



E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(6): 749-751
Received: 25-09-2017
Accepted: 27-10-2017

R Kavinilavan

M.V.Sc scholar, Department of Veterinary Pharmacology and Toxicology Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

P Mekala

Assistant Professor, Department of Veterinary Pharmacology and Toxicology Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

MJ Raja

Assistant Professor, Department of Veterinary Pharmacology and Toxicology Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

M Arthanari Eswaran

Assistant Professor and Head, Veterinary University Training and Research Centre, Tirupur Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

G Thirumalaisamy

Ph.D., Scholar, Division of Animal Nutrition, National Dairy Research Institute, Karnal Part of the M.V.Sc thesis of the first author approved by Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

Correspondence**P Mekala**

Assistant Professor, Department of Veterinary Pharmacology and Toxicology Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

Exploration of Immunomodulatory Effect of *Nilavembu Kudineer Chooranam* against Newcastle Disease Virus in Backyard Chicken

R Kavinilavan, P Mekala, MJ Raja, M Arthanari Eswaran and G Thirumalaisamy

Abstract

Nilavembu Kudineer Chooranam (NKC) was subjected to preliminary phytochemical screening and its immunomodulatory activity was assessed in sixty day-old unsexed backyard chicken divided into six treatment groups of ten each. T₁ served as control, T₂ was vaccine control, T₃ was positive control treated with levamisole, T₄ - T₆ were administered *Nilavembu Kudineer Chooranam* (NKC) @ 0.5, 1.0 and 2.0mL/kg body weight respectively for five days before vaccination. The birds (T₂ - T₆) were vaccinated with oral pellet vaccine (D58 strain) against Newcastle disease virus at the end of 1st, 4th and 8th week and HI titre was evaluated at the end of 4th, 8th, 12th and 16th week in all the groups. Throughout the experiment, the group pretreated with NKC @ 1.0mL/kg (T₅) had shown significantly higher titre when compared to vaccine control (T₂). The positive control (T₃) was numerically better than vaccine control (T₂) throughout the study. At the end of 12th and 16th week, the titre of positive control was significantly less than T₅. The results revealed that NKC when used @ 1.0mL/kg for 5 days in drinking water before immunization resulted in improvement in immune response in backyard chicken.

Keywords: *Nilavembu Kudineer Chooranam*, Namakkal Chicken-1, Immune response, Levamisole, Oral pellet vaccine

Introduction

Backyard poultry contributes significantly to poultry meat and egg production and despite their disease resistance outbreak due to Newcastle disease virus (NDV) are frequently reported wherein the mortality ranges from 75-100 per cent in unvaccinated flocks [1]. In the absence of effective treatment, prevention and control measures through vaccine strains such as LaSota and F are widely adopted because of low pathogenicity and induction of adequate immune response [2]. But vaccination in backyard poultry is a major problem because of difficulty in handling, maintenance of cold chain etc. Hence there is an increasing need for exploring new compounds with antiviral and immunomodulatory activity against poultry viral diseases. Benefits of various phytochemicals on innate and adaptive components of the immune system of poultry have been extensively reviewed [3]. *Nilavembu Kudineer Chooranam* (NKC), a poly herbal Siddha preparation containing an equal proportion of nine plants viz. *Andrographis paniculata*, *Piper nigrum*, *Santalum album*, *Zingiber officinale*, *Cyperus rotundus*, *Vetiveria zizanioides*, *Mollugo cerviana*, *Plectranthus vettiveroides* and *Trichosanthes cucumerina* was successful in the prevention and treatment of chikungunya and dengue viral fever in human and approved for use by the Government of Tamil Nadu. Instead of searching for immunomodulatory plants, an attempt was made to explore the available Siddha preparation for immunomodulation against Newcastle disease virus (NDV).

Materials and Methods

Nilavembu Kudineer Chooranam procured from Tamil Nadu Medicinal Plant Farms and Herbal Medicine Corporation Limited (TAMPCOL), Tampcol buildings, Anna Hospital Campus, Arumbakkam, Chennai was subjected to preliminary qualitative phytochemical screening [4] and used in the study.

Sixty day-old unsexed backyard chicks (Namakkal Chicken - 1) obtained from Poultry Farm Complex, Veterinary College and Research Institute, Namakkal divided into six treatment groups of ten each were used in this trial. Desi chick mash and grower mash, free of mycotoxins procured from Feed Manufacturing Unit, Department of Animal Nutrition, Veterinary College and Research Institute, Namakkal, Tamil Nadu were fed to the birds from day old to 8 Weeks and 8 to 16 weeks of age, respectively.

The birds were provided *ad libitum* feed and wholesome drinking water throughout the study period.

The design and protocol of the experiment were approved by Institutional Animal Ethics Committee, Veterinary College and Research Institute, Namakkal.

Experimental design

Group	Treatment
T ₁	Control (unvaccinated)
T ₂	Oral pellet vaccine (end of 1 st , 4 th and 8 th week of age)
T ₃	Positive Control (Levamisole @ 30mg/kg for 2 days before and 2 days after each oral pellet vaccination)
T ₄	Pretreatment with NKC @ 0.5mL/kg before oral pellet vaccination
T ₅	Pretreatment with NKC @ 1.0mL/kg before oral pellet vaccination
T ₆	Pretreatment with NKC @ 2.0mL/kg before oral pellet vaccination

Decoction of NKC was prepared by boiling 12.5gms of NKC powder with 240mL of water and concentrating to 60mL. It was filtered and administered to the birds at various dose levels. The required quantity of decoction was calculated, prepared freshly and mixed with 200mL of drinking water and provided in the morning. After drinking the medicated water, the birds were provided *ad libitum* wholesome drinking water for the rest of the day.

TANUVAS oral pellet vaccine (D58 strain) procured from Department of Veterinary Microbiology, Madras Veterinary College, Chennai was used for vaccinating the birds and

levamisole obtained from M/s. Himedia laboratories, Mumbai was used as a standard immunomodulator in this study. Blood samples were collected at the end of 4th, 8th, 12th and 16th week for assessing HI titre against NDV antigen by micro dilution technique^[5] using "V" bottom micro plates.

Statistical Analysis

Completely randomized design was followed for the experiment⁶ and the data collected were analyzed by One-way ANOVA procedure using SPSS[®] 20.0 software package for Windows. Post-hoc analysis was done by Duncan's significance difference test⁷.

Results

Preliminary phytochemical screening of NKC revealed the presence of alkaloids, carbohydrates, glycosides, flavonoids, phenols, tannins and terpenoids. The remaining phytochemicals *viz.* cardiac glycosides, proteins and amino acids, saponins, hydrolysable tannins, phlobatannins, volatile oils and vitamin C could not be detected by the present test.

The humoral immunity assessed by measuring HI titre against NDV is presented in the table 1. At the end of fourth week the mean HI titre (\log_2) was significantly higher ($p < 0.05$) in T₅ (6.00±0.33) and T₆ (5.70±0.01) which were pretreated with NKC @ 1.0 and 2.0mL/kg body weight, respectively for 5 days before oral pellet vaccine when compared to T₂ (4.80±0.33) which served as vaccine control. They were numerically higher than NKC pretreatment @ 0.5mL/kg (T₄) and positive control (T₃) treated with standard immunostimulant, levamisole (5.44±0.33).

Table 1: Effect of supplementation of NKC decoction on HI titre (\log_2) against NDV antigen in Namakkal Chicken-1

Treatment groups	Age (weeks)			
	4	8	12	16
T ₁	0.00 ^c ±0.00	0.00 ^c ±0.00	0.00 ^c ±0.00	0.00 ^c ±0.00
T ₂	4.80 ^b ±0.33	5.60 ^b ±0.22	5.60 ^b ±0.34	3.70 ^b ±0.42
T ₃	5.44 ^{ab} ±0.33	6.20 ^{ab} ±0.42	5.64 ^b ±0.28	3.90 ^b ±0.37
T ₄	5.40 ^{ab} ±0.28	7.00 ^a ±0.37	5.60 ^b ±0.31	4.10 ^{ab} ±0.42
T ₅	6.00 ^a ±0.33	6.20 ^{ab} ±0.36	6.20 ^a ±0.27	4.50 ^a ±0.36
T ₆	5.70 ^a ±0.01	6.20 ^{ab} ±0.42	5.70 ^b ±0.30	4.10 ^{ab} ±0.42

Each value in the table is mean of ten observations. Overall means within a column with different superscripts (a, b, c) differ significantly ($p < 0.05$).

At the end of eighth week significantly higher titre was recorded in T₄ pretreated with NKC @ 0.5mL/kg (7.00±0.37) when compared to vaccine control T₂ (5.60±0.22). The positive control (T₃) and groups pretreated with NKC @ 1.0 and 2.0mL/kg (T₅, T₆) recorded mean HI titre of 6.20 which did not differ from both T₂ and T₄. At the end of twelfth week HI titre was significantly higher in T₅ pretreated with NKC @ 1.0mL/kg (6.20±0.27) when compared to other vaccinated groups (T₂, T₃, T₄ and T₆) which did not differ among themselves.

At the end of sixteenth week, the mean HI titre was between 3.70±0.42 and 4.50±0.36 which was lower than the titre of twelfth week. The groups pretreated with NKC at different dose levels had higher HI titre of which the best titre of 4.50±0.36 was found at 1.0mL/kg dose level (T₅) which differed significantly from vaccine control (T₂) and levamisole group (T₃). In all the weeks the control group (T₁) which was not vaccinated did not show any titre against NDV.

In the present study, throughout the experimental period HI titre of the group treated with NKC @ 1.0mL/kg (T₅) was significantly higher than vaccine control (T₂). The positive

control (T₃) was numerically better than vaccine control (T₂) in all the weeks. At the end of 12th and 16th week the titre of positive control was significantly less than T₅. Hence it can be concluded that the optimum dose of NKC for modulating the immune response would be 1.0mL/kg.

Discussion

The results of the study reveal that oral pellet vaccine was capable of inducing protective immunity among free range chicken. Even though the titre was around 3.7 at 16th week the birds can resist challenge with virulent virus due to stimulation of mucosal immune response^[8].

The NKC treated groups had shown numerically better response than positive control group treated with levamisole. The results concerning the effect of levamisole on humoral immunity remain controversial. The drug was reported to have little or no effect on the production of specific antibody in normal individuals but was capable of restoring the immune response in immune suppressed individuals^[9]. In the present study the birds are reared under standard and uniform managemental condition hence are not exposed to any kind of stress or immunosuppression which might be the reason for

significantly low titre in the last two weeks reported. Some authors ^[10, 11] have reported similar findings wherein they could not record appreciable effect on the humoral immunity against NDV whereas others ^[12] observed significantly high titre in levamisole group but it is understood that the response of the birds to levamisole varies based on the type of vaccine administered ^[13]. As already discussed oral pellet vaccine could stimulate mucosal immunity better than humoral immunity and hence the response to levamisole was not significant in this study.

Perusal of literature revealed that out of nine ingredients in NKC, six of them possessed immunomodulatory property. The effect of *Piper nigrum* ^[14], *Santalum album* ^[15], *Zingiber officinale* ^[16], *Cyperus rotundus* ^[17] and *Vetiveria zizanioides* ^[18] on immune response was already reported in different species. The immunomodulatory effect of *Andrographis paniculata* in broilers was attributed to the diterpene, andrographolide ^[19]. Hence it can be construed that the combined effect of the ingredients in polyherbal formulation (NKC) would have resulted in better immune response.

Further, the polysaccharides present in the plants are considered to be biological response modifiers and they enhance the immune response by activation of complements, lymphocytes and macrophages ^[20]. Flavonoid which was found in higher concentration in NKC has more beneficial effect on the immune system. They function by stimulating interferon synthesis leading to immunomodulation ^[21]. Besides other secondary metabolites such as alkaloids, glycosides, tannins and phenols also possess immunomodulatory property ^[22]. Thus all these phytochemicals in NKC act synergistically and boost the immune system in backyard chicken when used at the rate of 1.0mL/kg in drinking water for 5 days before vaccination with oral pellet vaccine against NDV.

Acknowledgement

The authors acknowledge the financial assistance and facilities provided by Tamil Nadu Veterinary and Animal Sciences University, Chennai-51 to carry out the research.

References

- Alexander DJ. Gordon memorial lecture, Newcastle Disease. British Poultry Science. 2001; 42:5-22.
- Jagapur RV, Haribabu Y. Assessment of immune response in chicken vaccinated against Newcastle disease virus. Indian Veterinary Journal. 2006; 83:927-929.
- Hashemi SR, Davoodi H. Herbal plants as new immunostimulator in poultry industry: A review. Asian Journal of Animal and Veterinary Advances. 2012; 7(2):105-116.
- Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. Edn 1, Nirali Prakasan, Pune, 1990, 123.
- Manual of diagnostic tests and vaccines for terrestrial animals. www.fao.org/docrep/006/y5162e/y5162e03.htm, 2000.
- Snedecor GM, Cochran WC. Statistical method. Edn.8, Oxford and IBH Publishing Company. Mumbai, India, 2007.
- Duncan DB. Multiple range and F tests. Biometrics. 1955; 11:1-42.
- Jayawardane GWL, Spradbrow PB. Mucosal immunity in chickens vaccinated with the V4 strain of Newcastle disease virus. Veterinary Microbiology. 1995; 46:69-77.
- Symoens J, Rosenthal M. Levamisole in the modulation of the immune response: The current experimental and clinical state. Journal of the Reticuloendothelial Society. 1977; 21:175.
- Porchezian T, Punniarthy N. Effect of oral levamisole hydrochloride on feed intake and body weight of broiler chicks. Journal of Animal and Veterinary Advances. 2006; 5:847-848.
- Sanda ME, Anene BM, Owoade. Effect of levamisole as an immunomodulatory in cockerels vaccinated with Newcastle disease vaccine. International Journal of Poultry Science. 2008; 7(11):1042-1044.
- Gatne MM, Rajashree P, Ravikanth K, Shivi M, Rekhe, DS. Evaluation of immunomodulatory effect of Stresroak premix in broiler Chicks. Veterinary World. 2010; 3(3):122-125.
- Habibi M, Zandieh MA, Ghahri H, Zadeh RS, Yeganeh F. Effects of levamisole on the immune response of broilers against Newcastle disease vaccines. African Journal of Pharmacy and Pharmacology. 2012; 6(25):1860-1864.
- Abou-Elkhair R, Ahmed HA, Selim S. Effects of black pepper (*Piper nigrum*), turmeric powder (*Curcuma longa*) and coriander Seeds (*Coriandrum sativum*) and their combinations as feed additives on growth performance, carcass traits, some blood parameters and humoral immune response of broiler chickens. Asian-Australian Journal of Animal Science. 2014; 27(6):847-854.
- Gupta A, Chaphalkar SR. Immunopharmacological screening of aqueous root extract of *Santalum album*. Journal of Herbmmed Pharmacology. 2016; 5(1):7-11.
- Puri A, Srivastava A, Bharadwaj A, Tandon JS, Saxena KC. Immunomodulatory activity of certain plants used in Indian traditional medicine. Journal of Medicinal Plants Research. 2010; 7(44):3242-3246.
- Ediriweera PS, Kankanam KA, Gunasekara DKSC. Immunostimulative effect of *Cyperus rotundus*, *Alpinia calcarata*, *Solanum surattense*, *Clerodendrum infortunatum* and *Croton laccifer* extract combination on Cyclophosphamide-induced immunosuppression in rats. Journal of Experimental and Integrative Medicine. 2015; 5(2):110-114.
- Sunitha VS, Sunil MA, Radhakrishnan EK, Mathew J. Immunomodulatory activity of *Vetiveria zizanioides* extract on peritoneal macrophages of albino mice. International Journal of Pharmaceutical Research Scholars. 2015; 4:1-4
- Mathivanan R, Kalaiarasi K. *Panchagavya* and *Andrographis paniculata* as alternatives to antibiotic growth promoters on haematological, serum biochemical parameters and immune status of broilers. Journal of Poultry Science. 2007; 44(2):198-204.
- Sharma R, Rohilla A, Arya V. A short review on pharmacology of plant immunomodulators. International Journal of Pharmaceutical Sciences Review and Research. 2011; 9(2):126-131.
- Frankie T, Volgi M, Salobir J, Rezar V. Importance of medical herbs in animal feeding: A Review. Acta Agriculturae Slovenica. 2009; 92(2):95-102.
- Nagarathnam PK, Reena M, Reddy KS, Wesley J. Review on immunomodulation and immunomodulatory activity of some herbal plants. International Journal of Pharmaceutical Sciences Review and Research. 2013; 22(1):223-230.