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Mastitis Treatment And Prevention Of New Intramammary Infections With Topical Herbal Spray

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Abstract
Objective: The study was designed to study the potency of an herbal spray AV/AMS/15 in controlling mastitis.
Method: The 24 cows (HF × Sahiwal crossbred) found positive for specific mastitis in at least one quarter and not in late lactation were included in trial. The selected animals were divided randomly in two groups. The treatment group cows (n=12) were treated with Topical Herbal Spray, AV/AMS/15 spray (M/S Ayurvet Limited, Baddi, India) (every morning and evening milking for 5 days) and the other group of cows served as untreated control (n=12) group. Milk samples were collected and analyzed for various parameters viz. pathogens isolation and identification, California Mastitis Test, Electrical conductivity (EC), pH, Biochemical composition of the milk and Somatic cell count (SCC).
Result: The results showed elimination of intramammary infections, decrease in somatic cells and electrical conductivity in AV/AMS/15 spray treated group along with improvement in milk solids components.
Conclusion: AV/AMS/15 spray efficiently treated the mastitis in cows

Key words: Herbal spray, California mastitis test, quarter foremilk, cow composite milk

I. INTRODUCTION

Bovine mastitis is very common in cows all over the world. Mastitis is inflammation of mammary gland affecting all the species of domestic animals and is of great concern to dairy industry [1]. Bacteria are usually behind the udder infection, over 140 different microorganisms have been isolated from bovine intramammary infection and majority of infections are caused by Staphylococci, Streptococci and Enterobacteriacae [2, 3]. Intramammary infections cause huge economic losses. Approximately 70% of economic losses to diary industry occur due to SCM [4]. Apart from causing economic losses, this disease also posses the risk for the transmission of zoonotic diseases like tuberculosis, brucellosis, leptospirosis and streptococcal sore throat to human beings [5]. Antibiotics are used for the treatment and control of mastitis, but intramammary infusion of

antibiotics for mastitis therapy was cited as a major reason for milk contamination [6] and frequent use of antibiotic therapy leads to antibiotic resistance [7]. For this reason, alternative therapy to antibiotic therapy for mastitis treatment and control has to be developed like use of herbal preparation. Topical Herbal Spray AV/AMS/15 (*M/S Ayurvet Limited, Baddi, India*), a herbal spray usually applied on the udder, has been developed as an alternative method for controlling mastitis in ruminants. AV/ AMS/ 15 herbal spray active ingredients including herbal extracts of *Cedrus deodara* [8, 9], *Curcuma longa* [10, 11], *Glycyrrhiza glabra* [12, 13] and *Eucalyptus globulus* [14] are expected for their antibacterial, anti-inflammatory, analgesic, antihistaminic and immunomodulatory effects.

II. MATERIALS and METHODS

1. Experimental Plan

The study was carried out in HF × Sahiwal crossbred cows at University dairy farm. The farm followed the practice of semi loose housing system. The in-milk cows were screened for any evidence of clinical mastitis (udder/ milk examination), and a complete history particularly with respect to antibiotic treatment of animal if any during the last 21 days was noted. The cows found positive for clinical mastitis in any quarter and/ or treated with antibiotic during the last 21 days were not considered. The remaining cows were assessed for quarter health status as per the guidelines of International Dairy Federation (IDF). The 24 cows found positive for specific mastitis in at least one quarter and not in late lactation were included in trial. The selected animals were divided randomly in two groups (n=12). The cows in treatment group (n=12) were treated with Topical Herbal Spray, AV/AMS/15 spray (*M/S Ayurvet Limited, Baddi, India*) applied gently by spraying on the udder following every morning and evening milking for 5 days and the cows in other group were kept as untreated control (n=12).

1.1 Sampling and Parameters Studied

To assess the udder health status and milk quality, sampling was done pre-treatment (day 0), and at day 7th, 15th and 30th post-treatment. Two types of milk samples viz., Quarter foremilk (QFM) and cow composite milk (CC) were sampled. The milk samples were collected during the routine afternoon milking. Before collection of milk sample, it was ensured proper cleanliness and dryness of udder. The teat orifice was cleaned with a cotton swap soaked in 70% alcohol (spirit). First few streaks of milk were discarded and the individual quarter foremilk samples (about 5 ml) were collected in sterilized test tubes. Following this, about 20 ml of quarter foremilk were collected in clean plastic vials. On completion of cow milking, about 20 ml thoroughly mixed cow composite (CC) milk was taken in clean plastic vials [15]. The milk samples were transferred immediately to the laboratory and analyzed for various parameters viz. Isolation and identification of pathogens, California Mastitis Test, Electrical conductivity (EC) [16], pH,

Biochemical composition of the milk and Somatic cell count (SCC). The efficacy of herbal therapy in mastitis was evaluated in terms of elimination of intramammary infections and improvement of milk quality at quarter and cow level.

1.2 Statistical Analysis

The effect of therapy on udder health was analysed using Chi square analysis whereas Student's t-test distribution was applied to know the significance of results with respect to milk quality.

III. RESULTS AND DISCUSSION

1. Elimination of Intramammary Infections

The isolation and identification of microbial organisms from milk samples was done as per standard microbial procedures of National Mastitis Council [17]. The elimination of intramammary infections in cows treated with herbal spray was observed to be 60.0% (12/20) at day 30th post-treatment. The corresponding elimination of infection in control group was 28.57% (6/21) at day 30th (**table 1**).

Table 1: Elimination of intramammary infections with topical herbal spray therapy

Microorganisms	Number of intramammary infections			
	Present at 0 day (pre-treatment)		Eliminated at 30th day post-treatment	
	Treatment group	Control group	Treatment group	Control group
Coagulase positive staphylococci	14	14	08	04
Coagulase negative staphylococci	04	07	03	02
Corynebacteria	02	-	01	-
Overall	20	21	12 (60.0)	06 (28.57)

Figures in parentheses indicate percentage

*Significant differences existed in elimination of intramammary infections between treatment and control groups at 30th day post-treatment ($\chi^2 = 4.11$; 01df; $p < 0.05$)

Staphylococcus aureus and other coagulase-positive *staphylococci* are frequent contaminants of milk and dairy products, the most probable source being the udder of the milk animal [18], lead to production of enterotoxins. *S. aureus* and other CPS are the most frequently involved species in the etiology of bovine mastitis [19]. The decrease in number of microorganisms signifies the efficacy of herbal spray AV/AMS/15 in treating mastitis. The differences in elimination of intramammary infections in treatment vs. control were observed to be statistically significant ($p < 0.5$) with χ^2 value 4.11 on day 30th in herbal spray group. **Table 2** depicts the establishment of new intramammary infections in treatment groups and cows kept as untreated control. However, no significant differences were observed in occurrence of new intramammary infections with either of the therapy as compared to control yet low value was observed in herbal spray AV/AMS/15 (14.71%) treated group on day 30th (χ^2 always < 3.84, 01 df; $P > 0.05$).

Table 2: Establishment of new intramammary infections (IMI) in treatment and control groups

Observation	Herbal spray group	Control group
	At 30th day post-treatment	
Eligible quarters	34	28
New IMI with Coagulase positive staph	02	03
New IMI with Coagulase negative staph	02	04
New IMI with corynebacteria	01	0
Overall	05 (14.71)	07 (25.0)

Figures in parentheses indicate percent new IMI among eligible quarters
No Significant difference existed in occurrence of new intramammary infections between treatment and control groups at 30th day post-treatment ($\chi^2 < 3.84$; p.> 0.05)

In the absence of effective mastitis prevention and control measures, more quarters of the udder will be infected at calving during the dry period compared to the number infected at drying off. From the point of view of mastitis control, most new IMIs occur during the dry period [20]. By placing emphasis on prevention of new infections, udder health can be achieved more rapidly.

2. Effect of therapy on milk quality

2.1 Foremilk Quality

In untreated control group non significant variation in CMT score, EC (mS/cm) and pH was observed on day 7th and day 30th. SNF (%) and lactose (%) content also varied non-significantly (**table 3**). In cows treated with herbal spray, no significant difference (p > 0.05) was observed in CMT score (11 ± 0.80 vs. 0.86 ± 0.84) and EC (5.19 ± 1.21 vs. 5.16 ± 1.27 mS/cm) except pH (7.18 ± 0.19 vs. 7.00 ± 0.36) (p < 0.01) of quarter foremilk at 7th day post treatment (**table 4**).

Table 3: Foremilk quality at quarter level in cows kept as control

Parameters	Pre-Treatment (Mean ± SD)	7th day Post-treatment (Mean ± SD)	p-value	30th day Post-treatment (Mean ± SD)	p-value
CMT score	0.76 ± 0.63	0.76 ± 0.59	0.50	0.73 ± 0.57	0.40
EC (mS/cm)	4.45 ± 0.70	4.44 ± 0.62	0.48	4.59 ± 0.60	0.14
pH	6.93 ± 0.19	6.86 ± 0.19*	0.04	6.94 ± 0.35	0.39
Fat (%)	2.91 ± 1.04	-	-	3.30± 1.03*	0.03
SNF (%)	8.83 ± 0.81	-	-	9.03 ± 0.35	0.06
Lactose	5.20 ± 0.25	-	-	5.23 ± 0.27	0.30

*Significant difference (p<0.05); **significant difference (p<0.01)

However, therapy significantly (p<0.05, p< 0.01) restored the milk quality at 30th day post-treatment in terms of CMT score, EC, fat and lactose contents of milk (P<0.05). The corresponding pre and post therapy values were CMT: 1.11 ± 0.80 vs. 0.61 ± 0.64, electrical

conductivity (EC) 5.19 ± 1.21 vs. 4.7 ± 1.10 mS/cm, fat: 3.37 ± 1.09 vs. 3.67 ± 1.14 per cent and lactose: 4.89 ± 0.31 vs. 5.02 ± 0.34 per cent (**table 4**). California mastitis test scores as indicators of subclinical intra-mammary infections at the end of lactation in dairy cows [21]. Electrical conductivity has a high correlation with SCC in foremilk as the sodium and chloride concentrations increase in milk [22]. A decrease in both parameters in treated group indicted the efficacy of herbal spray AV/AMS/15 in mastitis control.

Table 4: Effect of therapy on foremilk quality at quarter level in cows treated with topical herbal spray

Parameters	Pre-Treatment (Mean ± SD)	7th day Post-treatment (Mean ± SD)	p-value	30th day Post-treatment (Mean ± SD)	p-value
CMT score	1.11 ± 0.80	0.86 ± 0.84	0.07	0.61 ± 0.64**	0.001
EC (mS/cm)	5.19 ± 1.21	5.16 ± 1.27	0.46	4.7 ± 1.10*	0.05
pH	7.18 ± 0.19	7.00 ± 0.36**	0.002	7.22 ± 0.22	0.17
Fat (%)	3.37 ± 1.09	-	-	3.67± 1.14**	0.11
SNF (%)	8.49 ± 0.67	-	-	8.36 ± 0.85	0.21
Lactose	4.89 ± 0.31	-	-	5.02 ± 0.34*	0.03

*Significant difference ($p<0.05$); **significant difference ($p<0.01$)

2.2 Composite Milk Quality

Composite milk samples containing milk from each of a cow's 4 mammary quarters are often collected in standard veterinary practice for diagnosis of IMI in cows with subclinical mastitis [23]. Somatic cell count ($\times 10^3$cells/ml) was non significantly increased in untreated control group from day 0 to day 30th (871 ± 800 vs. 921 ± 741). CMT score (0.96 ± 0.72 vs. 1.00 ± 1.00), EC (4.44 ± 0.36 vs. 4.60± 0.24) and pH (6.87 ± 0.14 vs. 6.95 ± 0.09) was also increased non significantly in untreated control group from day 0 to day 30th. Lactose content was also decreased non significantly from day 0th to day 30th (**table 5**).

Table 5: Cow composite milk quality in cows kept as control

Parameters	0 day (Mean ± SD)	15th day Post-treatment (Mean ± SD)	p-value	30th day Post-treatment (Mean ± SD)	p-value
CMT score	0.96 ± 0.72	1.04 ± 0.92	0.42	1.00 ± 1.00	0.45
EC (mS/cm)	4.44 ± 0.36	4.65 ± 0.40	0.09	4.60± 0.24	0.11
pH	6.87 ± 0.14	6.91 ± 0.11	0.23	6.95 ± 0.09	0.06
Fat (%)	3.73 ± 1.22	3.71 ± 0.54	0.48	4.38± 0.85	0.07
SNF (%)	8.70 ± 0.47	9.02 ± 0.56	0.07	8.91 ± 0.48	0.15

Lactose	5.18 ± 0.42	5.34 ± 0.15	0.16	5.06 ± 0.50	0.26
SCC (×10^{3}cells/ml)	871 ± 800	947 ± 828	0.41	921 ± 741	0.44

No Significant difference

Whereas in AV/AMS/15 herbal spray treated group the milk SCC (×10^{3}cells/ml) decreased from 1316 ± 726 to 880 ± 512 ($p<0.05$) (Table 6) at 30th day post-treatment. 30th day post-treatment CMT score was also decreased (1.21 ± 0.50 vs. 0.92 ± 0.82) non-significantly in AV/AMS/15 herbal spray treated group. Fat %, SNF % and lactose content was also non significantly improved in AV/AMS/15 herbal spray treated group (**table 6**). Milk pricing structures based on fat and protein yields cause contrasting interests for producers and processors [24]. Increase in milk solids components in AV/AMS/15 herbal spray treated group adds more economy to the milk.

Table 6: Effect of therapy on cow composite milk quality in cows treated with topical herbal spray

Parameters	Pre-Treatment (Mean ± SD)	15th day Post-treatment (Mean ± SD)	p-value	30th day Post-treatment (Mean ± SD)	p-value
CMT score	1.21 ± 0.50	1.17 ± 0.49	0.42	0.92 ± 0.82	0.15
EC (mS/cm)	5.04 ± 1.16	5.38 ± 0.90	0.22	5.37± 0.85	0.22
pH	6.96 ± 0.35	7.14 ± 0.22	0.07	7.10 ± 0.15	0.10
Fat (%)	3.49 ± 1.08	2.98 ± 0.71	0.09	4.23± 1.12	0.06
SNF (%)	8.25 ± 0.69	8.35 ± 0.73	0.38	8.33 ± 0.70	0.40
Lactose	4.77 ± 0.60	4.99 ± 0.43	0.16	4.80 ± 0.59	0.46
SCC (×10^{3}cells/ml)	1316 ± 726	1006 ± 488	0.13	880 ± 512*	0.05

*Significant difference ($p<0.05$)

IV. CONCLUSION

Results obtained from the study indicate beneficial effects of phyto-derived remedy AV/AMS/15 herbal spray against mastitis of lactating dairy cows, particularly against forms caused by staphylococci.

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