Cell Mediated Immune Response of Cow Urine with Withania Somnifera and Tinospora Cordifolia

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ABSTRACT

To study the immunomodulatory effect of cow urine ark(distillate) and medicinal herbs, evaluation of cell mediated immune (CMI) response by delayed type hypersensitivity(DTH) reaction to 2,4-dinitro chloro benzene (of experiment DNCB) in the broilers of group I(control), group II(ark treated), group III(ark and W.somnifera treated), group IV(ark and T.cordifolia treated), group V(ark, W.somnifera and T.cordifolia treated) and group VI(ImmuPlus-a polyherbal preparation treated) was carried out. The result indicated that DTH reaction in the broilers of group VI was most severe at 24 hours post challenge on 42nd day of experiment than that followed by group V, IV, III, II and I. DTH response indicates that cow urine ark and medicinal herbs are a potent immunomodulatory agent enhancing the cell mediated immune response. One of the explanations forwarded to justify the beneficial effect of cow urine and medicinal herbs in diseased stress is the non specific enhancement of immune status of the individual.

Keywords: Cell mediated immunity, Delayed type hypersensitivity, Withania somnifera, Tinospora cordifolia.

The “Cow Urine Therapy” is called the ‘Mother’ of Ayurvedic medicine. There are some medicinal plants reported to possess significant growth promoting, toxin counteracting, antistress and immunomodulating activity in broilers (Dahanukar et al., 2000). Two such important medicinal herbs are Withania somnifera
(Ashwagandha) and *Tinospora cordifolia* (Giloya). Several studies have reported an enhanced cell mediated immune response by supplementation of feed by cow urine, *W. somnifera* and *T. cordifolia* singly. Garg *et al.* (2005) found that oral administration of cow urine @ 1 ml/bird enhances cell mediated immune response in white leghorn layers. Sadekar *et al.* (1998) reported that *W. somnifera* is a better immunomodulator as compared to levamisole in cross bred calves. In comparison to control, administration of both levamisole and *W. somnifera* produced intense inflammatory response to DNBC skin contact, both physically and histopathologically. Kapil and Sharma (1997) demonstrated immunomodulatory activities of *T. cordifolia* with enhanced cell mediated immunity and macrophage activation. Singh *et al.* (2004) reported that ImmuPlus® (a polyherbal preparation containing both *W. somnifera* and *T. cordifolia*) significantly augmented the cell mediated immunity in birds as observed using DNBC as allergen. However, there is paucity of information regarding immune study in broilers using the cow urine along with *W. somnifera* and *T. cordifolia* in feed. Thus, the present study was designed to detect the cell mediated immunity in broilers using cow urine and both the medicinal herbs in the feed.

**MATERIALS AND METHODS**

*Selection of cow as donor:* Three apparently healthy pure Gir cows aged 3-4 years, maintained at Kasturbagram dairy farm, District Indore (M.P.) raised under standard feeding and management conditions, were selected as donor of the urine.

*Collection of urine:* Early morning cow urine was collected in sterilized plastic bottles by passing catheter aseptically and the samples were brought to the laboratory for preparation of ark (distillate).

*Preparation of ark:* It was prepared by boiling cow urine in an iron pot to which a vapour condensing device was attached, as per the method outlined by Khanuja *et al.* (2002). Sterility of cow urine was finally checked by inoculation on Blood Agar.

*Herbal powders:* Herbal powders of *W. somnifera* and *T. cordifolia* of Dabur Ltd. and ImmuPlus (a polyherbal preparation containing *W. somnifera* and *T. cordifolia*) of Indian Herbs was used for the experiment.

*Experimental broilers:* A total number of 144 broiler chicks, day old, 50-52 grams in body weight, vaccinated against Marek’s disease and of either sex, randomly selected from Poultry farm, Veterinary college Mhow, were used to examine the effect of cow urine ark alone and in combination with *W. somnifera* and *T. cordifolia*. These birds were raised and maintained under standard conditions.

*Grouping of broilers:* The broilers were randomly divided in to six groups with each containing 24 broilers. Each group included four replicates of six birds. They were raised for 45 days under standard managerial and feeding practices under following groups:
Group I (control), Group II (ark treated), Group III (ark and W. somnifera treated), Group IV (ark and T. cordifolia treated), Group V (ark, W. somnifera and T. cordifolia treated), Group VI (ImmuPlus—a polyherbal preparation treated).

Dose rate and route: Administration of cow urine ark @ 1 ml/broiler/day in drinking water as mentioned by Garg et al. 2005, herbal powders (W. somnifera and T. cordifolia) alone @ 1 gram/100 gram feed and in combination @ 0.5 gram/100 gram feed as mentioned by Kumar et al. (2003) with slight modifications, was started from day 0 of the experiment. ImmuPlus was given @ 75-100 mg/litre of drinking water.

Cell mediated immune response (CMI): CMI was adjudged on 28th and 42nd day of age using Contact Sensitivity Test (CST) (Kumari et al., 2011). The CMI response was studied by Delayed Type Hypersensitivity (DTH) reaction to 2, 4 dinitro-chloro benzene (DNCB) as per method adapted by Tiwary and Goel (1985). On 28th day six broilers from each group were selected for the test. A relative feather less elliptical area, approximately 20 to 30 square mm, was marked on the left lateral side of abdomen for topical application of DNCB, while a similar area on right side was marked as control. The area was cleaned with alcohol and then 0.25 ml of DNCB (10mg/ml) in a vehicle consisting of acetone and olive oil (4:1) mixture was applied within the marked area on the left side; on right side equal volume of only vehicle was applied. The sensitized chicks were challenged with 0.15 ml of DNCB (1mg/ml) on 42nd day of experiment. Skin thickness was measured by vernier caliper at zero (prior to challenge) and 24 hours post challenge to assess the reaction. Increase in mean skin thickness (MST) of broilers was recorded as the difference in thickness of skin at 24 hours and zero hours. The overall MST was obtained by taking the mean averages of individual broilers within a group.

Statistical analysis: With a view to arrive meaningful conclusion the data of observations were subjected to statistical analysis applying analysis of variance (ANOVA) in complete randomized design (CRD) and measured at 5% and 1% level of significance (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

CMI was assessed by DTH response, which is a direct correlate to CMI, was found to be increased in the treatment groups as compared to control. The mean values of skin thickness in the broilers of various groups are presented in the table.

ANOVA revealed highly significant increase (P<0.01) in the MST in between the groups and in between the intervals.

During CMI response, sensitized T-lymphocytes, when challenged by the antigen, are converted to lymphoblast and secretes lymphokines, attracting more scavenger cells at the site of reaction. The infiltrating cells are thus immobilized to promote defensive (inflammatory) reaction (Fulzele et al., 2002). In our study, the MST was much enhanced in groups treated with ark and medicinal herbs together as compared
to their individual treatments. This suggests a better immune enhancement on regular administration of ark and herbs together to broilers. Our study is also supported by Chauhan et al. (2001) who reported marked increase in CMI in mice without any side effect of feeding ark. The author further opined that this preparation of urine is a potent and safe immunomodulator. A glycoprotein named as uromodulin (18 KDa) has been shown to be a potent inhibitor of antigen induced lymphocyte proliferation (Brown et al., 1986). Cow urine has been reported to augment the CMI response by inducing B and T cells blastogenesis and increase in the level of IgG along with IL-1 and IL-2 in mice and rats in vivo studies (Chauhan et al., 2001 and Gupta, 2004). Chauhan (2003) concluded that cow urine has potent immunomodulatory effect and capable to enhance CMI. CMI was evaluated by DTH reaction against 1% DNCB. Agarwal et al. (1999) studied the immunomodulatory activities of Ashwagandha in mice for immune inflammation, active paw anaphylaxis and DTH. Similar immunomodulatory activities of Ashwagandha were stated by Milot, 2004; Gupta and Rana, 2007; Davis and Kuttan, 2002; Ziauddin et al., 2002; Gautam et al., 2004 and Owais et al., 2005. Khobragade et al. (2005) also concluded that the feed supplementation of T.cordifolia and Leptadenia reticulate improves the immune status in commercial broilers. All the above work done by various researchers suggested immunomodulatory activity of cow urine with medicinal plants. However, in the present study phytochemistry of the medicinal herbs was not performed to specify the active principle responsible for immunomodulation.

On the basis of above study it was concluded that practice of cow urine therapy with medicinal herbs can prove to be a boon for the poultry industry.

**Table 1:** Mean value of Mean Skin Thickness (MST) (mm)

<table>
<thead>
<tr>
<th>Period (Day)</th>
<th>Group I (C)</th>
<th>Group II (A)</th>
<th>Group III (AW)</th>
<th>Group IV (AT)</th>
<th>Group V (AWT)</th>
<th>Group VI (IP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>28</td>
<td>1.00 ±0.15</td>
<td>1.25 ±0.19</td>
<td>1.32 ±0.12</td>
<td>1.38 ±0.15</td>
<td>1.44 ±0.16</td>
<td>1.56 ±0.20</td>
</tr>
<tr>
<td>42</td>
<td>2.20 ±0.31</td>
<td>2.35 ±0.27</td>
<td>2.48 ±0.16</td>
<td>2.65 ±0.27</td>
<td>3.20 ±0.41</td>
<td>3.40 ±0.42</td>
</tr>
</tbody>
</table>

C=Control; A= Ark; AW = Ark and W. somnifera; AT =Ark and T.cordifolia; AWT =Ark; W. somnifera and T.Cordifolia; IP= ImmuPlus.

Values with different superscripts in capital letters in a row differ significantly in between groups.

Values with different superscripts in small letters in a column differ significantly in between intervals.
Table 2: Analysis of Variance for Mean Skin Thickness

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>d.f.</th>
<th>M.S.</th>
<th>F-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>5</td>
<td>59.60</td>
<td>129.07**</td>
</tr>
<tr>
<td>Between Intervals</td>
<td>6</td>
<td>6.05</td>
<td>13.09**</td>
</tr>
<tr>
<td>Error</td>
<td>60</td>
<td>0.46</td>
<td>-</td>
</tr>
</tbody>
</table>

** Highly Significant * Significant

REFERENCES


