



Effect of *Tinospora cordifolia* extract on immune response and serum biochemical profile in broilers

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Apparently healthy, day-old broiler chicks (100) were procured from a private poultry farm. All the chicks were maintained under standard conditions of feeding and management. The chicks were divided into 6 groups each comprising 15 birds each. The chicks of the first group were fed with aqueous extracts of *T. cordifolia* stem dried at high temperature @ 1 g/kg feed and were vaccinated against La Sota (lentogenic) strain of Newcastle disease (ND) virus as per the recommended schedule. The chicks of the second group were fed with aqueous extracts of *T. cordifolia*, stem dried at low temperature @ 1 g/kg feed and were vaccinated against ND as per the recommended schedules. The chicks of third group were fed *T. cordifolia* stem juice concentrate in *T. cordifolia* powder at low temperature @ 1 g/kg feed and were vaccinated against ND as per recommended schedule. The chicks of fourth group were not provided with *T. cordifolia* extract but were vaccinated accordingly against ND. The chicks of fifth group were not provided with an extract and they were also unvaccinated. The chicks belonging to the last (sixth) group were offered with levamisole @ 10 mg/kg body weight and were vaccinated with ND vaccination as per recommendation and were kept as control group.

Determination of maternal antibody level in chicks pre-vaccination was done in the chicks at first and seventh day age by hemagglutination (HA) and hemagglutination inhibition (HI) tests respectively, from the collected sera samples, except the fifth group of birds which remained unvaccinated throughout the present study. Blood was

collected from the wing veins of six chicks each on seventh day and the serum was separated. Hemagglutination (HA) test and HI test were carried out as per Buxton and Fraser (1977) with certain modifications.

For seeing the effects of different treatments on humoral immune response, analysis of variance (ANOVA) was done as per the method of Snedecor and Cochran (1967).

Total protein, albumen, globulin concentrations and A:G ratio were performed according to modified Biuret and Doumas method (Doumas 1971) with slight modifications. Hematological analyses for total leukocyte count and total erythrocyte count was done according to the method of Natt and Herrick (1952). Hemoglobin (Hb) concentration was measured by using Sahli's hemoglobinometer as per Schalm *et al.* (1975).

Cell-mediated immune response was studied based on delayed hypersensitivity reaction or the different experimental groups was measured by contact sensitivity test with 2,4-dinitrofluorobenzene (DNFB). The test was performed as per the method of Tiwary and Goel (1985) with slight modifications.

The mean hemagglutination antibody titre shows an overall increasing trend in the treated groups as the age of bird increased (Table 1). Similarly, the promising effects of treatments of *T. cordifolia* stem extract was shown in different groups were also analyzed for total protein concentration, serum albumen and serum globulin concentrations (mg/dl) and A:G ratio was also measured along with total leukocyte count ($10^3/\mu\text{l}$), total erythrocyte count ($10^4/\mu\text{l}$) and estimation of Hb concentration (g%). The effect on serum protein profile is represented in Table 2. It revealed a significant effect of treatment on the hematological profile of the broiler chicks (Table 3).

Kolte *et al.* (2007) studied the effect of feeding *T. cordifolia* in broiler birds which were immunosuppressed with cyclophosphamide. A significant rise was found in

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Table 1. Mean hemagglutination inhibition antibody titre ($\log_{10}$ value) of chick sera of different treatment groups during different periods of time

Age of chicks (days)	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
1	2.258 $\pm$ 0.067	2.258 $\pm$ 0.067	2.258 $\pm$ 0.067	2.258 $\pm$ 0.067	2.258 $\pm$ 0.067	2.258 $\pm$ 0.067
7	1.154 $\pm$ 0.093	1.154 $\pm$ 0.093	1.154 $\pm$ 0.093	1.154 $\pm$ 0.093	1.154 $\pm$ 0.093	1.154 $\pm$ 0.093
14	1.856 $\pm$ 0.121	1.806 $\pm$ 0.101 ^c	1.605 $\pm$ 0.063 ^{bc}	1.404 $\pm$ 0.063 ^b	0.903 $\pm$ 0.077 ^a	2.157 $\pm$ 0.121 ^d
21	2.207 $\pm$ 0.063 ^c	2.207 $\pm$ 0.063 ^c	1.957 $\pm$ 0.067 ^b	1.806 $\pm$ 0.109 ^b	0.652 $\pm$ 0.121 ^a	2.508 $\pm$ 0.063 ^d
28	2.056 $\pm$ 0.092 ^c	2.006 $\pm$ 0.063 ^c	1.806 $\pm$ 0.010 ^b	1.656 $\pm$ 0.067 ^b	0.00 ^a	2.307 $\pm$ 0.100 ^d
35	2.357 $\pm$ 0.093 ^c	2.360 $\pm$ 0.140 ^c	2.057 $\pm$ 0.093 ^b	1.957 $\pm$ 0.103 ^b	0.00 ^a	2.759 $\pm$ 0.093 ^d
42	2.709 $\pm$ 0.109 ^c	2.759 $\pm$ 0.050 ^c	2.408 $\pm$ 0.077 ^b	2.207 $\pm$ 0.100 ^b	0.00 ^a	3.161 $\pm$ 0.128 ^d
ANOVA value	27.462**	31.813**	34.355**	21.543**	142.931**	40.789**

(** P<0.01), Values bearing different superscripts in a row differed significantly. Each value is an average of 6 observations.

Table 2. Effect of treatment on mean total protein, total albumen, total globulin concentrations (g/dl) and A:G ratio in different groups of broiler birds (n=8)

Group	Total proteins	Serum albumen concentration	Serum globulin concentration	A:G ratio
1	3.073 $\pm$ 0.279	1.486 $\pm$ 0.062	1.586 $\pm$ 0.336	0.936:1
2	3.015 $\pm$ 0.271	1.451 $\pm$ 0.083	1.563 $\pm$ 0.299	0.927:1
3	2.989 $\pm$ 0.257	1.508 $\pm$ 0.047	1.478 $\pm$ 0.297	1.021:1
4	2.751 $\pm$ 0.243	1.471 $\pm$ 0.053	1.296 $\pm$ 0.257	1.146:1
5	2.612 $\pm$ 0.227	1.496 $\pm$ 0.036	1.120 $\pm$ 0.238	1.335:1
6	3.120 $\pm$ 0.249	1.493 $\pm$ 0.053	1.660 $\pm$ 0.263	0.899:1
ANOVA value	0.619 ^{NS}	0.127 ^{NS}	0.519 ^{NS}	NA

NS, Not significant; NA, Not applicable.

Table 3. Effect of treatment on Hb concentration (g %), mean total erythrocyte count ($10^4/\mu$ l) and total leukocyte count ($10^3/\mu$ l) in broiler birds of different groups (n=6) at the end of experiment

Group	Hb%	Total erythrocyte count ($10^4/\mu$ l)	Total leukocyte count ($10^3/\mu$ l)
1	8.61 $\pm$ 0.113 ^b	261.830 $\pm$ 7.162 ^b	26.166 $\pm$ 0.307 ^c
2	8.65 $\pm$ 0.123 ^b	258.00 $\pm$ 7.271 ^b	25.631 $\pm$ 0.401 ^c
3	8.58 $\pm$ 0.149 ^b	256.177 $\pm$ 7.125 ^b	24.833 $\pm$ 0.307 ^b
4	8.18 $\pm$ 0.090 ^a	223.667 $\pm$ 10.984 ^a	24.162 $\pm$ 0.170 ^{ab}
5	8.12 $\pm$ 0.108 ^a	219.333 $\pm$ 7.723 ^a	23.667 $\pm$ 0.421 ^a
6	8.33 $\pm$ 0.109 ^{ab}	242.167 $\pm$ 7.359 ^{ab}	28.166 $\pm$ 0.307 ^d
ANOVA value	3.952**	5.196**	24.452**

(**, P<0.01). Values bearing same superscripts in a row did not differ significantly.

Table 4. Mean skin thickness (in mm) of birds of different treatment groups at different hours post-challenge with DNFB.

Treatment period (in hour)	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	ANOVA value
0	0.521 $\pm$ 0.006	0.526 $\pm$ 0.012	0.523 $\pm$ 0.014	0.518 $\pm$ 0.014	0.513 $\pm$ 0.024	0.530 $\pm$ 0.010	0.154 ^{NS}
6	0.685 $\pm$ 0.036 ^a	0.693 $\pm$ 0.039 ^a	0.666 $\pm$ 0.050 ^a	0.635 $\pm$ 0.030 ^a	0.631 $\pm$ 0.028 ^a	0.805 $\pm$ 0.034 ^b	2.990*
24	0.945 $\pm$ 0.043 ^b	0.966 $\pm$ 0.068 ^b	0.938 $\pm$ 0.051 ^b	0.773 $\pm$ 0.035 ^a	0.756 $\pm$ 0.020 ^a	1.217 $\pm$ 0.055 ^c	8.029**
48	1.207 $\pm$ 0.062 ^b	1.218 $\pm$ 0.087 ^b	1.195 $\pm$ 0.070 ^b	0.998 $\pm$ 0.032 ^a	0.976 $\pm$ 0.046 ^a	1.561 $\pm$ 0.065 ^c	11.042**
72	1.102 $\pm$ 0.063 ^b	1.112 $\pm$ 0.075 ^b	1.074 $\pm$ 0.054 ^b	0.877 $\pm$ 0.026 ^a	0.863 $\pm$ 0.033 ^a	1.403 $\pm$ 0.059 ^c	13.160**

(*P<0.05), (** P<0.01). NS, Not significant. Values bearing different superscripts in a row differed significantly. Each value is an average of 6 observations.

antibody titer against ND virus with augmentation of inflammatory reaction to skin contact sensitivity test (Table 4), which is similar to the findings in the present study. There were insignificant differences in serum albumen and globulin concentrations between the groups. These findings are in correlation to Rege *et al.* (1989) and Bishavi *et al.* (2002) who have proved the hepatoprotective activities of *T. cordifolia*. In the present study, total lymphocyte count was increased in groups 1 and 2 as compared to group 3. Manjrekar *et al.* (1999) also had found that aqueous extract

of *T. cordifolia* is capable of increasing leukocyte count in mice. In the present study, the treated groups of broiler birds showed significantly higher Hb% and total erythrocyte count as similar to the findings of Mathew *et al.* (1999). Throughout the present study, no significant difference was observed among *T. cordifolia* stem extract fed group. The findings in the present study were in accordance with those of Kolte *et al.* (2007).

Significantly higher (P<0.01) values of mean humoral antibody titer was observed in group 6 in the present study

which is similar to the findings Soppi *et al.* (1979) where they concluded that levamisole influences both cellular and humoral immune responses. Significantly higher total protein and serum globulin concentrations in levamisole treated group of broiler birds comply with the findings of Vyas *et al.* (1987) and Karnataka *et al.* (1993). Higher Hb concentration in levamisole fed group may be due to anti-parasitic activities of levamisole allowing better absorption of the food particles in intestinal lumen.

SUMMARY

The present study was done to determine the effect of *Tinospora cordifolia* stem extract in enhancing and strengthening the humoral and cell-mediated immune response in broiler chicks against Newcastle disease along with its impact on serum protein and hematological profile. The study indicated marked overall promising and significant ($P < 0.01$) improvement in the concentration of both mean hemagglutination antibody titre and cell-mediated immune status along with significantly ($P < 0.01$) encouraging response reflected by the hematological profile and biochemical parameters in serum in the treated groups. The control birds were administered with levamisole, a potent and proven immunomodulator and a comparative study was laid down between the treated and control groups in the present study for the better analysis of the immunomodulatory effects of *T. cordifolia* stem in relation to the promising effects of levamisole.

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