IMMUNOMODULATORY EFFECTS OF GARLIC AND TULSI IN REPEAT BREEDING CROSSBRED COWS*

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ABSTRACT

Role of crude extracts of garlic and tulsi as an agent to stimulate uterine defense mechanism was studied by examining total protein and immunoglobulins in uterine flushing of repeat breeding cows and compared with most sensitive antibiotic (enrofloxacin), selected on the basis of antibiogram. Forty crossbred repeat breeding cows with endometritis were selected on the basis of history, appearance of cervical mucus and white side test after thorough per-rectal examination. Cows were divided randomly into five groups viz. A, B, C, D and E (control). 30 ml crude extracts of garlic, tulsi, garlic+tulsi, diluted with distilled water (1:5) were infused intrauterine, thrice, at 24 hrs interval starting on day of estrum in cows of group A, B and C, respectively. Similarly, enrofloxacin, selected on the basis of antibiogram (30 ml) and PBS (30 ml) were infused in cows of group D and E, respectively. Uterine flushings were collected at 0 hrs (day of estrum before treatment) and considered as "0" hour collection of uterine flushing. Second uterine flushing was also collected at 72 hrs of first flushing. These flushings were used for estimation of total protein and immunoglobulins. Results revealed a non significant rise in total protein after garlic, tulsi and garlic+tulsi treatment. On the other hand, there was significant increase ($p<0.05$) in immunoglobulins concentration after treatment. However, a significant decline ($p<0.05$) in these values was observed after treatment in antibiotic group.

Keywords: Repeat breeding cows; Herbal extracts; Immunomodulators

INTRODUCTION

Uterine fluid proteins have been reported to increase during genital infections (Brochart and Mascarenhas, 1990; Ahmad *et al.*, 1993). The serum immunoglobulins might pass into the uterus either by passive diffusion or by leakage of serum (Mitchell *et al.*, 1982). IgG and complement (C3b) were considered to be the most important opsonin in uterine defense mechanism (Asbury *et al.*, 1984).

Plant based immunomodulators such as rasayans could stimulate humoral immunity without side effect (Praveen kumar *et al.*, 1999). Garlic and tulsi have been reported to show good immunomodulatory activity (Mediratta and Sharma 2000; Ghazanfari *et al.*, 2002). Therefore, in the present paper, attempts have been made to evaluate the immuomodulatory effects of crude extract of garlic and tulsi treatment in repeat breeding crossbred cows.

MATERIALS AND METHODS

The study was carried out in 40 repeat breeder crossbred cows stationed at Instructional Dairy Farm, Nagla, G. B. Pant Univ. of Agriculture and Technology, Pantnagar- 263 145, District Udham Singh Nagar (Uttarakhand). Animals, with the history of repeat breeding, were thoroughly examined per-rectally to rule out any anatomical defect of genitalia and ovarian abnormalities. The animals, manifesting signs of heat regularly after 20-21 days and positive for white side test were included in the study.

Fresh cloves of garlic and fresh leaves of tulsi were taken in separate sterilized pestle-mortar and crushed properly. The crushed material was filtered through muslin cloth twice and finally through Whatman's filter paper No. 41. The filtrate was centrifuged at 3000 revolution per minute (rpm) for 15 minutes in a refrigerated centrifuge.

Fresh leaves of tulsi were dried in shed for a period of about 5-6 days. The dried material was pulverized by a mechanical mixer grinder and passed through a 40-

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mesh siew to obtain fine power, which was stored in a clean, wide mouthed, air tight bottle at room temperature for further use.

100 gm herbal power was dissolved in 500 ml solvent (acetone or methanol) and 500 ml distilled water and left for a period of 24 hrs. Mixed with the help of glass rod for 5 minutes at every 2 hrs then filtered twice through muslin cloth. The filtrate was centrifuged at 3000 rpm for 30 minutes in a refrigerated centrifuge. Supernatant evaporated in vacuum evaporator at 65°C temperature and 600 mm Hg pressure. Extract so obtained was stored in a clean vial at 4°C for further use.

Cows were divided randomly into five groups viz. A, B, C, D and E. 30 ml crude extract of garlic, tulsi and garlic+tulsi (1:1) diluted with distilled water (1:5) was infused intrauterine, thrice at 24 hrs interval starting on day of estrum in cows of group A, B and C, respectively. Similarly, enrofloxacin, selected on the basis of antibiogram (30 ml, 10%) and PBS (30 ml) were infused in cows of group D and E, respectively.

Uterine flushings were collected twice by two way Foley catheter i.e. one before giving the treatment at 12 hrs after onset of estrus and second at 72 hrs of first flushing. The collection technique involved aspiration of uterine flushing. Uterine flushings were collected in vials and kept in a thermocol box containing ice till further processing. 10ml uterine flushing was centrifuged at 3000 rpm for 15 minutes. Supernatant was used for the estimation of total proteins and immunoglobulin concentration.

Total protein concentration in the cell free uterine flushing was estimated with Folin phenol reagent method (Lowry *et al.*, 1951) using bovine serum albumin (BSA) as a standard. Total immunoglobulin concentration was estimated as per method of McEvan *et al.* (1970).

The data so generated were analyzed statistically (Snedecor and Cochran, 1989) using analysis of variance (ANOVA) and Chi-square test to test the significant differences of means.

RESULTS AND DISCUSSION

Total protein concentration (mg/dl) varied non-significantly (146.94±9.28 to 161.40±8.96) among all five groups prior to treatment (Table 1) indicating similar immune status of the experimental animals. A non significant increase in total protein values was observed from pre-treatment (0 hours) to post-treatment (72 hours) in uterine flushing of group A, B, C and group E cows The increase in total protein concentration might be due to stimulation of uterine immune responses after herbal extract treatment. Several workers have demonstrated a marked increase in protein concentration and PMNs infiltration in uterine flushings following intrauterine infusion of garlic (Savic 2002; Seong and Dong, 2006). Herbal extracts used in the study appeared to cause influx of serum derived proteins. A significant decline ($p<0.05$) was seen from pre-treatment (0 hours) to post-treatment (72 hours) in uterine flushing of group D cows.

Infusions of sensitive antibiotic after in-vitro antibiogram would kill the bacteria effectively as a result, inflammatory response in uterus might have declined and so the protein influx towards the uterine lumen had reduced (Prasad, 2006). In control group cows, non significant rise seen in total protein concentration might be due to natural uterine defense mechanism.

Total immunoglobulin concentration (mg/dl) varied non-significantly and ranged from (37.67±2.25 to 41.07±2.63) in all five groups prior to treatment as shown in Table 2. A significant increase ($p<0.05$) in total immunoglobulin value was observed from pre-treatment (0 hours) to post-treatment (72 hours) in uterine flushing of group A, B and group C cows. However, there was almost no change (41.00±2.40 to 41.09±2.51) in immunoglobulin values of group E (control) cows prior and after treatment. A significant decline ($p<0.05$) was seen in immunoglobulin values from pre-treatment to post treatment in uterine flushing of group D cows. Total immunoglobulin concentration in the present study significantly increased after infusion of garlic, tulsi and garlic + tulsi in comparison to concentration before treatment indicating the stimulation of uterine immune response. The serum immunoglobulin may probably pass into the uterus either by passive diffusion or leakage (Mitchell *et al.* 1982). An influx of specific antibodies from serum in to the uterine endometrium along with neutrophilic infiltration after infection in cows has been reported by Schulz *et al.* (1979). The immunoglobulin contents in the uterine exudates reflect accurately the seriousness of the uterine inflammatory process and serve the criterion of clinical recovery (Aknarzov, 1988). The interaction between immunoglobulins and neutrophil is of great importance for better immunity against infection than concentration of immunoglobulins alone (Leu and Cheung, 1986; Watson and Stokes, 1990).In antibiotic group a significant decline ($p<0.05$) was found

in immunoglobulin concentration as compared to pre-treatment. Kumar *et al.*, (2008) has reported significant ($p<0.05$) effect of garlic & tulsi on recovery rate of endometritis (87.5 ± 7 Vs 25 ± 2) in comparison to control. No citation could be traced in the literature for comparison of results.

Table 1: Mean ± SE of total protein concentration (mg/dl) in uterine flushing cows of in different group before (0 hours) and after (72 hours) treatment.

Groups	No. of animals	Total protein (mg/dl) before treatment	Total protein (mg/dl) after treatment
Group A (Garlic)	8	157.19±9.66^{a}	170.22±9.56^{a}
Group B (Tulsi)	8	146.94±9.28^{a}	157.59±6.31^{a}
Group C (Garlic+Tulsi)	8	161.40±8.96^{a}	179.29±9.73^{a}
Group D (Antibiotic)	8	161.10±7.80^{a}	134.30±4.11^{b}
Group E (Control)	8	161.32±8.61^{a}	166.99±9.20^{a}

Means with different superscripts in a row vary significantly ($p<0.05$).

Table 2: Meant SE of total immunoglobulin (Igs) concentration (mg/dl) in uterine flushing in cows of different group before (0 hours) and after (72 hours) treatment.

Groups	No. of animals	Total protein (mg/dl) before treatment	Total protein (mg/dl) after treatment
Group A (Garlic)	8	39.93±3.26^{a}	52.75±3.03^{b}
Group B (Tulsi)	8	37.67±2.25^{a}	47.31±1.50^{bc}
Group C (Garlic+Tulsi)	8	39.90±1.45^{a}	57.10±2.07^{b}
Group D (Antibiotic)	8	41.07±2.63^{a}	33.49±1.40^{d}
Group E (Control)	8	41.00±2.47^{a}	41.09±2.51^{ac}

Means with different superscripts in a row vary significantly ($p<0.05$).

On the basis of present investigation it may be concluded that extract of garlic + tulsi is having a very good antibacterial and immunomodulatory activity and further proved to be useful. It may become the drug of choice in future for bacterial endometritis leading to repeat breeding condition in crossbred cows.

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