This medium was used for the separation of mononuclear leukocyte cells from peripheral blood, obtained from Biochrom AG Cat No. L 6113 Lot No. 729B, stored at +2±25°C. Culture medium for *C. albicans*: Sabouraud dextrose agar medium containing chloramphenicol 40 g mL^{-1} was kindly supplied from Dept. of Mycology, Animal Health Research Institute, Dokki, Egypt and used for cultivation of *Candida albicans*.

Fetal Calf Serum (FCS): Biochrom AG, Cat No. S 0113, Lot No. 224 B inactivated at 56°C for 30 min and preserved at -20°C. This serum was added to the medium at a final concentration of 20%.

Haemagglutination-Inhibition (HI) methods test: HI test was done according to Majiyagbe and Hitchner (1977).

Challenge test: The chickens were challenged intramuscularly with 0.2 mL suspension containing 10^6 NDV/chicken (Velogenic strain).

Phagocytic activity and percentage of chicken peripheral monocyte using *C. albicans*: According to Richardson and Smith (1981), as modified by El-Enbaway (1990) and Saif (2004). The phagocytic activity was calculated according to the following equations:

$$\text{Percentage of phagocytosis} = \frac{\text{No. of ingesting phagocytes}}{\text{Total No. phagocytes including non ingesting cells}} \times 100$$

$$\text{Phagocytes index} = \frac{\text{Total No. phagocytes with more than 3 blastospores}}{\text{Total No. phagocytes ingesting blastospores}}$$

RESULTS AND DISCUSSION

Some immunosuppressive agents like NDV and MDV play an important role in exposing chickens to contact dangerous viral or bacterial diseases even if their agents are of low virulence. Newcastle disease virus inducing fatal disease in young chicks and respiratory, nervous disorders besides decreasing in egg production in adults (Aldous and Alexander, 2001; Office Internationale

Table 1: Mean heamagglutination Inhibition antibody titer against NDV vaccines

	Mean HI titer/days of age						
Groups*	10th day	14th day	17th day	21st day	28th day	35th day	42nd day
2	256	204.8	430.4	716.8	179.2	230.4	204.8
4	460.8	409.6	430.4	409.6	204.8	204.8	153.6
6	409.6	204.8	716.8	819.2	358.4	179.2	409.6

*G2: NDV vaccine only. G4: NDV vaccine+MDV vaccine. G6: NDV vaccine+MDV vaccine+Curcuma

des Epizooties, 2001; Ali *et al*., 2004). To modulate the immunosuppressive effect of these agents, some immunostimmulants either natural or synthetic were used. In this study *Curcuma longa* powder is used as feed additives for studying its effects against the immunosuppression of Marek's disease virus vaccine and infection with very virulent Newcastle disease virus. To achieve the main goal of this study three hundreds young one day old Hubbered chicks were divided into 6 groups (G1-G6, 50 chicks/each). The G1 (control neither vaccinated nor treated), G2 (vaccinated with NDV), G3 vaccinated with MDV Rispen strain. Group 5 vaccinated with MDV Rispen strain and treated with *Curcuma longa* powder. Groups 4 vaccinated with MDV and NDV while group 6 (vaccinated with MDV and NDV) and treated with *Curcuma longa* powder. All these 6 groups were challenged with vvNDV at 42nd day of age.

In this study, natural immunostimmulants as *Curcuma longa* powder, was purchased from popular supper market for aromatic and medicinal herbal plants and used as feed additives in a percentage of 1% and used as an immunostimulant in chicken.

Groups 3-6 were vaccinated with MDV vaccine Respin strain as one dose 0.2 mL/chick S/C in the first day of life while groups 2, 4 and 6 received two doses of NDV vaccine at 7 (Hitchner B1 by eye drop inoculation) and 14 day of life (LaSota by drinking water). Groups 5 and 6 fed on ration containing *Curcuma longa* powder as 1%. Group 1 was left unvaccinated and untreated group. The HI antibody titers that determined in all groups vaccinated with NDV vaccines showed gradual increase and reached the peak at the third week of age. On comparing these groups according to their treatment, the obtained results reaveled that group 2 that only received the NDV vaccine showed the highest HI antibody titer at 21st day of age then declined at day 28th and elevated again from day 35th of age while group 4 that vaccinated with MDV and NDV showed decrease in HI antibody titer from the 21st day of age onward tell the end of the experiment and this declare the immunosuppressive effect of MDV vaccine. The highest HI antibody at 21st day of age 819.2 was detected in group 6 that vaccinated with MDV and NDV and treated with Curcuma longa and this denotes to the immuno-stimulatory effect of Curcuma longa (Table 1).

Regarding the phagocytic activity of macrophages in these groups, group 1 (not MDV vaccinated) showed higher percentage of phagocytosis (34 and 24%) than group 3 (only MDV vaccinated) (24 and 17%) at 10th and 14th day of age. While, group 3 showed lower percentage of phagocytosis (24, 17, 55 and 49) other than group 5 (MDV vaccinated and Curcuma treated) (44, 58, 75 and 71) at 10, 14, 17 and 21 days post vaccination (Fig. 1).

However, the phagocytic indexes in vaccinated and treated groups were higher than group 4 (vaccinated but not treated with any *Curcuma longa*). Group 1 (not MDV vaccinated) and group 3 (only MDV vaccinated) showed lower index (0.324 and 0.333, respectively) than groups 5 that showed 0.523 (MDV vaccinated and treated with Curcuma) at 10th day of age. The same picture was found at 14th, 17th and 21st day of age. Group 6 (MDV and NDV vaccinated and Curcuma treated) showed higher index (0.567 and 0.544) at 17th and 21st day of age than the other groups (Fig. 2).

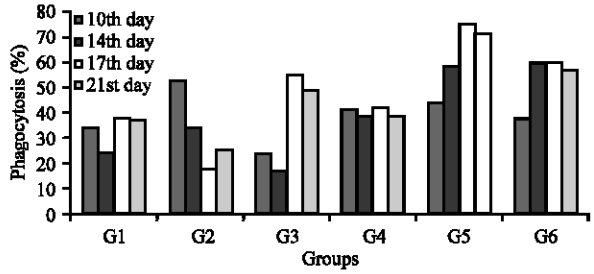

Fig. 1: Phagocytosis % for all groups

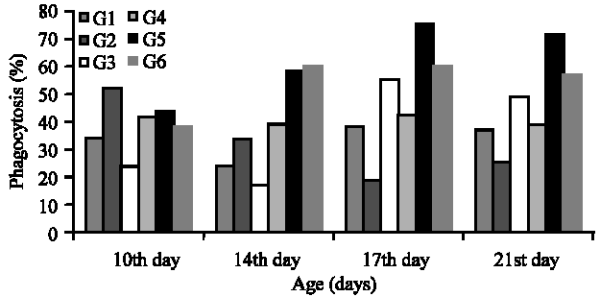

Fig. 2: Phagocytosis Index for all periods

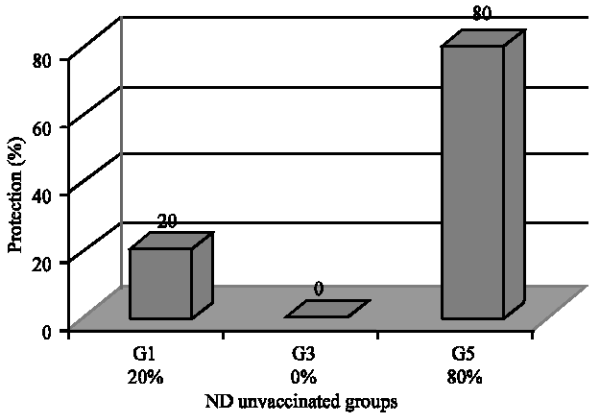

Fig. 3: Protection level against experimental infection with vvNDV in unvaccinated groups

On the protection level against experimental infection with vvNDV, Group 5 treated with Curcuma and vaccinated with MDV vaccine but not with NDV vaccine and challenged with vvNDV showed 80% protection. Group 6 treated with Curcuma and vaccinated with both MDV and NDV vaccines and challenged with vvNDV showed 100% protection. While group 2 (only NDV vaccinated) showed 80% protection but some birds showed severe symptoms of ND in group 4 then survived and this may be attributed to the immunosuppressive effect of MDV vaccine (Fig. 3, 4).

The surprising immunostimulatory effect of Curcuma in induction of protection level (80%) (in treated but not NDV vaccinated group) equivalent to that group vaccinated with NDV vaccine only (but not treated) needs further studies for confirming these obtained results. The main effect

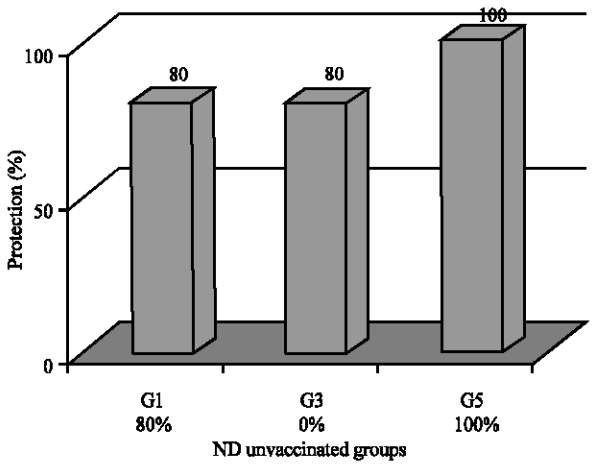

Fig. 4: Protection level against experimental infection with vvNDV in vaccinated groups

of protecting these chicks from infection with vvNDV may be attributed to the anti inflammatory effect of *Curcuma longa*. Different molecules involved in inflammation that are inhibited by curcumin including phospholipase, lipooxygenase, cyclooxygenase 2, leukotrienes, thromboxane, prostaglandins, nitric oxide, collagenase, elastase, hyaluronidase, monocyte chemoattractant protein-1 (MCP-1), interferon-inducible protein, tumor necrosis factor (TNF) and interleukin-12 (IL-12). Curcumin has been demonstrated to be safe in six human trials (human trials using 1125-2500 mg of curcumin per day and up to 8000 mg of curcumin per day for 3 months found no toxicity from Curcumin, Chainani-Wu, 2003). Curcumin's reported beneficial effects in arthritis, allergy, asthma, atherosclerosis, heart disease, Alzheimer's disease, diabetes and cancer might be due in part to its ability to modulate the immune system (Natarajan and Bright, 2002; Aggarwal *et al*., 2003; Chan *et al*., 2003; Chendil *et al*., 2004; Adams *et al*., 2005; Fang *et al*., 2005; Furness *et al*., 2005). Together, these findings warrant further consideration of curcumin as a therapy for immune disorders (Jagetia and Aggarwal, 2007). Modern science has revealed that curcumin mediates its effects by modulation of several important molecular targets, including transcription factors (e.g., NF- kappaB, AP-1, Egr-1, beta-catenin and PPAR-gamma), enzymes (e.g., COX2, 5-LOX, iNOS and hemeoxygenase-1), cell cycle proteins (e.g., cyclin D1 and p21), cytokines (e.g., TNF, IL-1, IL-6 and chemokines), receptors (e.g., EGFR and HER2) and cell surface adhesion molecules. Because it can modulate the expression of these targets, curcumin is now being used to treat cancer, arthritis, diabetes, Crohn's disease, cardiovascular diseases, osteoporosis, Alzheimer's disease, psoriasis and other pathologies (Shishodia *et al*., 2005). On the other hand Curcuma longa may be involved in retarding the replication pathway of NDV by preventing its entry to the host cells, replication of viral nucleic acid and /or releasing of the progeny virus particles from the infected cells. A third explanation in our opinion may be to the synergistic effect of the curcuma longa as anti inflammatory and antiviral. The antiviral effect of the curcuma longa was existed in different studies contributing to different viruses. Curcumin significantly reduced Coxsackievirus RNA expression, protein synthesis and virus titer and protected cells from virus- induced cytopathic effect and apoptosis. Si *et al*. (2007) and Mazumber *et al*. (1995) demonstrated that curcumin has an antiviral activity, being a HIV-1 integrase inhibitor (IC_{50} = 40 μM) and suggested that curcumin analogs may be developed as anti- Aids drugs. Data showed that curcumin inhibited the replication of HIV-1 integrase protein. Eigner and Scholz (1999) reported that curcumin was claimed for anti- HIV-1 and HIV-2 activities in a recent patent application.

From this study, it could be concluded that MDV vaccine has an immunosuppressive effect on chicks and this could be antagonized by immunostimulants as *Curcuma longa*.

From these results we recommend the use of Curcuma powder in poultry ration for enhancing the immune response against either field infection or vaccination.

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