

## SUPPRESSION OF THE CELLULAR RESPONSE IN BUFFALO CALF SKIN BY CYPROHEPTADINE DRUG

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### ABSTRACT

This study was conducted on 3-6 month old 12 buffalo calves. The buffalo calves were randomly divided into two groups- a control group (1) and an experimental group (2) Each group were divided into subgroups i.e. 1A,1B and 2A, 2B respectively. Experimental calves were pretreated with cyproheptadine intramuscularly 30 minutes prior to intradermal injection of Staph. Epidermis (Group 2A) and turpentine (Group 2B). Lesions of different time intervals were obtained for the sequential study of cellular responses. Maximal suppression of leukocytes was observed at 3 h in both types of inflammation. In both subgroups neutrophils were markedly suppressed at 3 h as compared to others cells.

**Keywords:** buffalo calf, *Bubalus bubalis*, cellular response, *Staphylococcus epidermidis*, turpentine

### INTRODUCTION

In the buffalo, few reports are available on the bacteriological, epidemiological and cellular responses in clinical and subclinical spontaneous cases of mastitis (Chaudhry *et al.*,

1982; Muhammad *et al.*, 1996). However, the information on the cellular responses occurring in experimentally induced inflammation in the buffalo seems to be lacking in the literature. Further, the mediation of the inflammatory response has been studied by number of workers using the anti-inflammatory drugs in experimental animals and birds. However, this aspect in the buffalo has also remained unexplored. Thus, this work was done on buffalo calves to study the suppression of cellular response in buffalo calf skin by cyproheptadine in response of the chemical (turpentine) and bacterial injury (*Staphylococcus epidermidis*).

### MATERIALS AND METHODS

Healthy male buffalo calves (12), 3-6 month-old were divided into two groups: a control and an experimental group, for the study of the cellular response in the buffalo calf skin. Each group comprised six calves. All calves were maintained under standard hygienic conditions. The site of the cutaneous reaction was prepared according Zarrilli and Calhoun (1970). **Control Group 1** - The six calves of the control group were again equally divided into two subgroups, i.e., Subgroup 1A and Subgroup 1B. Each subgroup had three calves.

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Each calf of Subgroup 1A received two intradermal injections of *Staphylococcus epidermidis* (0.1 ml) suspension in normal saline and each calf of subgroup 1B received two intradermal injections of turpentine for each time interval of 0-2 minutes, 30 minutes, 1 h, 3 h, 6 h, 12 h, 24 h and 48 h on both side (left and right) of thoraco-abdominal region. Each calf received in all sixteen injections, two for each time interval and making six lesions per time interval. The calves were euthanized by saturated solution of magnesium sulphate given intravenously .

**Experimental Group 2-** Six calves of experimental group were again equally divided into two subgroups, i.e., Subgroup 2A and Subgroup 2B. Each calf of Subgroup 2A was pretreated with cyproheptadine i/m at the dose rate of 1 mg/kg body weight 30 minutes prior to i/d *Staph epidermidis* (0.1 ml) and this was repeated every 12 h. The rest of the procedure was same as in control group. The skin specimen were collected and fixed in Cornoy's fluid for histopathological studies as described for chicken by Shrivastava *et al.* (1997). Sections were cut at 4-5  $\mu$ m thickness. Skin sections were stained with haematoxylin and eosin, and with 0.05 percent solution of toluidine blue in acetate buffer (pH 3-8) for basophils as described for chickens by Dhodapkar *et al.* (1984).

## RESULTS AND DISCUSSION

**Control Group 1A-** Vesicular changes were noticed at initial stage (0-2 minutes, and 30 minutes) Hyperaemia of blood vessels and oedema of the dermis were marked at 1 h and gradually increased. More marked hyperaemia and extensive oedema were noticed at 6 h. Necrosis of dermis was noticed at 24 h. Leukocyte emigration was

also noticed at the 30 mininterval and gradually significantly increased up to 12 h and then the number of cells decreased considerably and no cells were noticeable at 48 h in the buffalo skin. The maximal infiltration of neutrophils was at 6 h, mononuclear cells at 12 h, lymphocytes at 24 h basophils at 1 h and total leukocytes were observed at 12 h. Infiltration of eosinophils was not noticed in any one stage (Table 1).

**Subgroup 1B-** Vascular changes of blood vessels and oedema of dermis were not evident at the 0-2 minutes time interval. These changes were observed from 30 minutes onward. More marked hyperemia and oedema were observed at 6 h. Leukocyte migration was noticed at 0-2 minutes along with few neutrophils in small blood vessels. The significantly highest number of neutrophils, monocytoids, lymphocytes and basophils were observed at the 6, 12, 48 and 3 h time intervals, respectively. Infiltration of eosinophils was not noticed at any time (Table 2)

**Experimental Group 2- Subgroup 2A-** Thirty minutes before injection of the *Staph epidermidis* suspension in the skin, the calves were pretreated with cyproheptadine intramuscularly and the lesion as per non-pretreated group were obtained and processed for histopathological examination.

Cellular changes were not noticed at the initial stage (0-2 minutes, and 30 minutes). The hyperaemia of the blood vessels and oedema of the dermis were marked at 1 h and gradually increased. More marked hyperaemia and extensive oedema were noticed at 6 h but less marked as compared to Subgroup 1A. The maximal suppression of leukocytes was observed at 3 h. A few neutrophils were seen intravascularly at 0-2 minutes; the number of neutrophils increased significantly gradually up to 6 h and revealed maximal rate of suppression being 29.39 percent at 3 h. Monocytes,

Table 1. Tissue leukocytosis in response to *Staphylococcus epidermidis* in control and cyproheptadine pretreated buffalo calves.

Time Interval	Number of leukocytes (Mean $\pm$ S.E.) / high power (x400) microscopic field														
	Neutrophils			Monocytes			Lymphocytes			Basophils*			Total leukocytes		
	Staph. epi.	Cyproheptadine	Percent Suppression %	Staph. epi.	Cyproheptadine	Percent Suppression %	Staph. epi.	Cyproheptadine	Percent Suppression %	Staph. epi.	Cyproheptadine	Percent Suppression %	Staph. epi.	Cyproheptadine	Percent Suppression %
0.2 min	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
30 min	0.433 $\pm$ 0.221	0.400 $\pm$ 0.105	7.621	00	00	00	00	00	00	00	00	19.982	1.6 $\pm$ 0.278	1.333 $\pm$ 0.278	16.68
1 h	10.600 $\pm$ 0.992	8.366 $\pm$ 0.991	21.075	1.533 $\pm$ 0.564	1.400 $\pm$ 0.149	8.675	1.100 $\pm$ 0.174	0.866 $\pm$ 0.125	21.272	2.633 $\pm$ 0.245	2.00 $\pm$ 0.149	24.04	15.966 $\pm$ 1.368	13.2 $\pm$ 0.912	17.32
3 h	30.166 $\pm$ 1.996	21.300 $\pm$ 1.870	29.390	3.033 $\pm$ 0.784	2.600 $\pm$ 0.623	14.276	1.933 $\pm$ 0.554	1.366 $\pm$ 0.267	29.332	2.033 $\pm$ 0.531	1.600 $\pm$ 0.149	21.298	37.233 $\pm$ 2.582	26.933 $\pm$ 2.269	27.66
6 h	59.466 $\pm$ 4.676	47.600 $\pm$ 3.914	19.995	15.766 $\pm$ 1.579	12.133 $\pm$ 0.735	23.043	10.000 $\pm$ 0.527	7.400 $\pm$ 2.084	26.00	1.566 $\pm$ 0.194	1.300 $\pm$ 0.139	16.666	85.966 $\pm$ 3.250	70.166 $\pm$ 3.947	18.37
12 h	50.266 $\pm$ 1.206	42.133 $\pm$ 2.529	16.179	27.366 $\pm$ 1.883	21.160 $\pm$ 1.257	20.828	14.333 $\pm$ 1.763	13.533 $\pm$ 2.083	5.581	0.8 $\pm$ 0.149	0.700 $\pm$ 0.125	12.500	92.833 $\pm$ 2.969	78.066 $\pm$ 3.045	15.90
24 h	10.066 $\pm$ 3.023	9.5 $\pm$ 1.143	5.622	5.066 $\pm$ 1.530	4.566 $\pm$ 0.571	9.369	18.366 $\pm$ 1.747	17.366 $\pm$ 1.285	5.444	00	00	00	32.400 $\pm$ 3.508	31.966 $\pm$ 1.976	1.339
48 h	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00

\*Toluidine blue sections

Table 2. Tissue leukocytosis in response to turpentine in control and cyproheptadine pretreated buffalo calves.

Time Interval	Number of leukocytes (Mean $\pm$ S.E.) / high power(x400)microscopic field														
	Neutrophils			Monocytoids			Lymphocytes			Basophils*			Total leukocytes		
	Turpentine	Cyproheptadine	Percent Suppression %	Turpentine	Cyproheptadine	Percent Suppression %	Turpentine	Cyproheptadine	Percent Suppression %	Turpentine	Cyproheptadine	Percent Suppression %	Turpentine	Cyproheptadine	Percent Suppression %
0.2 min	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
30 min	10.200 $\pm$ 1.206	7.633 $\pm$ 0.705	15.181	0.433 $\pm$ 0.194	0.400 $\pm$ 0.210	7.621	00	00	00	1.733 $\pm$ 0.360	1.666 $\pm$ 0.194	5.770	12.5 $\pm$ 0.905	9.633 $\pm$ 0.323	22.93
1 h	24.500 $\pm$ 1.336	17.066 $\pm$ 3.325	30.34	2.300 $\pm$ 0.699	2.060 $\pm$ 0.434	10.434	1.800 $\pm$ 0.502	1.40 $\pm$ 0.272	22.222	3.033 $\pm$ 0.618	2.766 $\pm$ 0.655	8.803	32.433 $\pm$ 1.904	23.133 $\pm$ 1.136	28.67
3 h	137.996 $\pm$ 6.762	78.566 $\pm$ 1.934	43.054	18.300 $\pm$ 2.267	15.933 $\pm$ 0.880	12.934	7.400 $\pm$ 0.958	5.200 $\pm$ 0.688	29.729	5.666 $\pm$ 0.750	4.800 $\pm$ 0.404	15.300	169.333 $\pm$ 5.120	103.333 $\pm$ 1.695	38.97
6 h	244.833 $\pm$ 5.002	148.133 $\pm$ 3.522	39.496	71.530 $\pm$ 2.837	50.900 $\pm$ 2.561	28.841	44.766 $\pm$ 4.769	34.800 $\pm$ 1.082	22.262	4.133 $\pm$ 0.360	3.966 $\pm$ 0.356	4.040	365.066 $\pm$ 6.947	235.633 $\pm$ 4.855	35.95
12 h	196.866 $\pm$ 3.32	141.733 $\pm$ 0.730	28.005	95.833 $\pm$ 4.980	75.100 $\pm$ 1.642	21.634	64.466 $\pm$ 2.484	57.466 $\pm$ 0.723	10.858	2.600 $\pm$ 0.520	2.500 $\pm$ 0.434	3.846	358.900 $\pm$ 6.841	276.533 $\pm$ 5.966	22.94
24 h	134.600 $\pm$ 2.995	111.266 $\pm$ 3.707	17.335	72.566 $\pm$ 3.089	67.566 $\pm$ 1.240	6.896	131.666 $\pm$ 3.210	120.733 $\pm$ 1.746	8.303	1.466 $\pm$ 0.172	1.433 $\pm$ 0.235	2.251	341.333 $\pm$ 5.455	297.266 $\pm$ 5.971	12.92
48 h	94.933 $\pm$ 4.086	88.433 $\pm$ 1.710	6.846	48.633 $\pm$ 2.466	46.766 $\pm$ 1.627	3.836	142.266 $\pm$ 3.803	139.300 $\pm$ 5.232	2.084	00	00	00	285.833 $\pm$ 4.372	272.00 $\pm$ 4.404	4.839

\*Toluidine blue sections

lymphocytes and basophils cells showed maximum suppression at 6 h, 3 h and 1 h respectively (Table 1).

**Subgroup 2B-** Thirty minutes before injection of turpentine in the skin, the calves were pretreated with cyproheptadine intramuscularly, and the lesion as per non-pretreated group were obtained and processed for histopathological examination.

Vascular changes of the blood vessels and oedema of dermis were not evident at 0-2 minutes time interval. These changes were observed from 30 minutes onward. More marked hyperemia and oedema were observed at 6 h but less than Subgroup 1B. The significantly maximal suppression of leukocytes, neutrophils, lymphocytes and basophils was recorded at 3 h while maximal suppression of monocytes was noticed at 6 h (Table 2).

The emigration of the leukocytes was suppressed in cyproheptadine pretreated calves in *Staphylococcus epidermidis* and turpentine-induced inflammations. Maximal suppression of the leukocyte infiltration was recorded at 3 h in both stimuli. In bacterial inflammation cyproheptadine caused maximal suppression of the neutrophils at 3 h, monocytes cells at 6 h, and lymphocytes and basophiles at 1 h. Whereas, in turpentine injury, maximal suppression of the neutrophils, lymphocytes and basophiles at 3 h, and of monocytes at 6 h interval. In both stimuli neutrophils were more extensively suppressed as compared to mononuclear cells. The effect of non-steroidal anti-inflammatory drugs on cell migration has not been extensively investigated. Furthermore, to our knowledge, the selected inhibition of 5-HT by use of its antagonist and the resultant effect on cellular response remains totally unexplored in mammals. However, Khare (2000) studied the suppression of cellular response in birds pretreated with reserpine, a known 5-HT antagonist. The

worker reported a significant suppression of leukocyte infiltration at site of punch wounding. However, in birds, monocytoids cells were reduced in greater number as compared to heterophils. The findings differed from our observations in buffalo, where the infiltration of neutrophils was suppressed more than that of monocytes cells. Gupta *et al.* (2007, 2008) reported in the buffalo, antihistamine also suppressed neutrophil infiltration more significantly than monocytes and leukocytes taken together. It can be suggested that antagonists of vasoactive amine have more suppressive effect on neutrophil infiltration in buffaloes. However, further investigation needs to be in conducted.

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