STUDY ON THE IMMUNO-MODULATORY EFFECT OF HERBAL EXTRACT OF *ASPARAGUS RACEMOSUS* WILDL. IN BROILER CHICKS

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Abstract

The present study was carried out to determine the immuno-modulatory effects of *Asparagus racemosus* extract treated feed and to analyze the role of T and B cells in host defense against Newcastle disease in one week old normal and immuno-compromized broiler chicks. After the treatment significant (P<0.01) positive effects were observed in both humoral and cell mediated immune responses of the birds which was found to be evident by increased antibody titer after Hemagglutination inhibition (HI) test. The variation in skin thickness was significantly (P<0.01) more among the herbal treated groups rather than the non-treated groups which was a clear marker for immuno-stimulation among the birds.

Key words: Immuno-modulatory, *Asparagus racemosus*, host defense, humoral, cell mediated
Introduction

Poultry rearing is the fastest growing industry in livestock sector currently which is benefiting us from production and advantages in prices as well as providing with healthy food. In India, poultry industry is recognized as an important cottage as well as fast growing, large commercial agriculture industry. Poultry farming is always prone to a heavy risk of increased disease incidences leading to high mortality even if scheduled mass vaccination programmes are implemented. Certain diseases, pesticides and chemicals may lead to immune-suppression in the birds. Large numbers of report are available on outbreak of Newcastle disease (ND) resulting in alarming economic losses mainly due to ‘Vaccine failure state’ even after programmed vaccination schedules have been used (Chakraborty and Chatterjee 1998).

Many plant drugs have been categorized under ‘Rasayana Dravyas’ and are prescribed in Ayurveda to hasten host resistance (Thatte and Dahanukar 1986). Asparagus racemosus fall in this category along with many other useful plants. They exhibit immuno-modulatory activities. The root of Asparagus racemosus (commonly called ‘Satavar’) possesses 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).

In the present study, judgment of the immuno-modulatory effects of Asparagus racemosus root extract has been attempted by monitoring their effects on various non-specific and specific humoral and cellular immunological response parameters.

Materials and Methods

Fifty (50) days old broiler chicks were procured from a private hatchery and were maintained under standard hygienic conditions of feeding and housing. On the 7th day, they were divided into three groups (Group 1-3) comprising of fifteen (15) chicks in each group. They were provided with ration as broiler starter (0-2 weeks), broiler grower (3-4 weeks) and broiler finisher (5-6 weeks). Group 1 consisted of treated chicks fed with A. racemosus extract treated feed, Group 2 was kept as vaccinated control comprising of chicks administered with Newcastle disease (ND) vaccine as per recommended schedule but without being fed with A. racemosus extract treated feed, Group 3 was the non-vaccinated control which consisted of untreated and unvaccinated chicks respectively.

Monitoring of humoral immune response

Hemagglutination (HA) test and Hemagglutination inhibition (HI) tests were performed according to Buxton and Frazer (1977) with certain modifications. Fowls were screened for suitable RBCs required for HA test. Their blood was collected aseptically in Alsever’s solution and was centrifuged at 1500 g for 10 min. The solution was washed and centrifuged three times in phosphate buffer saline (PBS) solution. The supernatant was discarded and packed blood cells were re-suspended in PBS solution, pH 7.2 to make 1% v/v RBC suspension. La Sota strain of Newcastle disease virus vaccine was procured and was used as virus stock solution for conducting HA test. The readings of antibody titer were converted into log10 values and the converted values were subjected to statistical analysis. For seeing the effect of different treatments on humoral immune response, analysis of variance (ANOVA) was done as per the method of Snedecor and Cochran (1967).

Monitoring of 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 2,4 dinitrofluorobenzene (DFNB) by single percutaneous application of 0.25 ml DFNB @ 10 mg/ml in the vehicle consisting of acetone and olive oil (4:1) mixture as per the method described by Tiwary and Goel (1985). Featherless area of about 20 cm² 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 with Vernier Calipers before challenge and post-challenge on 0, 6, 24 and 48 days age of the birds. 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 analysis as per standard methods and techniques laid down by Snedecor and Cochran (1967).

**Results and Discussion**

Mean hemagglutination inhibition (MHI) antibody titer of chick sera has been presented in Table 1 and their analysis of variance indicated an overall significant effect of treatment with herbal preparation on HI antibody titer at all the days post-vaccination at weekly intervals up to 42nd day post-vaccination. There was significant decrease in mean antibody titer from Day 1 to Day 7 in chicks.

The extent of cell mediated immune reaction was observed by increase in mean skin thickness of sensitized broiler chicks (Table 2). The cell mediated immune response showed by the increase in skin thickness was more among chicks treated with herbal preparation than the other groups which did not differ significantly between themselves.

In the present study it was seen that administration of *A. racemosus* dried root powder might have significantly stimulated both humoral and cell mediated immune responses. This finding simulates with the findings of Kuttan and Kuttan (1992) who also reported the same observations in Swiss albino mice. The previous studies have demonstrated that *A. racemosus* extracts increase phagocytic activities of macrophages *in vitro* (Rege and Dahanukar 1993). There have been studies on the immunomodulatory activities of *A. racemosus* in mice with myelo-suppression induced by cyclophosphamide, azathioprim or prednisolone. This finding supports our result in the current investigation that *A. racemosus* root extracts stimulate immune response activities in broiler chicks. Extracts of *A. racemosus* have also shown immuno-potentiating effects in cyclophosphamide 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. This was attributed to natural passive immunity in young chicks as demonstrated by Hellar (1975). In the present study, the highest antibody level as detected by HI test on 1st day of age decreasing up to 21st day with no detectable antibody titer on 28th day which is similar to the findings of Deka et al. (2002) and Kalita and Dutta (1999). In the present study, non-significant effect of treatment on skin thickness in contact sensitivity test with DNFB at 0 as well as 72 hour post-challenge. These results were similar to Kumari (2005) than the treated group with *Withania somnifera* which revealed significantly higher results at 48 and 72 hour post-challenge with DNFB. Muruganadan et al. (2001) reported the effects of ethanol extracts of *A. racemosus* on humoral immune system which was assessed by humoral immune response and cell mediated immune response in mice. Kumari Rita et al. (2011) showed the determinative role of extracts of Ashwagandha (*W. somnifera*) and Satavar (*A. racemosus*) as herbal feed additives in obtaining higher humoral and cell mediated immune responses providing better protection level against infections in protecting the immuno-deficient broiler chicks against infections. The reports simulated to the findings in the present study which showed the immuno-modulating effects of dried root powder of *A. racemosus* on responses of humoral immune system assessed by rise in HI antibody titer and by cell mediated immune responses in broiler chicks.
Conclusion

The use of *A. racemosus* dried root powder in a specific dose during the scheduled vaccination regimen may be helpful in obtaining higher protective antibody against different vaccinations including more effective cell mediated immune response for protection against various bacterial, viral and other diseases. Herbal formulations containing extracts of *A. racemosus* may be therefore recommended for use as positive immunomodulator in normal and immuno-compromized broiler chicks. The present study also indicated the determinative roles of herbal feed additives in effective augmentation of humoral and cell mediated immune responses providing better protection level against infections.

Acknowledgement

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References


Table 1. Mean hemagglutination inhibition (MHI) antibody titer (log_{10} values) in different groups of chick.

<table>
<thead>
<tr>
<th>Age of chicks (in day)</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>ANOVA-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.154 ± 0.050</td>
<td>1.154 ± 0.050</td>
<td>1.154 ± 0.050</td>
<td>NS</td>
</tr>
<tr>
<td>7</td>
<td>0.502 ± 0.100</td>
<td>0.502 ± 0.100</td>
<td>0.502 ± 0.100</td>
<td>NS</td>
</tr>
<tr>
<td>14</td>
<td>1.154 ± 0.092</td>
<td>1.003 ± 0.063</td>
<td>0.301 ± 0.134</td>
<td>0.771^{NS}</td>
</tr>
<tr>
<td>21</td>
<td>1.355 ± 0.067^a</td>
<td>1.153 ± 0.092^a</td>
<td>0.100 ± 0.100^b</td>
<td>59.195*</td>
</tr>
<tr>
<td>28</td>
<td>1.304 ± 0.063^a</td>
<td>1.054 ± 0.067^b</td>
<td>0.000^c</td>
<td>169.058*</td>
</tr>
<tr>
<td>35</td>
<td>1.606 ± 0.126^a</td>
<td>1.204 ± 0.077^b</td>
<td>0.000^c</td>
<td>95.181*</td>
</tr>
<tr>
<td>42</td>
<td>1.957 ± 0.128^a</td>
<td>1.505 ± 0.109^b</td>
<td>0.000^c</td>
<td>110.47*</td>
</tr>
</tbody>
</table>

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

Table 2. Average skin thickness (in mm) of broiler chicks at different hours post DNFB challenge.

<table>
<thead>
<tr>
<th>Age of chicks (in hour)</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>ANOVA-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.261 ± 0.014^a</td>
<td>0.244 ± 0.012^a</td>
<td>0.216 ± 0.100^a</td>
<td>3.48^{NS}</td>
</tr>
<tr>
<td>6</td>
<td>0.418 ± 0.020^a</td>
<td>0.367 ± 0.050^b</td>
<td>0.307 ± 0.019^ab</td>
<td>7.5**</td>
</tr>
<tr>
<td>24</td>
<td>0.619 ± 0.036^a</td>
<td>0.586 ± 0.018^a</td>
<td>0.478 ± 0.032^b</td>
<td>6.00*</td>
</tr>
<tr>
<td>48</td>
<td>1.090 ± 0.061^a</td>
<td>0.983 ± 0.047^a</td>
<td>0.815 ± 0.026^b</td>
<td>8.846**</td>
</tr>
<tr>
<td>72</td>
<td>1.392 ± 0.168^a</td>
<td>1.217 ± 0.135^a</td>
<td>0.920 ± 0.040^a</td>
<td>3.55^{NS}</td>
</tr>
</tbody>
</table>

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