



***Oroxylum indicum* possess a potential effect on humoral and cell mediated immune response in broiler chicks**

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Immunomodulatory properties of *syonaka* (locally called *Sonapatha* and *hathipanjari*), *Oroxylum indicum* were assessed through estimation of humoral and cell mediated immune response in broiler chicks. Day-old broiler chicks (100) were procured, grown up to 7 days and divided into T1, T2, T3, T4 and T5 comprising 20 chicks each. The chicks were vaccinated against Ranikhet disease (RD) virus on day 7 (F1 strain) and day 28 (R₂B strain) except those in fifth group which served as unvaccinated control group. All the groups of chicks were vaccinated against infectious bursal disease (IBD) on day 14 (Georgia stain). The birds were maintained under similar managemental conditions. Chicks of T1 and T2 were fed *O. indicum* stem bark and root bark powder respectively each @ 250 mg/kg b. wt. Group T3 chicks were fed levamisole @ 10 mg/kg b.wt to compare the immunomodulatory effect of the preparations of *O. indicum* with that of levamisole. Groups T4 and T5 included in the experimental design served as the vaccinated and unvaccinated control group respectively.

Hemagglutination (HA) test and HI test were carried out as per Buxton and Frazer (1977) with certain modifications. Blood was collected from 6 chicks each on seventh day and serum was separated. Hemagglutination inhibition (HI) test was performed to show the status of maternal antibody titre against ND virus. Total protein, albumen, globulin concentrations in serum and albumen-globulin (A: G) ratio were assessed on day 1 and 7 before vaccination of all the chicks. All the chicks were re-vaccinated with La Sota strain of ND virus on 28th day and serum was collected on 35th and 42nd days of vaccination. Post-vaccination sera samples, collected at weekly intervals on 14th, 21st, 28th, 35th and 42nd days were put to HI test to monitor the antibody status of the birds against ND virus. HA and HI tests were performed according to Buxton and Frazer (1977).

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Cell mediated immune response was studied on the basis of delayed hypersensitivity reaction or the different experimental groups, measured by contact sensitivity test with 2, 4-dinitrofluorobenzene (DNFB). The test was performed as per Tiwary and Goel (1985) with slight modifications. The featherless areas on 20 cm² on both sides of upper abdomen were marked for inoculation. The sites were properly sterilized. Six chicks from each group were sensitized with DNFB by single percutaneous application of 0.25 ml of DNFB (10 mg/ml) in a vehicle consisting of acetone and olive oil in the ratio 4:1 on day 28. Sensitized chicks (10) were challenged after 14 days i.e. on 42nd day by application of 0.25 ml DNFB solution @ 1 mg/ml on left demarcated area, while right side was painted with vehicle only. The skin thickness was measured with screw-gauge pre-challenge dose and post-challenge doses on 0, 6, 24, 48 and 72 hours. All data were subjected to statistical analysis as per Snedecor and Cochran method (1994).

Effect of treatments on humoral immune response against NDV vaccine: A significant decrease in mean hemagglutination inhibition (MHI) antibody (Ab) titre (log₁₀ value) was observed from day 1 to day 7. On 14 d significantly (P<0.001) higher MHI Ab titre was observed in levamisole treated group T₃ as compared to groups T₁, T₄ and T₅. The MHI Ab titre of the *O. indicum* stem bark and root bark powder treated groups differed nonsignificantly from that of the vaccinated control group (T₄). No significant difference was observed between MHI Ab titres of groups T₂ and T₃. The antibody titre of the unvaccinated control group T₅ was significantly lower than all other groups. On day 21 value of MHI Ab titre of group T₃ was found significantly higher as compared to all other groups. T₂ showed a significantly higher MHI titre than the vaccinated control group T₄ but no significant difference was observed in the MHI Ab titre values of groups T₁ (1.706±0.100) and T₂. T₁ showed a higher MHI titre than T₄ but the difference was nonsignificant. MHI titres of all the groups were significantly higher than that of T₅. On day 28 i.e. 21 days

post ND vaccination the trend of MHI Ab titre between groups was similar to that on day 21. Significantly highest value of MHI titre was observed in T₃ (2.057±0.093) followed by T₂ (1.756±0.093), T₁ (1.555±0.093) and T₄ (1.455±0.093). No HI Ab titre was observed in T₅ on day 28 and the following days of observations. Observations of day 35, i.e. a week after vaccination with the booster dose of NDV vaccine again showed the significantly highest value of MHI Ab titre in the levamisole treated group T₃ (2.508±0.100). T₃ was followed by group T₂ (2.157±0.093) whose MHI titre was significantly higher than those of groups T₁ (1.906±0.063) and T₄ (1.806±0.110). MHI antibody titre against NDV in serum samples on day 42 showed significantly highest MHI titre in group T₃ (2.91±0.100) followed by groups T₂ (2.559±0.067), T₁ (2.258±0.067) and T₄ (2.157±0.093). The MHI titre of all the treatment groups differed significantly from each other except a nonsignificant difference between the *O. indicum* stem bark treated group T₁ and the vaccinated control group T₄.

Increase in antibody titre might be due to increased proliferation and transformation of B lymphocytes into plasmocytes, suggestive of humoral immune response. A significantly (P<0.001) higher and longer lasting response was observed after the booster dose of vaccine. These features of secondary immune response indicated the ability of the antibody forming system to remember previous exposure to the antigen (Tizzard 1998).

O. indicum stem bark powder treated group showed a moderately higher antibody titre than the vaccinated control but no significant effect of the herb was observed on the

MHI Ab titre of the chicks in our study. The observations indicated that *O. indicum* stem bark in its crude powder form can not be suggested as an enhancer of humoral immunity for broiler chicks. However since the herb gave a persistently higher antibody titre than the control, right from day 14 to day 42, it may be presumed that some extract of the herb may have immunomodulatory properties.

Effect of *O. indicum* stem bark and root bark powder on the MHI titre of the chicks did not differ significantly from each other before the booster dose of RD vaccine but the secondary immune response against the antigen was significantly (P<0.001) higher in the chicks fed with the root bark powder. However none of the 2 herbal fed groups gave a humoral immune response as high as levamisole, during our 42 days study period.

Zaveri *et al.* (2006) observed that treatment with n-butanol fraction, in response to SRBC, caused a significant rise in circulating HA titres during secondary antibody responses, indicating a potentiation of certain aspects of the humoral response. The treatment also resulted in a significant rise in paw oedema formation, indicating increased host DTH response.

Effect of treatments on cell mediated immune response: The normal mean skin thickness (MST) recorded before the sensitization of chicks with DNFB on day 28 was significantly lower (Table 2) than the MST values of all the groups post sensitization. At 0 hour, before the challenge application of DNFB on day 42, i.e. 14 days post sensitization, the highest value of MST was observed in group T₃ followed by T₂, T₁, T₄ and T₅ (but the difference between

Table 1. MHI antibody titre (log 10) value of chicks' sera of different treatment groups at following ages (in days) of the birds

Days	T ₁	T ₂	T ₃	T ₄	T ₅
Day 14	1.455±0.100 ^b	1.605±0.100 ^{bc}	1.906±0.100 ^c	1.304±0.100 ^b	0.552±0.093 ^a
Day 21	1.706±0.100 ^{bc}	1.856±0.093 ^c	2.207±0.100 ^d	1.555±0.093 ^b	0.301±0.078 ^a
Day 28	1.555±0.093 ^{ab}	1.706±0.100 ^b	2.057±0.093 ^c	1.455±0.093 ^a	ND
Day 35	1.906±0.063 ^a	2.157±0.093 ^b	2.508±0.100 ^c	1.806±0.110 ^a	ND
Day 42	2.258±0.067 ^a	2.559±0.103 ^b	2.91±0.100 ^c	2.157±0.093 ^a	ND

Values in the same row bearing different superscripts differ significantly; ND, the serum antibody level was non-detectable through HI test, *P<0.01.

Table 2. Effect of treatments on skin thickness (mm) of chicks of various treatment groups (hours pre-challenge and post-challenge with DNFB)

Days	T ₁	T ₂	T ₃	T ₄	T ₅
Normal	0.525±0.046	0.525±0.046	0.525±0.046	0.525±0.046	0.525±0.046
0 hr pre	1.458±0.100	1.542±0.100	1.583±0.154	1.292±0.077	1.333±0.105
6 hr post	1.850±0.048 ^a	2.042±0.077 ^{ab}	2.208±0.100 ^b	1.875±0.099 ^a	1.800±0.100 ^a
24 hr post	2.250±0.112 ^a	2.833±0.105 ^b	2.875±0.085 ^b	2.417±0.124 ^a	2.333±0.124 ^a
48 hr post	2.042±0.100 ^a	2.500±0.112 ^b	2.542±0.119 ^b	2.125±0.085 ^a	2.042±0.164 ^a
72 hr post	1.708±0.100 ^a	1.75±0.0914 ^a	2.292±0.208 ^b	1.833±0.124 ^a	1.75±0.145 ^a

The normal mean skin thickness of the chicks before DNFB sensitization was 0.525±0.046 mm. Values in the same row bearing different superscripts differ significantly; NS, nonsignificant, * P<0.01; ** P<0.05.

the groups was statistically nonsignificant). Six hours post DNFB challenge a significant rise in MST was observed in all the treatment groups. The highest MST was observed in the levamisole treated group T₃ followed by group T₂. The MST of groups T₁, T₄ and T₅ was significantly lower than that of group T₃. The peak value of mean skin thickness in all the groups was observed at 24 h post DNFB challenge. The levamisole treated group T₃ (2.875±0.085) and *O. indicum* root bark powder treated group T₂ showed significantly higher MST value than groups T₁, T₄ and T₅ which differed nonsignificantly from each other in their mean skin thickness. A similar trend was observed at 48 h post DNFB challenge. Highest MST was observed in group T₃ (2.542±0.119) followed by group T₂. The MST of these 2 groups was significantly higher than groups T₁, T₄ and T₅. At 72 h post DNFB challenge, the levamisole treated group T₃ showed significantly highest MST value than all other treatment groups. However, groups T₁, T₂, T₄ and T₅, showed a significant difference in their MST value 72 h post DNFB challenge.

In this study, a significant rise in skin thickness was observed in all the groups 6 h, post challenge. It indicated that DNFB is a potent agent for initiating CMI response. No significant change in the MST was observed among the groups, 14 days post sensitization i.e. 0 h pre challenge. This finding might be suggestive of the fact that neither *O. indicum* nor levamisole has a significant effect on the primary, cell mediated, immune response to DNFB application. The finding is in tune with the findings of Bhardwaj (2008) who observed that feeding extracts of *Tinospora cordifolia* stem showed no effect on the MST of broiler chicks 0 h post challenge. Singh (2008) had a similar finding while working with broiler chicks to see the effect of his polyherbal preparation on their CMI response.

In our study, feeding of *O. indicum* stem bark and root bark powder showed no significant effect on the MST of the chicks at 6 h post challenge. However, the MST of levamisole treated group was significantly higher than the vaccinated and non-vaccinated control at this time of observation. Feeding of *O. indicum* stem bark powder showed no significant effect on the MST of the broiler chicks at 6, 24, 48 and 72 h post challenge. This finding might be attributed to the fact that *O. indicum* stem bark powder might not be a positive modulator of cell mediated immune response in broiler chicks. Slightly lower value of MST in the stem bark powder treated group than the control is not well understood. This might be attributed to presence of some factor in the

stem bark powder of *O. indicum* responsible for lowering the cell mediated DTH function. Contrary to this, we observed that *O. indicum* root bark powder introduced per os to the broiler chicks significantly increased their MST at 24 and 48 h post-challenge. The finding may be suggestive of the fact that root bark powder of the herb potentiates the cell mediated immunity of broiler chicks during the secondary response.

Our result was supported by the findings of Zaveri *et al.* (2006) who evaluated the activity of n-butanol fraction of root bark of *O. indicum* on the cell mediated immune response of rats, using delayed type hypersensitivity reaction. The treatment resulted in a significant rise in paw edema formation, indicating increased host DTH response. In our study however, significantly steep rise and fall was observed in the MST of the group treated with root bark powder of *O. indicum*.

SUMMARY

Levamisole treatment was found to have superior stimulatory effect on both humoral and cell mediated immune response of chicks compared to *O. indicum* on these parameters. *O. indicum* root bark powder showed a better response over its stem bark counterpart. Thus, *O. indicum* root bark powder may be recommended as safe and commercially beneficial immunomodulator as is evident from better humoral immune response against NDV vaccine for better protection against diseases.

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