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Immunomodulatory effect of herbal feed supplement in normal and immunocompromised broiler chicks

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Diseases, pesticides and chemicals may lead to immunosuppression in the birds. Large numbers of reports are available on outbreak of Newcastle disease (ND) resulting in alarming economic losses mainly due to vaccine failure even after programmed vaccination schedules have been used (Chakraborty and Chatterjee 1998). Many plant preparations are prescribed in Ayurveda to hasten host resistance (Thatte and Dahanukar 1986). Roots of *Asparagus racemosus* (commonly called 'Satavar') possess anti-diarrheal, anti-ulcerative, anti-spasmodic, aphrodisiac, galactogogue and other properties and has therefore gained its importance in Ayurveda, Siddha and Unani systems of medicine (Nadkarni 1954). *Withania somnifera* (commonly called 'Ashwagandha') possess anti-estrogenic, adaptogenic, anti-cancer and anabolic activities having beneficial effects in the treatment of arthritis, geriatric problems and stress.

In the present study, immunomodulatory effects of *W. somnifera* and *A. racemosus* plants were attempted by monitoring their effects on various non-specific and specific humoral and cellular immunological response parameters.

Day-old chicks (100) were procured from a private hatchery and were maintained under standard management conditions. On the seventh day, they were divided into 8 groups (group 1-8) comprising 10 chicks in each group. They were fed standard broiler starter (0–2 weeks), broiler grower (3–4 weeks) and broiler finisher (5–6 weeks) ration.

Thymocytes and bursacytes were procured from 2-week-old chicken thymus lobes and bursa of fabricius was collected aseptically in pre-chilled Hank's balanced salt solution (HBSS). The suspensions of thymocytes and bursacytes were prepared by teasing the tissue against sterile plastic mesh in HBSS. Cell suspension was passed repeatedly through stainless steel wire gauze. Cell suspension thus obtained was washed twice in HBSS by centrifugation. After the last centrifugation at 1500 rpm for 10 min, the supernatant was discarded and the cells were resuspended in HBSS. The viable count was then done by trypan blue dye exclusion method.

Both ABS and ATS were raised in apparently healthy ducks as per Mishra and Jaiswal (1984) with certain modifications. Eight ducks of 16 weeks age were used for raising ATS and ABS respectively, each of which were administered with 10^9 thymocytes or bursacyte cell suspension in doses as per the schedule given below:

Day	1	10	20	28
Dose (ml)	1	1	1	1
Route	Subcutaneous (SC)	Intravenous (IV)	Intravenous (IV)	Intravenous (IV)

The ducks were bled after 1 week of last injection. Their sera were collected, heat inactivated at 56°C for 30 min and were stored at –20°C for further use. Normal duck sera were also collected from the same birds at 0 day before inoculation of thymocytes and bursacytes.

The ATS and ABS were absorbed separately as per Albini and Wick (1974) with certain modifications. Viable bursacytes in the concentration of 3–5 × 10^6 cells/ml of ATS and thymocytes in the concentration of 4–5 × 10^7 cells/ml of ABS were added and absorbed overnight at 4°C. ATS and ABS were clarified by centrifugation at 2500 rpm for 15 min.

Cytotoxicity test was done to determine activities of ATS and ABS respectively. The test was performed in 96 well flat bottom tissue culture plate.

Immunosuppression was done by administration of DATG and DABG in chicks as per the method of Cameron *et al.* (1974) with slight modifications. The following schedule of administration of DATG and DABG was followed:

Day post Vaccination	2	5	8	11
Dose (ml)	0.5	0.5	2	2
Route	Subcutaneous (SC)	Intravenous (IV)	Intravenous (IV)	Intravenous (IV)

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Monitoring the humoral immune response: HA and HI tests were performed according to Buxton and Frazer (1977).

Monitoring the cellular immune response by contact *sensitivity test:* Six chicks were randomly picked up from each experimental group on 28th day of experiment for standardization with DFNB by single percutaneous application of 0.25 ml of DFNB (2,4 dinitrofluorobenzene) @ 10 mg/ml in the vehicle consisting of acetone and olive oil (4:1) mixture as per Tiwary and Goel (1985). Featherless area of about 20 cm^2 was chosen on left and right lateral abdomen for DFNB application.

The virus challenge was done on 14th day post-application (DPA) with 0.25 ml DNFB 1 mg/ml solution on left marked area and the right side was painted with vehicle only. The skin reaction was measured before challenge and post-challenge on 0, 6, 24 and 48 days post-challenge with vernier calipers. An average of three consecutive measurements was made to find out the mean skin thickness of individual chicks within the groups.

All data obtained were subjected to statistical analyses (Snedecor and Cochran 1967) and Systat software.

The mean hemagglutination inhibition (MHI) antibody titer of chick sera are presented in Table 1. There was significant effect of treatment with herbal preparations on HI antibody titer at all the days post-vaccination at weekly intervals up to 35th day post-vaccination. There was significant decrease in mean antibody titer from day 1 to 7 in normal as well as immunocompromised chicks.

Similarly, mean antibody titers determined by indirect ELISA of ND vaccinated chick sera in different groups showed a significant increase in antibody titer in treated groups administered with herbal preparations (Table 2).

The extent of cell mediated immune reaction was observed by increase in mean skin thickness of sensitized normal and immunocompromized chickens (Table 3). The cell mediated immune response as shown by the increase in skin thickness was higher among chicks treated with herbal preparations than the other groups, which did not differ significantly between themselves. The purpose of thymectomy and bursectomy supplemented with DATG and DABG was to create immunosuppressive conditions.

In the present study it was seen that administration of *W. somnifera* and *A. racemosus* dried root powder might have significantly reduced lymphocytopenia in immunosuppressed

Table 1. Mean hemagglutination inhibition (MHI) antibody titer ((log10 values) in different groups of chick

Age (in days)	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
1	2.258±0.068^a	2.258±0.068^a	2.258±0.068^a	2.258±0.068^a	2.258±0.068^a	2.258±0.068^a	2.258±0.068^a	2.258±0.068^a
7	0.752±0.067^a	0.752±0.067^a	0.752±0.067^a	0.752±0.067^a	0.752±0.067^a	0.752±0.067^a	0.752±0.067^a	0.752±0.067^a
14	0.652±0.05^a	1.304±0.064^a	0.702±0.064^a	1.655±0.067^c	0.852±0.050^a	1.153±0.050^b	0.752±0.067^a	1.003±0.064^a
21	0.551±0.050^a	1.404±0.064^b	0.702±0.064^a	1.755±0.050^c	0.953±0.050^a	1.454±0.050^b	0.802±0.064^a	1.103±0.064^a
28	0	1.204±0.000^b	0.301±0.000^c	1.705±0.064^d	0.752±0.067^e	1.404±0.064^f	0.501±0.064^g	1.003±0.064^h
35	0	1.705±0.064^b	0.200±0.064^c	2.608±0.064^d	1.053±0.067^e	2.207±0.064^f	0.551±0.050^g	1.655±0.067^b
42	0	2.257±0.067^b	0.100±0.064^a	2.960±0.050^c	1.103±0.064^d	2.307±0.064^b	0.652±0.050^e	1.956±0.067^f

Values bearing different superscripts in a row differed significantly; each value is the average of 6 observations±SE;NA, not applicable (**P<0.001).

Table 2. Mean antibody titers (log_{10} values) as determined by ELISA in different groups of chick

Age (in days)	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
1	33324±4.8^a	33324±4.8^a	33324±4.8^a	33324±4.8^a	33324±4.8^a	33324±4.8^a	33324±4.8^a	33324±4.8^a
7	9924.84 ±6.32^a	27316.03 ±7.17^b	9755.19 ±37.58^c	35113.91 ±6.22^d	8230.19 ±13.50^e	27317.03 ±6.72^b	6855.25 ±3.0^f	26918.09 ±4.49^g
14	5412.88 ±6.03^a	35117.41 ±5.61^b	5732.5 ±5.14^c	38714.77 ±5.23^d	9625.17 ±10.53^e	35125.38 ±2.8^b	8257.76 ±1.7^f	27310.69 ±6.7^g
21	5412.88 ±6.03^a	35117.41 ±5.61^b	5732.5 ±5.14^c	38714.77 ±5.23^d	9625.17 ±10.53^e	35125.38 ±2.8^b	8257.76 ±1.7^f	27310.69 ±6.7^g
28	841.65 ±2.73^a	27321.38 ±6.76^b	1136.54 ±6.37^c	35085.22 ±26.95^d	7836.77 ±5.302^e	27921.83 ±6.27^f	5243.33 ±8.67^g	24122.54 ±7.13^h
35	100±0^a	27921.08 ±4.96^b	422.84 ±7.22^c	38737.93 ±5.39^d	8237.63 ±12.0^e	29326.67 ±5.33^f	5754.87 ±6.99^g	26911.14 ±9.45^h
42	100±0^a	30120.36 ±6.44^b	143.22 ±9.50^c	39373.6 ±5.32^d	9613.5 ±3.80^e	30133.79 ±3.24^b	6334.43 ±5.24^f	27929.49 ±6.60^g

Values bearing different superscripts in a row differed. Each value is the average of 6 observations±SE.

Table 3. Average skin thickness (mm) of broiler chicks at different hours post DNFB challenge

Period (h)	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
0	1.642±0.015^{a}	1.758±0.020^{b}	1.625±0.011^{a}	1.817±0.021^{b}	1.742±0.020^{b}	1.250±0.018^{c}	1.633±0.017^{d}	1.208±0.008^{c}
6	2.325±0.017^{a}	2.625±0.011^{b}	2.750±0.171^{b}	3.308±0.015^{c}	3.150±0.026^{c}	1.825±0.017^{d}	2.750±0.018^{e}	1.650±0.022^{d}
24	2.425±0.025^{a}	3.283±0.021^{b}	2.767±0.017^{c}	3.583±0.031^{d}	3.400±0.047^{e}	1.917±0.028^{f}	2.958±0.027^{g}	1.408±0.015^{h}
48	1.875±0.025^{a}	3.067±0.04^{b}	2.167±0.053^{c}	3.292±0.015^{d}	3.058±0.027^{b}	1.583±0.011^{c}	2.408±0.058^{f}	1.275±0.036^{g}
72	1.725±0.011^{a}	1.908±0.015a	1.833±0.011^{a}	2.108±0.049^{b}	2.050±0.032^{b}	0.950±0.022^{c}	1.783±0.031^{d}	0.933±0.011^{c}

Values bearing different superscripts in a row differed significantly; each value is the average of 6 observations±SE (**P<0.001).

bursectomized and thymectomized broiler chicks supplemented with DABG and DATG respectively. This finding simulates with the findings of Kuttan and Kuttan (1992) who also reported the same observations in swiss albino mice. The previous studies demonstrated that *W. somnifera* and *A. racemosus* extracts increase phagocytic activities of macropahages *in vitro* (Kuttan and Kuttan 1992). There are studies on the immunomodulatory activities of *W. somnifera* and *A. racemosus* in mice with myelosuppression induced by cyclophosphamide, azathioprim or prednisolone. This finding supports our result in the current investigation that both *W. somnifera* and *A. racemosus* extracts stimulate immune response activities in immunosuppressed busectomized and thymectomized broiler chicks supplemented with DABG and DATG respectively. Extracts of *W. somnifera* and *A. racemosus* also showed immunopotentiating effects in cyclophasphamide treated mouse with ascetic sarcoma (Diwanay *et al.* 2004). The findings in the present study simulate with those reported by Kalita and Dutta (1999) who also reported that maternal antibody was persistently found in sera samples tested against ND virus during the first week of age. In the present study, the highest antibody level as detected by ELISA on 42nd day of age i.e. 14th day of booster immunization in all vaccinated groups. This increase in the level of antibodies was in accordance with the findings of Padamanabhan (1976) and Kaiser *et al.* (1998). Among the various species of animals, ducks were found to be suitable subjects for production of effective chicken antithymocyte and antibursacyte globulin (Jaiswal *et al.* 1981). ATS and ABS derived from duck was successfully used by Cameron *et al.* (1974) and Tiwary and Goel (1985) to raise cell mediated immune response in humoral immune deficient chicken as similar to the present study. The present investigation showed that both DATG and DABG are strong immunosuppressant and are similar to the reports of various other workers (Gupta *et al.* 1999, Ara 2001). In the present study, nonsignificant effect of treatment on skin thickness in contact sensitivity test with DNFB at 0, 48 and 72 h post-challenge. These results were similar to Kumari (2005) than the treated group with *W. somnifera* which revealed significantly higher results at 48 and 72 h post-challenge with DNFB. Muruganadan *et al.* (2001) reported the effect of ethanolic extracts of *A. racemosus* humoral cell mediated immune response in mice. The reports simulate to our findings in immunocompromized broiler chicks showing the effects of dried root powder of *W. somnifera* and *A. racemosus* on humoral immune system of chicks assessed by HI and ELISA antibody titer responses and by cell mediated immune responses in normal and immunocompromized broiler chicks.

SUMMARY

The use of *W. somnifera* and *A. racemosus* dried root powder in a specific dose during the scheduled period showed significant positive effects on both humoral and cell mediated immune responses of the birds. The bursectomized and thymectomized birds showed a decline in the antibody titer. The variation in skin thickness was significantly more among the herbal treated. Herbal formulation containing extracts of *W. somnifera* and *A. racemosus* may be therefore recommended for use as positive immunomodulator in normal and immunocompromized broiler chicks. The present study also indicated the determinative roles of herbal fed additives in helping the immunodeficient subjects in obtaining higher humoral and cell mediated immune responses providing better protection level against infections.

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