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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 DNCB 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 DNCB 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 managemental 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 0.15ml 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)

Period (Day)	Group I(C) 6 broilers	Group II(A) 6 broilers	Group III(AW) 6 broilers	Group IV(AT) 6 broilers	Group V(AWT) 6 broilers	Group VI(IP) 6 broilers
28	1.00^{Aa}	1.25^{Ba}	1.32^{Ca}	1.38^{Da}	1.44^{Ea}	1.56^{Fa}
	±0.15	±0.19	±0.12	±0.15	±0.16	±0.20
42	2.20^{Ab}	2.35^{Bb}	2.48^{Cb}	2.65^{Db}	3.20^{Eb}	3.40^{Fb}
	±0.31	±0.27	±0.16	±0.27	±0.41	±0.42

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

Source of Variation	d.f.	M.S.S.	F-Value
Between Groups	5	59.60	129.07**
Between Intervals	6	6.05	13.09**
Error	60	0.46	-

** Highly Significant * Significant

REFERENCES

Agarwal, R., Diwanay, S., Patki, P. and Patwardhan, B. 1999. Studies on immunomodulatory activity of *W. somnifera* extracts in experimental immune inflammation. *Journal of Ethnopharmacol*ogy, **67**(1): 27-35.

Brown, M.K., Muchmore, V.A. and Rosenstreich, L.D. 1986. Immunomodulation and immunosuppressive protein derived from pregnancy urine is an inhibitor of IL. *Proc. Natl. Acad. USA*,**83**:9119-9123.

Chauhan, R.S. 2003. Enhancement of body resistance by cow's urine. *In*: National Symposium on – The directions in the research in veterinary field in the next decade. February, 27-28, 2003, IVRI, Izatnagar.

Chauhan, R.S., Singh, B.P. and Singhal, L.K. 2001. Immunomodulation with kamdhenu ark in mice. *Journal of Immunology and Immunopathology*, **3**:74-77.

Dahanukar,S.A., Kulkarni,R.A. and Rege,N.N. 2000. Pharmacology of Medicinal Plants and Natural Products. *Indian Journal of Pharmacology*.**32**:S81-S118.

Davis, L. and Kuttan, G. 2002. Effect of *W. somnifera* on cell mediated immune responses in mice. *Journal of Experimental and Clinical Cancer Research*, **21**(4):585-590.

Fulzele, S.V., Satturwar, P.P., and Dorle, A.K. 2002. Study of the immunomodulatory activity of haridradi ghrita in rats. *Indian Journal of Pharmacology*, **35**: 51-54.

Garg, N., Kumar, A., Shukla, G. and Chauhan, R.S. (2005). Effects of Indian cow urine on the egg production and quality. *In*: XI th European Symposium on the quality of eggs and egg products, Doorwerth, The Netherlands, 23 -26 May 2005.

Garg, N., Chauhan, R.S. and Kumar, A. 2005. Assessing the effect of cow urine on immunity of white leghorn layers. *http://www. Isah-soc.org/documents/2005/sections/17-vol-2.pdf*.

Gautam, M., Diwanay, S.S., Gairola, S., Shende, V.S., Jadhav, S.S. and Patwardhan, B. 2004. Immune response modulation to DPT vaccine by aqueous extract of *W. somnifera* in experimental system. *International Immunopharmacology*, **4**(6):841-849.

Gupta, A. (2004). Effect of cow's urine on the health of rats. M.V.Sc. thesis, College of Veterinary Science (Mathura), CSA University of Agriculture and Technology, Kanpur (U.P.), India.

Gupta, G.L. and Rana, A.C. 2007. *W. somnifera* (ashwagandha): a review. *Pharmacognosy Reviews*, **1** (1):129-136.

Kapil, A. and Sharma, S. 1997. Immunopotentiating compounds from *T. cordifolia*. *Journal of Ethnopharmacology*, **58**(2):89-95.

Khanuja, S.P., Kumar, S.S., Arya, A.K., Jai, S.D. and Mahendra, P. 2002. A pharmaceutical composition containing cow urine distillate and an antibiotic, CSIR, New Delhi (India, U. S., Patent No. 6410059, June 25th, 2002).

Khobragade, R.S., Sarag, A.N., Rekhate, D.M. and Dhok, A.P. 2005. Effect of herbal feed supplementation of medicinal plants *T. cordifolia* and *L. reticulata* on haemo- immuno-biochemical profile of broilers. *Indian Veterinary Medical Journal*, **29**:280-282.

Kumar, R., Singhal, L.K., Singh, B.P., Rana, N., Singh, D.D. and Chauhan, R.S. 2003. ImmuPlus up regulates immune response to FMD vaccine in calves. *Livestock International*. **7**(10):11-15.

Kumari, R., Tiwary, B.K., Prasad, A. and Ganguly, S. 2011.Immunomodulatory effect of herbal feed supplement in normal and immunocompromised broiler chicks. *Indian Journal of Animal Sciences*, **81**(2):158-161.

Milot, B. 2004. *W. somnifera. Alternative Medicine Review*, **9** (2):211-214.

Owais, M., Sharad, K.S., Shehbaz, A. and Saleemuddin, M. 2005. Antibacterial efficacy of *W. somnifera* (ashwagandha) an indigenous medicinal plant against experimental murine salmonellosis. *Phytomedicine*, **12**(3):229-235.

Sadekar, R.D., Bhad, P.D., Bhandarkar, A.G., Ali, S.Z. and Kolye, A.Y. 1998. Comparative immunomodulatory effects of *W.somnifera* (ashwagandha) and levamisole in crossbred calves. *In*: 5th Annual Conference of IAAVR and National symposium on –Challenges for the advancement of veterinary science in 21st century, Indore, 18-19 February.

Singh, B., Singh, G.K. and Chauhan, R.S. 2004. Effect of ImmuPlus on cell mediated immune responses of chicks. *Journal of Immunology and Immunopathology*, **6** (1):49-53.

Snedecor, G.W. and Cochran, W.G. 1994. Statistical Method. 8th edn. The Iowa state College Press, Inc. Amer, Iowa, USA.

Tiwari, B.K. and Goel, M.C. 1985. Contact sensitivity to DNCB in normal and cell mediated immunity deficient chicken: *In vitro* detection and correlation with lymphocyte transformation and graft versus host reaction. *Journal of Veterinary Immunology and Immunopathology*, **8**(4): 329-339.

Ziauddin, M.M., Phansalkar, N., Patki, P., Diwanay, S. and Patwardhan, B. 2002. Studies on the immunomodulatory effects of ashwagandha. *Journal of Ethno pharmacology*, **50**(2): 69-76.