

## COW URINE UPREGULATES LYMPHOBLASTOGENESIS IN CHICKS

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### Introduction

Cowpathy is an old system of medicine mentioned in ancient Indian literature (Ayurveda) as *Panchgavya Chikitsa*. The ayurvedic medicines of animal origin are mainly prepared from *Panchgavya* (five things from Indian cow viz., urine, dung, milk, butter oil and curd), which boost up the body immune system and makes body refractory to various diseases (Chauhan *et al.*, 2004). The specificity of immune system depends upon the number and activity of lymphocytes. Chauhan *et al.*, (2001) studied the immunomodulatory effect of cow urine in mice and found that cow urine enhances both T- and B-cell blastogenesis and also increases the level of IgG. Kumar (2001) and Chauhan *et al.*, (2004) reported increase in both cellular and humoral immune responses due to cow urine. The present study was planned to investigate the blastogenic activity of lymphocytes and effect of *in-vivo* cow urine treatment on it so as to find out their potential to mount protective immune response against diseases.

### Materials and methods

**Experimental birds:** Sixty chicks were procured on the day of hatch and randomly divided into two groups of 30 each. The chicks were raised up to 28 day of age under ideal and uniform husbandry conditions. The group I (control) was given plain water and the group II (treated) was given distilled cow urine @ 10 ml / liter of drinking water. Lymphoid organs thymus, bursa and spleen was collected aseptically from 6 birds of each group at 7 days interval i.e., on day 0, 7, 14, 21 and 28 lymphoid organs (thymus, bursa and spleen) and lymphocytes isolated separately in RPMI-1640 media.

**Cell culture medium:** One vial of tissue culture medium RPMI-1640 (HiMedia, India) was dissolved in one liter of triple glass distilled water and filtered through 0.22 µ membrane filter. The filtrate was distributed in aliquots of 100 ml and kept at 4°C until use. Before use, pH was adjusted to 7.2±0.1 with the help of sterile 0.89 M sodium bicarbonate solution. Then

the medium was supplemented with 10 ml of sterile fetal calf serum (Sigma), 1000 IU of penicillin, 100 µg of streptomycin and 2000 IU of nystatin were added to check the contamination of fungus and bacteria in the culture medium.

**Mitogen:** Concanavalin-A (Con-A) was used as mitogen for T-cell responses while lipopolysaccharide (LPS) was used for B-cell responses in 5 µg/ml and 4 µg/ml concentration, respectively.

**Isolation of lymphocytes:** Thymus, bursa and spleen, collected from the experimental birds under aseptic conditions, were cut into small pieces and suspended in media for separation of lymphocytes. Suspension was filtered through sterile muslin cloth and the cells were counted in filtrate using trypan blue (0.5%) dye exclusion test. Finally, the lymphocyte counts were adjusted as  $10^6$  cells / ml in RPMI-1640 medium and 2 ml suspension of lymphocytes each from thymus, bursa and spleen were prepared.

**Test procedure:** Lymphocyte proliferation assay was performed using Con A (Concavallin A) as mitogen for T-cells and LPS (lipopolysaccharide) for B-cells following the method of Rai-el-Balho *et al.*, (1987) with slight modifications (Chauhan 1995 and 1998). Triplicate cultures were made using 100 µl of cell suspension and 50 µl of medium alone or medium containing Con A/LPS in flat bottom sterile micro titer plates (Corning, USA). Plates were sealed with cello tape and were incubated at 37°C in CO<sub>2</sub> chamber for 68 hr. After incubation supernatant fluids from the wells was discarded and 50 µl MTT (5 mg/ml) (Sigma) was added to all wells followed by reincubation at 37°C in CO<sub>2</sub> chamber for 4 hr. 100 µl of acid isopropanol (0.04 N HCl in isopropanol) was then added to each well and the absorbance of each well was measured in computerized micro scan ELISA reader at wave length of 570 nm.

The values of triplicate wells were averaged and the mean optical density of mitogen stimulated cultures was obtained. The mean OD of control wells was subtracted from mean OD of wells with mitogen and presented as mean delta OD.

## Results and discussion

The mean delta OD for T- and B-cell proliferation and effect of cow urine on it has been presented in Table 1. The increase in lymphocyte proliferation activity was maximum during first two weeks of development. During the experimental period cow urine enhanced the T- and b-cell blastogenesis by 1.81% and 2.21% respectively. However, Chauhan *et al.*, (2001) reported that cow urine significantly enhances T- and B-cell proliferative activity in mice. The results of the experiment showed cow urine marginally upregulates

lymphoblastogenesis in developing stages of chicks. This means immune system develops at an early stage and neonatal mortality can be reduced with the use of cow urine.

**Table 1. Age wise lymphoblastogenesis of bursal lymphocytes and effect of Cow urine (Mean delta OD±SE)**

Age (days)	T-cell blastogenesis		B-cell blastogenesis	
	Control	Treated	Control	Treated
0	0.205±0.036	0.205±0.036	0.242±0.034	0.242±0.034
7	0.316±0.016	0.321±0.028	0.356±0.014	0.361±0.023
14	0.382±0.014	0.391±0.023	0.421±0.043	0.426±0.029
21	0.397±0.029	0.403±0.022	0.429±0.022	0.437±0.056
28	0.407±0.010	0.417±0.019	0.441±0.028	0.448±0.040

Further research in the field can be carried out by assessing the blastogenic response of lymphocytes and identifying various cell surface markers following treatment with different doses and formulations of cow urine to develop an immunomodulator based on Ayurveda system of medicine. The agent will not only boost up immune system of livestock, birds and human beings but will also be environment friendly.

## References

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