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R. Kavinilavan
Veterinary Assistant Surgeon, Veterinary Dispensary, Perambalur- 621212, Tamil Nadu, India

P. Mekala
Professor and Head, Department of Veterinary Pharmacology and Toxicology,Veterinary College and Research Institute, Udumalpet- 642205, Tamil Nadu, India

M.J. Raja
Professor, Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Namakkal- 637002, Tamil Nadu, India

M. Arthanari Eswaran
Professor & Head, Department of Veterinary Microbiology, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Udumalpet- 642205, Tamil Nadu, India

S. Nagajothi
Veterinary Assistant Surgeon, Veterinary Dispensary, Sholur- 643005, Tamil Nadu, India

Correspondence:
Prof. P. Mekala
Professor and Head, Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Udumalpet- 642205, Tamil Nadu, India
Email: mekskathir@gmail.com

Immunomodulatory effect of Nilavembu Kudineer Choornam in backyard chicken

R. Kavinilavan, P. Mekala, M.J. Raja, M. Arthanari Eswaran, S. Nagajothi

ABSTRACT

Aim and Objective: The study aimed to evaluate the immunomodulatory effects of Nilavembu Kudineer Choornam (NKC) on humoral, cell-mediated, and nonspecific immunity in unsexed backyard chickens. Materials and Methods: Seventy-two unsexed backyard chickens were randomly assigned to six groups (n=12 per group): control, vaccine control (Newcastle disease oral pellet vaccine), positive control (levamisole at 30 mg/kg), and three treatment groups receiving NKC decoction at 0.5, 1.0, and 2.0 mL/kg. All groups, except the control, were vaccinated at the end of the 1st, 4th, and 8th weeks. Levamisole and NKC were administered via drinking water for five days prior to immunological assessments. Humoral immunity was assessed by immunizing the birds with sheep red blood cells at the end of the 11th and 12th weeks, followed by haemagglutination titre measurements at the end of the 13th and 16th weeks. Cell-mediated immunity was evaluated using a delayed hypersensitivity reaction to phytohemagglutinin-P at the 16th week. Nonspecific immunity was assessed via a carbon clearance test at the 16th week. Results: The haemagglutination titres at the 13th week ranged from 5.80 ± 0.49 to 6.60 ± 0.60, and at the 16th week, from 4.50 ± 0.27 to 5.05 ± 0.20. The highest titres were observed in the NKC 1.0 mL/kg group, followed by the levamisole group. For cell-mediated immunity, the delayed hypersensitivity test showed significantly greater skin thickness (0.20 ± 0.01 to 0.34 ± 0.04 mm) in the treatment groups compared to the control. The carbon clearance test demonstrated enhanced phagocytic activity in all treatment groups (0.024 ± 0.001 to 0.013 ± 0.001) relative to controls. Conclusion: Administration of NKC at 1.0 mL/kg for five days before vaccination significantly enhanced humoral, cell-mediated, and nonspecific immune responses, comparable to levamisole. NKC shows potential as a natural immunomodulator in poultry, enhancing immune responses and offering a viable alternative to synthetic immunostimulants.

Keywords: Humoral immunity, Cell mediated immunity, Phagocytic activity, Carbon Clearance Index.

INTRODUCTION

Backyard poultry rearing is a vital source of nutrition and income for rural communities, with chicken meat contributing significantly to protein intake [1]. However, challenges such as disease outbreaks, predator attacks, and limited awareness of scientific rearing practices hinder productivity. Among these, Newcastle disease (ND) is the most significant constraint due to its rapid spread and high mortality rates, posing a persistent threat to both backyard and commercial poultry [2]. While vaccination is a preventive measure, factors such as limited farmer awareness, vaccine availability, and cold chain maintenance reduce its effectiveness [3]. Enhancing immune function in birds could serve as an additional strategy to mitigate ND and other disease related losses.

Herbal immunomodulators have been widely explored in traditional medicine systems for their ability to enhance immune responses. Nilavembu Kudineer Choornam (NKC), a polyherbal Siddha formulation containing *Andrographis paniculata*, *Piper nigrum*, *Zingiber officinale*, and other medicinal plants, has demonstrated antiviral and immunostimulatory effects in humans, particularly against viral infections like dengue and chikungunya [4,5]. The active compounds in these herbs influence immune cell activation, cytokine modulation, and antibody production, indicating their potential application in poultry immunomodulation [6]. However, limited research has been conducted on their efficacy in poultry, necessitating further evaluation.

This study evaluates the immunomodulatory effects of NKC in Namakkal Chicken 1, a backyard poultry breed developed for enhanced egg production, focusing on its impact on humoral, cell-mediated, and nonspecific immunity.

MATERIALS AND METHODS

Preparation of Nilavembu Kudineer Choornam (NKC) decoction

The NKC powder used in the study was procured from Tamil Nadu Medicinal Plant Farms and Herbal Medicine Corporation Limited (TAMPCOL), Chennai, India and the composition is given in Table 1. The decoction was prepared by boiling 12.5 g of NKC powder in 250 mL of water and concentrating it to 60 mL. The decoction was filtered and administered to the birds in drinking water during morning hours as a breakfast regimen. The daily requirement was calculated and freshly prepared on the day of administration.

Experimental birds and management

Seventy-two-day-old, unsexed backyard chicks (Namakkal Chicken 1) were obtained from the Poultry Farm Complex, Veterinary College and Research Institute, Namakkal. The birds were weighed, wing banded, and divided into six groups (n=12 per group). They were reared in a deep litter system under standard and uniform management conditions. The birds were fed desi chick mash (0–8 weeks) and grower mash (8–16 weeks), both free of mycotoxins, with ad libitum access to feed and drinking water. The experimental design and protocol was approved by the Institutional Animal Ethics Committee of VCRI, Namakkal.

Experimental design

Group	Treatment
T1	Control
T2	Vaccine control (Oral pellet vaccine)
T3	Positive control - Levamisole @ 30 mg/kg in drinking water for 5 days
T4	NKC decoction @ 0.5 mL/kg in drinking water for 5 days
T5	NKC decoction @ 1.0 mL/kg in drinking water for 5 days
T6	NKC decoction @ 2.0 mL/kg in drinking water for 5 days

All birds, except the control group, were vaccinated with an oral pellet vaccine (D58 strain) against Newcastle disease virus at the end of the 1st, 4th, and 8th weeks of age.

Immunological Assessments

Humoral immunity against Sheep Red Blood Cells (SRBC)

Birds in all groups, except the control, were inoculated with 0.1 mL of 1% SRBC via the brachial vein at the 11th week, followed by a booster dose at the 12th week. The control group received 0.1 mL of phosphate-buffered saline (PBS). Levamisole and NKC decoction were administered for five days before the primary SRBC injection. Blood was collected from the brachial vein at the end of the 13th and 16th weeks, and the haemagglutinin antibody (HA) titer against SRBC was estimated [7]. The HA titer was expressed as $\log_2$ of the reciprocal of the last dilution showing macroscopic agglutination.

Cell-Mediated Immunity (CMI) by PHA-P skin test

CMI was assessed at the end of the 16th week in six birds per group [8]. Levamisole and NKC decoction were administered for five days before the test. A total of 100 µg of phytohaemagglutinin-P (PHA-P) in 0.1 mL of PBS was injected intradermally into the interdigital space between the 2nd and 3rd toes of the right foot. The left foot received 0.1 mL of PBS as a control. Skin thickness was measured before and 24 h post-injection using a digital micrometer (Mitutoyo, Japan), and the web index was calculated as:

Web Index (mm) = (Post PHA-P – Pre PHA-P) – (Post PBS – Pre PBS)

Nonspecific immunity by carbon clearance test

Nonspecific immunity was assessed by evaluating phagocytic ability at the 16th week in six birds per group by carbon clearance test [8]. Birds were pretreated with levamisole and NKC decoction for five days before testing. A total of 1.0 mL/kg of sterile black Indian ink was injected into the wing vein, and blood samples were collected from the opposite wing before (0 min) and at 3 and 15 minutes post-injection. Blood samples (100 µL) were mixed with 2 mL of 10% sodium carbonate solution and centrifuged at 50 x g for 4 minutes. The relative carbon particle concentration in the supernatant was measured using a UV-Vis spectrophotometer at 650 nm, and the carbon clearance index (K) was calculated as:

$$K = (\log OD1 - \log OD2) / (t2 - t1)$$

Where, OD1 and OD2 represent optical densities at 3 minutes (t1) and 15 minutes (t2), respectively.

Statistical analysis

The experiment followed a completely randomized design [9]. Data were analyzed using one-way ANOVA in SPSS® 20.0 software. Post-hoc analysis was performed using Duncan's significance difference test.

RESULTS

Humoral immunity against Sheep Red Blood Cell (SRBC)

The mean hemagglutination (HA) titre against SRBC at the end of the 13th and 16th weeks is presented in Table 2. At the 13th week, the mean HA titre ranged between 5.80 ± 0.49 and 6.60 ± 0.60. The highest HA titre was observed in the group pretreated with NKC at 1.0 mL/kg (T5) and in the levamisole-treated group (T3), with significant differences ($p<0.05$) compared to the control group (T1). No significant differences were noted among the treatment groups (T2 - T6).

At the 16th week, the mean HA titre ranged from 4.50 ± 0.27 to 5.05 ± 0.20, with the highest value in T5. However, compared to the 13th week, a slight decline in HA titre was observed in all groups. The control group (T1) injected with PBS did not show any HA titre against SRBC antigen at both time points.

Cell-mediated immunity by PHA-P skin test

Cell-mediated immunity was assessed using the phytohemagglutinin-P (PHA-P) skin test, with the mean skin thickness values presented in Table 3. The skin thickness response at the 16th week ranged from 0.20 ± 0.01 to 0.34 ± 0.04 mm. The highest PHA-P response was observed in the T5 group (1.0 mL/kg NKC), followed by the positive control (T3). All treatment groups (T2 - T6) exhibited significantly higher ($p<0.05$) responses than the control group (T1).

Non-specific immunity by carbon clearance test

The mean carbon clearance index measured at the 16th week ranged from 0.024 ± 0.001 to 0.013 ± 0.001 (Table 3). The highest carbon clearance index was observed in T5 and T6, which received 1.0 and 2.0 mL/kg NKC, respectively, and these values were numerically superior to the levamisole-treated group (T3). The T4 group (lower NKC dose) did not show significant differences from the control (T1) or vaccine control (T2).

DISCUSSION

Humoral immunity

SRBC immunization studies indicate that the primary antibody response peaks at 5-7 days post-immunization, with a subsequent

rapid decline in antibody titre two weeks later [10]. The present study aligns with this trend, showing higher HA titres at the 13th week, followed by a decline at the 16th week. However, the observed titres remained relatively high, likely due to the booster SRBC dose and NKC's immunostimulatory effects. Previous studies have reported a peak anti-SRBC antibody titre of 5.7 in probiotic-treated groups, followed by a decline to 2.0 and 1.5 over the subsequent two weeks [11].

Table 1: Composition of Nilavembu Kudineer Choornam

S. No.	Ingredients	Percentage
1	*Zingiber officinale* (rhizome)	11.1%
2	*Piper nigrum* (dried fruit)	11.1%
3	*Cyperus rotundus* (root)	11.1%
4	*Mollugo cerviana* (plant)	11.1%
5	*Andrographis paniculata* (plant)	11.1%
6	*Trichosanthes cucumerina* (plant)	11.1%
7	*Vetiveria zizanioides* (root)	11.1%
8	*Plectranthus vettiveroides* (root)	11.1%
9	*Santalum album* (wood)	11.1%

Table 2: Effect of supplementation of Nilavembu Kudineer Chooranam decoction on HA titer (Log2) against SRBC antigen in backyard chicken

Treatment groups	HA titer (Log_2)	
	13th Week	16th Week
T1 - Control	$0.00^{c} \pm 0.00$	$0.00^{b} \pm 0.00$
T2 - Vaccine control	$5.85^{ab} \pm 0.30$	$4.50^{a} \pm 0.27$
T3 - Positive control (Levamisole @ 30mg/kg)	$6.20^{a} \pm 0.57$	$4.80^{a} \pm 0.22$
T4 - NKC @ 0.5ml/kg	$5.80^{ab} \pm 0.49$	$5.00^{a} \pm 0.39$
T5 - NKC @ 1.0ml/kg	$6.60^{a} \pm 0.60$	$5.05^{a} \pm 0.20$
T6 - NKC @ 2.0ml/kg	$5.80^{ab} \pm 0.51$	$4.70^{a-} \pm 0.27$

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).

Figure 1: Injection of PHA-P solution between 2nd and 3rd interdigital space on right foot web

Figure 2: Measurement of web thickness after 24 h of PHA-P injection

Figure 3: Injection of Indian ink in the wing vein at the dose rate of 1ml/kg body weight

Levamisole, used as the positive control, is known to enhance antibody responses to non-natural antigens [12]. The enhanced humoral immunity observed in the NKC-treated groups could be attributed to bioactive compounds such as polysaccharides, flavonoids, and phenols, which stimulate interferon synthesis. Individual NKC components like *Andrographis paniculata*, *Zingiber officinale*, and *Cyperus rotundus* have been reported to augment antibody responses against SRBC [13-15]. Furthermore, the combined effect of multiple immunostimulant herbs in NKC likely contributed to the observed increase in HA titres [16].

Cell- mediated immunity

The PHA-P test is a reliable method for assessing T-cell-mediated immune responses. The significant increase in web thickness in NKC-treated groups suggests enhanced cellular immunity. Similar improvements in PHA-P responses have been reported in birds supplemented with *Artemisia annua*, where increased lymphocyte proliferation was observed [17]. Levamisole, a known T-cell activator, also induced a strong response in the positive control group [18, 19].

Natural products are known to stimulate immune responses by directly enhancing lymphatic tissue activity and modifying gut microflora [20-22]. The immunomodulatory effect of NKC could be due to its influence on cytokine production and leukocyte activation. The observed improvement in T-cell response in the NKC-treated groups suggests a positive impact on adaptive immunity.

Non- specific immunity

The carbon clearance test evaluates the activity of mononuclear phagocyte system, reflecting innate and adaptive immune responses [23]. The significantly higher clearance index in T5 and T6 indicates enhanced phagocytic activity, likely due to NKC's bioactive compounds. NKC has been reported to contain several key phytochemicals, including alkaloids, carbohydrates, glycosides, flavonoids, phenols, tannins, and terpenoids. These compounds play a critical role in antioxidant defense, supporting the body's immune function and overall health [16]. Similar findings were reported in birds supplemented with flavonoid-rich extracts, where enhanced phagocytosis and reduced oxidative stress were observed [24].

Phagocytic cells generate reactive oxygen species (ROS) during immune responses, and an imbalance between ROS production and antioxidant defenses can lead to oxidative stress [24]. The high phenolic and flavonoid content in NKC is likely responsible for reducing oxidative stress, thereby improving phagocytic function. Comparable improvements in phagocytic indices have been reported for fenugreek and *Arachis hypogaea* [25, 26]. The ability of NKC to enhance non-specific immunity may be attributed to the synergistic action of its herbal components.

CONCLUSION

The present study demonstrated that NKC decoction at 1.0 mL/kg body weight effectively enhances both humoral and cell-mediated immunity in Namakkal Chicken-1. The highest HA titre against SRBC and PHA-P skin test response were observed in the NKC-treated group, comparable to levamisole, indicating its potent immunostimulatory effects. Additionally, NKC significantly improved phagocytic activity, as evidenced by the carbon clearance index, suggesting an enhancement of nonspecific immunity. The immunomodulatory effects of NKC can be attributed to the synergistic action of its bioactive phytochemicals. These findings highlight NKC as a natural alternative to synthetic immunostimulants, with potential applications in poultry health management.

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Conflict of interest

The authors declared no conflict of interest.

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ORCID ID

P. Mekala: https://orcid.org/0000-0002-1600-3498

R. Kavinilavan: https://orcid.org/0009-0005-1858-6593

M.J. Raja: https://orcid.org/0000-0001-5530-7913

M. Arthanari Eswaran: https://orcid.org/0000-0003-4679-8364

S. Nagajothi: https://orcid.org/0009-0003-8262-7922

REFERENCES

1. Kumar R, Gupta I, Bisht A, Kumar A, Mishra S, Gupta VK, et al. Occurrence of Newcastle disease in backyard poultry: A pathomorphological study. Indian J Vet Pathol. 2022;46(4):339-41.
2. Joshi VG, Chaudhary D, Bansal N, Singh R, Maan S, Mahajan NK, et al. Prevalence of Newcastle disease virus in commercial and backyard poultry in Haryana, India. PLoS One. 2022;17(2):e0264028.
3. Kumar R, Dey S, Sharma P, Bhardwaj K, Kumar A, Bhatia A, et al. A study of risk factors associated with Newcastle disease and molecular characterization of Newcastle disease virus isolated from backyard and commercial poultry flocks in Assam, India. Trop Anim Health Prod. 2022;54(3):179. doi:10.1007/s11250-022-03159-8.
4. Srivastava A, Rengaraju M, Srivastava S, Narayan V, Gupta V, Upadhayay R. A double-blinded placebo-controlled comparative clinical trial to evaluate the effectiveness of Siddha medicines, Kaba Sura Kudineer (KSK) and Nilavembu Kudineer (NVK), along with standard allopathy treatment in the management of symptomatic COVID-19 patients—a structured summary of a study protocol for a randomized controlled trial. Trials. 2021;22(1):130.
5. Maideen NMP. Efficacy of Nilavembu Kudineer for Dengue Fever Management – An Overview of Clinical and Preclinical Evidences. South East Eur J Immunol. 2023;6(1):109-14.
6. Amalorpavanaden ND, Mani J. Antipyretic, anti-inflammatory and analgesic properties analysis by GC-MS, FT-IR and phytochemical screening of *Carica papaya* L. and Nilavembu Kudineer Choornam. Int J Bot Stud. 2021;6(2):392-403.
7. Revagade AYH, Ali D, Singh P, Rajnarayan. Impact of Naked neck (Na) and Frizzle (F) gene on general immunocompetence in egg-type chicken. Indian J Poult Sci. 2013;48(1):101-4.
8. Cheng S, Lamont SJ. Genetic analysis of immunocompetence measures in a White Leghorn chicken line. Poult Sci. 1988;67(7):989-95.
9. Snedecor GM, Cochran WC. Statistical methods. 8th ed. Mumbai: Oxford and IBH Publishing Company; 2007.
10. Nagase F, Nagashima I, Ikato. Studies on the immune response in chickens IV: Generation of hapten-specific memory and absence of increase in carrier-specific helper memory in antibody response to sheep red blood cell antigen and its hapten conjugate. Dev Comp Immunol. 1983;7:127-37.
11. Haghighi HR, Gong J, Gyles CL, Hayes MA, Sanei B, Parvizi P, et al. Modulation of antibody-mediated immune response by probiotics in chickens. Clin Diagn Lab Immunol. 2005;12(12):1387-92.
12. Porchezhian T, Punniamurthy N. Effect of oral levamisole hydrochloride on feed intake and body weight of broiler chicks. J Anim Vet Adv. 2006;5:847-8.
13. Puri A, Saxena R, Saxena P, Saxena KC, Srivastava V, et al. Immunostimulant agents from *Andrographis paniculata*. J Nat Prod. 1993;56(7):995-9.
14. Mathivanan R, Kalaiarasi K. Panchagavya and Andrographis paniculata as alternatives to antibiotic growth promoters on hematological, serum biochemical parameters, and immune status of broilers. J Poult Sci. 2007;44(2):198-204.
15. Puri A, Srivastava A, Bharadwaj A, Tandon JS, Saxena KC. Immunomodulatory activity of certain plants used in Indian traditional medicine. J Med Plants Res. 2010;7(44):3242-6.
16. Kavinilavan R, Mekala P, Raja MJ, Arthanari Eswaran M, Thirumalaisamy G. Exploration of immunomodulatory effect of Nilavembu Kudineer Choornam against Newcastle disease virus in backyard chicken. J Pharmacogn Phytochem. 2017;6(6):749-51.
17. Soppi E, Lassila O, Viljanen MK, Lehtonen OP, Eskola J. *In vivo* effect of levamisole on cellular and humoral immunity in normal chickens. Clin Exp Immunol. 1979;38:609-14.
18. Hassan AB, Atta AH, Solman RT, Nehal, Afifi A. Effect of levamisole hydrochloride on the immune response to Newcastle disease virus vaccine in chickens. Indian Vet J. 1989;66:389-94.
19. Sani L, Mohammadi M, Sendi JJ, Abolghasemi SA, Mehr RA. Extract and leaf powder effect of *Artemisia annua* on performance, cellular and humoral immunity in broilers. Iran J Vet Res. 2013;14(1):15-20.
20. Frankic T, Volgi M, Salobir J, Rezar V. Importance of medicinal herbs in animal feeding: a review. Acta Agric Slov. 2009;92(2):95-102.
21. Sharma R, Rohilla A, Arya V. A short review on pharmacology of plant immunomodulators. Int J Pharm Sci Rev Res. 2011;9(2):126-31.
22. Hashemi SR, Davoodi H. Herbal plants as new immuno-stimulators in the poultry industry: a review. Asian J Anim Vet Adv. 2012;7(2):105-16.
23. Yamate J, Yohinda H, Tsukamoto Y, Ide M, Kuwamura M, et al. Distribution of cells immunopositive for AM-3K, a novel monoclonal antibody recognizing human macrophages, in normal and diseased tissues of dogs, cats, horses, cattle, pigs, and rabbits. Vet Pathol. 2000;37(2):168-76.
24. George A, Chinnappan S, Choudhary Y, Bommu P, Sridhar M. Immunomodulatory activity of an aqueous extract of

Polygonum minus Huds on Swiss albino mice using carbon clearance assay. Asian Pac J Trop Dis. 2014;4(5):398-400.
25. Tripathi S, Maurya AK, Kahrana M, Kaul A, Sahu RK. Immunomodulatory property of ethanolic extract of *Trigonella foenum*-graecum leaves on mice. Der Pharm Lett. 2012;4(2):708-13.
26. Babu NL, Ashok P, Surana YS, Srivastava R, Madharkhandi A, et al. Immunomodulatory activity of aqueous extract of *Arachis hypogaea* seeds (Fabaceae) in rats. Int J Pharm Sci Rev Res. 2015;34(2):130-4.

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