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Research Article

A new herbal teat dip for preventing mastitis

S. Kapoor[1*], D. K. Gupta[1], R. S. Singh[2] and D. Narang[3]

[1]Department of Veterinary Medicine, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana- 141 044, Punjab, India; [2]Department of Teaching Veterinary Clinical Complex, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana- 141 044, Punjab, India; [3]Department of Veterinary Microbiology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana- 141 044, Punjab, India

Abstract

The present study was conducted to prepare a novel herbal teat dip formulation which could be feasible for the farmer to prepare by himself and to evaluate its efficacy in preventing mastitis. The herbal teat dip is comprised of Aloe vera, Tulsi and Turmeric as the main ingredients. Animals were divided into 3 groups: group I (control group) animals where no teat dipping was done, group II animals where chemical teat dipping (Betadine) was done and group III animals where herbal teat dipping was practised for a period of 6 months. Milk quality parameters like somatic cell count (SCC), electrical conductivity (EC), pH and incidence of intra-mammary infection were noted and compared between all 3 groups throughout the study period. Microbiological culture was performed to isolate mastitis pathogens. SCC and EC were highest in animals of the control group, followed by the chemical group and lowest in the herbal teat dip group throughout the study period. The pH value did not differ significantly between 3 groups. The incidence of intra-mammary infection was highest in the control group, followed by the chemical group and lowest in the herbal teat dip group throughout the study period. Herbal teat dip showed healing properties on teat end lesions and no side-effects, which were evident in the chemical teat dip group (teat end score>2). Therefore, herbal teat dip was found to have better efficacy than conventional teat dip in preventing mastitis. However, herbal teat dip did not eliminate mastitis pathogens from milk, and latent infection persisted in animals.

Keywords: Efficacy, Herbal teat dip, Mastitis, Post-milking teat dipping

Highlights

- The farmer can easily prepare the post-milking herbal teat dip formulation as the ingredients are readily available, and the procedure is very simple and feasible to follow at home.
- Herbal teat dip proved to be as effective as commercial chemical teat dip in preventing mastitis.
- Herbal teat dip formulation had an advantage over chemical teat dip formulation because there were no side effects like teat end lesions, and it had a healing effect on teat skin.
- One drawback was the persistence of latent infection because mastitis pathogens were not eliminated from milk.

INTRODUCTION

Post-milking teat disinfection is recognised as the most important and fundamental component of a successful mastitis control programme. However, a number of drawbacks are associated with most commercial teat disinfectants currently available. One important drawback is the irritation of teats due to low or high pH, high titratable acidity or alkalinity, or both (Yanuartono *et al.*, 2020). To avoid teat irritation and enhance skin conditioning, emollients such as glycerin or lanolin are frequently included in teat dip formulations, but teat dips' germicidal effectiveness may reduce if emollient concentrations are substantially higher than 10 to 12 percent. Besides, there is a problem of teat dip residues shed in milk, which is of public health concern. To mitigate these issues, alternative medicines are being used nowadays. Herbal medicines have gained importance due to their lower toxicity, fewer side effects, and no residues in the milk.

Globally, many studies have been conducted on these herbs and have revealed unique active constituents that can be used as herbal biocides. Plants like Neem (*Azadirachta indica*) and Tulsi (*Ocimum sanctum* L.) have been used for medicinal purposes in India for centuries (Subapriya and Nagini, 2005). Tulsi, often described as "Queen of plants", has been shown to have insecticidal and antibacterial properties. Based on traditional knowledge, the crude plant and its extracts are used to treat various infections (Singh *et al.*, 2010). The antimicrobial activity of Turmeric (*Curcuma*

*Corresponding Author, E-mail: smrititholang@gmail.com

longa L.) was first reported by Schraufstätter and Bernt (1949). Despite its poor solubility in water, poor bioavailability, and pharmacokinetic profile, modern studies have proven curcumin's strong antimicrobial potential (Kotha *et al.*, 2019). Aloe vera has been tested for its antibacterial activity and found effective against clinically isolated bacterial pathogens such as *Enterococcus bovis*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Proteus mirabilis* and *Pseudomonas aeruginosa* (Pandey and Mishra, 2010).

Use of these herbs as active ingredients in herbal disinfectants may provide efficient protection against common pathogens and can reduce our dependence on commercial chemical disinfectants. Herbal disinfectants, if proven to be equally effective, may also replace chemical disinfectants in future. There are many *in vitro* studies on herbs to analyse their antibacterial activity, but their efficacy as herbal teat dip in an *in vivo* study has not been thoroughly investigated. Besides, there are very few herbal teat dips available in the market, and very few studies have assessed their efficacy in comparison to chemical teat disinfectants. Lago *et al.* (2016) studied the efficacy of a novel glycolic acid-based (GA) post-milking barrier teat disinfectant and compared the results with those of commercial iodine-based post-milking teat disinfectant. As per his findings, GA-based teat disinfection was not inferior to commercial iodine-based disinfectant. The study concluded that a teat disinfectant based on GA was safe for teat conditioning and might be used as an efficient post-milking teat disinfectant. Another study (Yu *et al.*, 2017) compared the efficacy of a novel probiotic lactic-acid-bacteria (LAB) based teat disinfectant to a commercial disinfectant in managing mastitis through a change in somatic cell count (SCC). During the study, the SCC of the LAB group gradually decreased and was somewhat lower than that of the animals of the commercial disinfectant group. The results of the study suggested that the LAB disinfectant has the potential to eliminate mastitis-associated bacteria and might be used instead of chemical pre- and post-milking teat disinfectants to control mastitis. Punniamurthy *et al.* (2017) investigated the antibacterial efficacy of an ethnoveterinary preparation containing Aloe vera and Curcuma longa against key field isolates (*E. coli*, *S. aureus* and *P. aurogenosa*). The herbal preparation was found quite effective with variable zones of inhibition, and the maximum efficacy against the isolates was obtained with ethanol and aqueous extracts.

Keeping these facts in view, the current study aimed to develop a novel herbal teat dip formulation and evaluate its efficacy as a post-milking teat disinfectant in preventing intramammary infection.

MATERIALS AND METHODS

Experimental design: This study was conducted at an organized private dairy farm in Shahkot city, at district Jalandhar, Punjab, India. Lactating cows in mid-lactation were screened for intra-mammary infection by somatic cell count (SCC) and bacteriological examination. A total of 30 cows with no intra-mammary infection were selected for the study and divided into three groups: Group I served as the control group having 6 cows which did not receive post-milking teat dipping, Group II and III were the treatment groups having 12 cows each, subjected to different post-milking teat dips. In Group II, post-milking teat dipping was done with a commercial chemical teat antiseptic (Betadine) and in Group III, with a herbal teat dip formulation. The experiment was carried out for 6 months (February to July). The presence of latent infection was not taken into consideration while grouping the animals, because uniformity of animals in 3 groups was difficult to achieve as very few animals were free of latent infection. In treatment groups, teats of cows were dipped in post-milking teat disinfectant, twice daily immediately after milking, with a minimum contact time of 20-30 seconds.

Collection of milk samples: Both the quarter foremilk and cow composite milk samples were collected at monthly intervals for 6 months, the time of regular afternoon milking. While collecting milk samples, it was ensured that the animal udder and teats were clean and dry. The tip of the teat orifice was disinfected with a spirit/ 70% alcohol swap. The first few streaks of milk were discarded, and approximately 5 mL of quarter foremilk (QFM) from every quarter was obtained in a sterilized glass test tube. At the end of milking, another 20 mL of milk representing cow composite milk (CCM) was collected in clean plastic vials from the milking machine/ hand bucket after thorough mixing. The milk samples so obtained were ice packed and moved to the research lab for further investigation.

Parameters observed in control and treatment groups: 1. Quarter health status (specific and non-specific mastitis) at monthly interval; 2. Composite SCC, EC and pH (Schalm *et al.*, 1971) at monthly interval.

The quarter health status was defined on the basis of SCC and bacteriology of quarter foremilk samples according to the International Dairy Federation (1987) criteria as detailed below:

Milk SCC ($\times 10^3$ cells/mL)	Microbial pathogen	
	Not detected	Detected
≤ 200	Healthy	Latent infection
> 200	Non-specific mastitis	Specific mastitis

Somatic Cell Count: The milk SCC was measured by an automatic somatic cell counter (SomaScope Smart, Delta instruments, The Netherlands). The principle of the instrument is that the cells are labeled with a fluorescent dye. The fluorescent molecules are absorbed by the cell substance of interest, e.g., DAPI (4', 6- Diamidine-2'-phenylindole dihydrochloride) by the nuclear DNA. For each single cell, the fluorescent light intensity is measured. The more +light the cell emits, the more cell substance of interest, it contains. Fluorescent cell in this volume is counted. From that, the concentration of cells per mL is determined. Each single measurement took approximately 40 seconds. Results were expressed in $\times 10^3$ cells/mL of milk.

Electrical conductivity: A handheld digital mastitis detector named Milk Checker (AR Brown Co., Ltd, Toyko, Japan) was used for measuring the electrical conductivity of milk samples. The equipment uses the most innovative technology to deliver consistent outcomes on EC of milk at 3-40°C with automatic compensation to 25°C. The results were expressed in Milli Siemens per cm (ms/cm).

pH: The pH of milk was measured using a digital pH meter (Mettler-Toledo FiveEasy™ Plus).

Detection of mastitis pathogens: The quarter milk samples were subjected to microbiological examination. First, the samples were streaked on blood agar and incubated at 37°C for 12-18 hours. The primary colonies derived on blood agar were then sub-cultured on freshly prepared non-selective media, i.e., BHI agar plate. Finally, the pure culture of bacteria was then identified through MALDI-TOF MS available in the Department of Veterinary Microbiology. The MALDI-TOF MS works on the principle of analyzing the chemical compound and measures the m/z (mass to charge) ratio for microbial identification. The sample to be analyzed was coated with an energy-absorbent organic compound solution called matrix. This matrix crystallizes upon drying, which co-crystalizes the sample and causes ionization with a laser beam and produces singly protonated ions. These protonated ions accelerate at a fixed potential and separate from each other as per their m/z ratio, which are then detected and measured using a TOF analyser. In MALDI-TOF MS, m/z ratio of an ion is a measurement of the time required by an ion to travel the length of flight tube. On this basis of TOF information, PMFs, i.e., peptide mass fingerprint (mass spectrum), is developed for analytes in the sample. Identification of pathogens is done by comparing the unknown organisms peptide mass fingerprints (PMFs) with the PMFs contained in the database. For species level identification a m/z of 2-20 kilodalton (kDa) was utilized which mainly represented ribosomal proteins which comprise about 60-70% weight of a microbial cell. The solvents penetrated the microbial cell wall to extract out the intracellular proteins. When matrix evaporates, crystallization of protein molecules takes place within the matrix.

Herbal teat dip preparation: The herbal teat disinfectant was prepared from 3 main ingredients: i) Aloe vera (*Aloe barbadensis miller*), ii) Tulsi leaves/ Holy Basil (*Ocimum sanctum*) and iii) Turmeric (*Curcuma longa*).

For preparing 1 litre of herbal teat dip (which would suffice for 12 cows for 30±2 days), the composition of ingredients was as follows: (i) Aloe vera juice 850 mL, (ii) dried Tulsi leaves powder 75 gm, (iii) Turmeric powder 50 gm, and (iv) glycerine 150 mL (to achieve desired consistency).

Freshly plucked 3-4 large Aloe vera leaves, each weighing approximately 250 gm, were taken and washed thoroughly to remove all soil and dirt from the leaves. Aloe vera gel was removed completely and was mixed thoroughly in a mixer/grinder such that the thick viscous Aloe vera gel transformed into watery clear light green coloured pure Aloe vera juice. Then about 75 gm of dried Tulsi leaf powder and 50 gm of Turmeric powder was added to the Aloe vera juice. All 3 ingredients were mixed thoroughly in a mixer/grinder for 4-5 minutes. Then it was collected in a jar and allowed to rest overnight in a refrigerator. Next day it was sieved through a muslin cloth. Then 150 mL of glycerine was added to achieve the desired consistency with 850 mL Aloe vera mixed solution. Finally, a clear, homogenous liquid solution of herbal teat dip was ready to be used (Fig. 1).

Statistical analysis: Data was analyzed by MINITAB statistical software (release 14.2) using statistical procedures like ANOVA (Analysis of variance) for comparing means between 3 groups. The level of significance was set at 5% ($P<0.05$).

RESULTS

Somatic Cell Count: When the experiment began in February, the SCC in the control (group I), chemical teat dip (group II), and herbal teat dip (group III) groups were not significantly different and were comparable in March as well. Milk samples collected in April showed a significant ($p<0.05$) difference in SCC of the herbal teat dip group with the chemical and control groups. Thereafter in May, June and July, there was a significant

Fig. 1 Herbal teat dip solution

difference in SCC, primarily between the control group and the herbal teat dip group, with the control group having significantly higher SCC than the herbal group, but the difference between the control and chemical teat dip groups was not significant. In all three groups marked increase in SCC was seen in the month of July as compared to the SCC values of previous months. When the SCC of six months was averaged, there was a significant difference in SCC of both the test groups compared to the control group (Table 1), where the control group had the highest SCC, followed by the chemical teat dip group, and the herbal group having the lowest.

EC and pH: In February, the EC values did not differ significantly between three groups (Table 2). EC showed significant variation in March, April and May, primarily between the control group and the herbal teat dip group, while the EC of the chemical teat dip group was not significantly different from the herbal and control groups. In June, EC of the control group was significantly higher than EC of both treatment groups, while EC of the chemical and herbal group was comparable. In July, there was no significant difference in EC between the three groups. Overall, the control group had significantly higher EC, followed by the chemical teat dip group, and the herbal group had the lowest.

The pH of milk followed a similar pattern, with the control group having the highest overall pH, followed by the chemical group, and the herbal group having the lowest, but the values were not significantly different between groups throughout the study period (Table 3).

Quarter Health Status and efficacy of teat dips: In February, at the beginning of the study, there was no intra-mammary infection (zero specific and non-specific mastitis) in the control and treatment groups. In the following months, no. of quarters affected with specific

Table 1. SCC (× 10^3 cells/mL) in control (Group I), chemical (Group II) and herbal (Group III) teat dip group

Month	Group I (n=6) Mean ± S.E.	Group II (n=12) Mean ± S.E.	Group III (n=12) Mean ± S.E.	Significance p-value
Feb	96.17 ± 45.74	78.58 ± 30.99	47.83 ± 12.47	0.4053
March	138.67 ± 48.02	124.17 ± 44.93	48.75 ± 6.60	0.1739
April	169.33 ± 27.50^b	156.67 ± 48.12^b	44.5 ± 6.48^a	0.0308
May	198.33 ± 45.10^b	121.5 ± 35.78^{ab}	48.08 ± 6.34^a	0.0106
June	238.17 ± 58.09^b	141.5 ± 45.54^{ab}	72.83 ± 29.45^a	0.0380
July	504.33 ± 177.87^b	174.25 ± 65.66^{ab}	142.25 ± 82.29^a	0.0410
Total	225.83 ± 38.70^b	132.77 ± 18.64^a	67.37 ± 14.88^a	0.0000

Values in rows with at least one similar superscript do not differ significantly ($p<0.05$)

Table 2. EC (mS/cm) in control (Group I), chemical (Group II) and herbal (Group III) teat dip group

Month	Group I (n=6) Mean ± S.E.	Group II (n=12) Mean ± S.E.	Group III (n=12) Mean ± S.E.	Significance p-value
February	4.68±0.11	4.63±0.08	4.61±0.05	0.8532
March	4.84±0.11^b	4.77±0.08^{ab}	4.46±0.06^a	0.0151
April	4.87±0.10^b	4.78±0.10^{ab}	4.43±0.10^a	0.0145
May	4.87±0.15^b	4.66±0.08^{ab}	4.41±0.09^a	0.0159
June	5.14±0.09^b	4.72±0.11^a	4.60±0.08^a	0.0082
July	5.15±0.18	4.85±0.10	4.70±0.12	0.0963
Total	4.90±0.05^c	4.73±0.03^b	4.53±0.03^a	0.0000

Values in rows with at least one similar superscript do not differ significantly ($p<0.05$)

and non-specific mastitis was recorded in herbal, chemical and control group and intra mammary infection (IMI) incidence was compared between three groups (Table 4, Fig. 3). Overall, the incidence of mastitis (specific and non-specific) was highest in the control group, i.e., 27.1% (15.3%; 11.8%), followed by the chemical teat dip group 10.7% (4.2%; 6.6%) and least in herbal teat dip group 3.8% (2.4%; 1.4%). The efficacy of chemical post-milking teat dip in reducing new IMI was 60.52%, and efficacy of herbal post-milking teat dip was 85.97% (Table 5).

Mastitis pathogens isolated from control and treatment groups: The mastitis pathogens isolated from each group are illustrated in Table 6. *S. xylosus*, *S. chromogenes*, *S. haemolyticus*, *S. uberis*, *E. coli*, *C. amycolatum* and *S. agalactiae* were the most frequently isolated pathogens.

E. coli, *C. amycolatum* and *S. agalactiae* were not isolated from animals of the herbal teat dip group. From the herbal group, mostly coagulase negative staphylococcus (CNS) was isolated, and from other two groups, different environmental and contagious mastitis

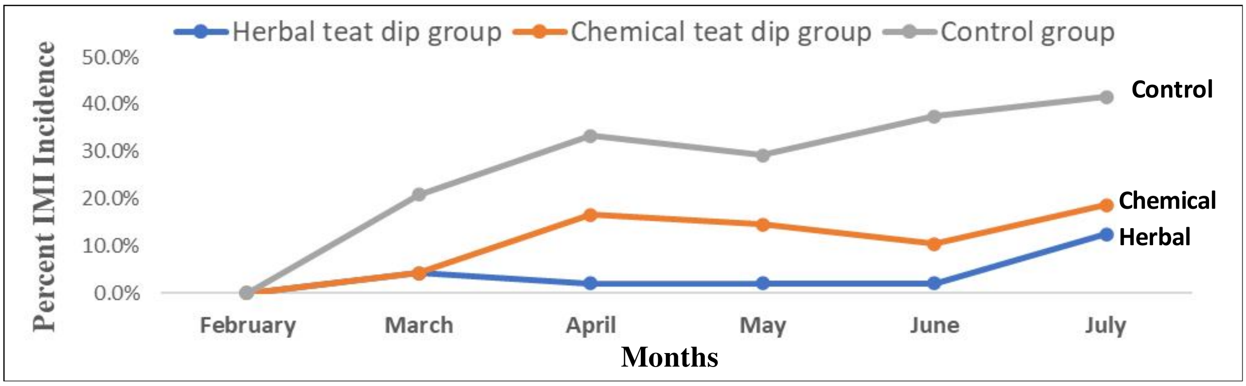

Fig. 3. Intra-mammary infection in group I (control), II (chemical) and III (herbal) during 6 months study period

Table 3. pH in control (Group I), chemical (Group II) and herbal (Group III) teat dip group

Month	Group I (n=6) Mean ± S.E.	Group II (n=12) Mean ± S.E.	Group III (n=12) Mean ± S.E.	Significancep P-value
Feb	6.54±0.03[a]	6.54±0.03[a]	6.51±0.02[a]	0.6683
March	6.54±0.04[a]	6.56±0.02[a]	6.53±0.02[a]	0.5609
April	6.55±0.02[a]	6.56±0.02[a]	6.52±0.02[a]	0.2571
May	6.57±0.03[a]	6.56±0.02[a]	6.54±0.02[a]	0.6587
June	6.60±0.04[a]	6.58±0.02[a]	6.55±0.03[a]	0.5732
July	6.64±0.04[a]	6.58±0.03[a]	6.57±0.03[a]	0.4169
Total	6.57±0.01[a]	6.56±0.01[a]	6.53±0.01[a]	0.0510

Values in rows with at least one similar superscript do not differ significantly ($p<0.05$)

Table 4. Intra-mammary infection in control (Group I), chemical (Group II) and herbal (Group III) teat dip group

	IMI	Feb n(%)	Mar n(%)	April n(%)	May n(%)	June n(%)	July n(%)	Total n(%)
Group I (Control group) (N=24)	Specific	0	3	5	3	5	6	22 (15.3%)
	Non-specific	0	2	3	4	4	4	17 (11.8%)
	Total	0 (0.0)	5 (20.8)	8 (33.3)	7 (29.2)	9 (37.5)	10 (41.6)	39 (27.1%)
Group II (Chemical teat dip) (N=48)	Specific	0	1	3	3	1	4	12 (4.2%)
	Non-specific	0	1	5	4	4	5	19 (6.6%)
	Total	0 (0.0)	2 (4.2)	8 (16.6)	7 (14.6)	5 (10.4)	9 (18.7)	31 (10.7%)
Group III (Herbal teat dip)(N=48)	Specific	0	1	0	1	1	4	7 (2.4%)
	Non-specific	0	1	1	0	0	2	4 (1.4%)
	Total	0 (0.0)	2 (4.2)	1 (2.1)	1 (2.1)	1 (2.1)	6 (12.5)	11 (3.8%)

N= Total no. of quarters at risk, n= no. of quarters affected

Table 5. **Efficacy of herbal and chemical post-milking teat dips**

	Percent new IMI in treatment groups (A)	Percent new IMI in control group (B)	Efficacy of treatments = (B-A/B) × 100
Group II (chemical) n=48	10.7	27.1	60.52%
Group III (herbal) n=48	3.8	27.1	85.97%

Table 6. Frequency of mastitis pathogens isolated from milk samples over a six months period

		Feb	March	April	May	June	July
Herbal teat dip group	Total samples	48	48	48	48	48	48
	S. xylosus	1	1	1	0	1	1
	S. chromogenes	2	2	2	4	4	3
	S. haemolyticus	1	2	2	1	0	0
	S. uberis	0	0	0	0	1	2
	Total culture positive	4 (8.33%)	5 (10.4%)	5 (10.4%)	5 (10.4%)	6 (12.5%)	6 (12.5%)
Chemical teat dip group	Total samples	48	48	48	48	48	48
	S. xylosus	1	1	1	0	0	0
	S. chromogenes	1	2	1	3	4	3
	S. haemolyticus	1	1	0	0	0	0
	S. uberis	0	0	2	2	2	2
	E. coli	0	0	1	0	0	1
	C. amycolatum	0	0	0	1	1	1
	Total culture positive	3 (6.25%)	4 (8.3%)	5 (10.4%)	6 (12.5%)	7 (14.5%)	7 (14.5%)
Control group	Total samples	24	24	24	24	24	24
	S. xylosus	0	1	1	0	0	0
	S. chromogenes	2	2	2	1	1	1
	S. haemolyticus	2	2	1	1	1	1
	S. uberis	0	0	1	1	2	1
	E. coli	0	0	1	0	0	2
	C. amycolatum	0	0	0	2	2	1
	S. agalactiae	0	0	0	1	1	2
	Total culture positive	4 (16.6%)	5 (20.8%)	6 (25.0%)	6 (25.0%)	7 (29.2%)	8 (33.3%)

pathogens were isolated. The total number of quarters with positive cultures were highest in the control group, followed by the chemical and herbal teat dip group. However, both herbal and chemical teat dip did not prove to be effective enough in reducing the no. of mastitis pathogens secreted in milk. The pathogens did not get eliminated from the milk samples and were frequently isolated throughout the study period from all groups.

DISCUSSION

An increase in SCC is the most important indicator of subclinical mastitis (Mukharjee and Das, 2002). Previous studies have shown that teat dipping effectively reduces somatic cell counts, pH and EC of milk, suggesting that teat dipping has a beneficial effect on the quality of milk (Singh, 2002). SCC variations are primarily determined by the recruitment of leukocytes from blood to tissue and then to milk and occur due to a response to an inflammatory reaction in the mammary tissue caused by bacterial invasion of the mammary gland (Rainard *et al*., 2018). In the present study, mean SCC in the chemical and herbal teat dip group remained <200 (×10^3 cells/mL) throughout the study period, whereas, SCC in the control group was comparatively higher throughout but increased two-fold times in the month of July and increased SCC in the control group is suggestive of subclinical mastitis. It could be because of heat stress and high relative humidity in July which makes the environment favourable for mastitis outbreak. Overall, SCC was highest in the control group, followed by the chemical teat dip group and least in the herbal group indicating higher no. of mastitis cases in the

A B

Fig. 2 A. Post-milking teat dipping with commercial chemical teat dip (Betadine); B: Post-milking teat dipping with herbal teat dip formulation

Fig. 4a. Before starting post-milking teat dipping with herbal teat dip solution

Fig. 4b. After 1 month of post-milking teat dipping with herbal teat dip solution

Fig. 5a. Teat ends in animals with commercial chemical post-milking teat dipping

Fig. 5b. Teat ends in animals with herbal post-milking teat dipping

control group as compared treatment group. Our findings are in agreement with other studies (Thangadurai *et al.*, 2017), which reported lower SCC in animals who received herbal combinations of Aloe vera, Turmeric and lime as a teat dip. Researchers have used herbal preparation comprising 95% Aloe vera gel mixed with 2.5% olive oil and 2.5% glycerine for mastitis management and found reduced SCC in animals subjected to the herbal preparation (Sharma *et al.*, 2014). Similar findings were reported by Waghmare *et al.* (2013), who evaluated the efficacy of herbal teat dip Mastidip liquid in subclinical mastitic cows and found that SCC was normalized after treatment with Mastidip liquid.

EC values showed significant variation mainly between the control group and herbal teat dip group in April, May and June corresponding with SCC variation as mentioned earlier. SCC and EC hold a positive correlation (Mukharjee and Das, 2002), and an increase in SCC is followed by an increase in EC value suggestive of subclinical mastitis. Changes in electrical conductivity may be caused by the ionic changes during inflammation caused by higher sodium and chloride concentrations in mastitis milk (Popovic, 2004). Our findings corroborate with other researchers (Maiti *et al.*, 2004) who observed that post-milking teat dipping with herbal teat dip solution for 30 consecutive days significantly reduced EC in the treatment group as compared to the control group where no teat dipping was done. Studies have used herbal preparation composed of Aloe vera, curcuma longa rhizome and calcium hydroxide on affected quarters and found that SCC and EC restored to normal and proved quite effective in mastitis management (Balakrishnan *et al.*, 2017). In our study, there was no significant difference in pH values of control and treatment groups. No significant difference in pH could be because milk pH is not a clinically helpful tool for diagnosing sub clinical mastitis in cattle (Kandeel *et al.*, 2019). Our results resonate with Ogola *et al.* (2007), who found that subclinical mastitis had no significant impact on the pH values of milk samples, and the mean pH values remain within the normal milk pH range. Our findings also resonate with Singh *et al.* (2019), who observed that herbal treatment in combination with standard treatment was most effective in reducing SCC, EC and pH of mastitic milk as compared to standard treatment alone.

Studies have proved that herbal preparations containing herbs like Aloe vera, Neem, Tulsi and Turmeric can be effective in mastitis management because of their antimicrobial, anti-inflammatory and immunomodulatory activity (Chattopadhyay *et al.*, 2004; Mukherjee *et al.*, 2005; Olaleye and Michael, 2005; De and Mukherjee., 2009). In the present study, the incidence of mastitis was lowest in animals that received herbal teat dipping, indicating that it is as effective as chemical teat dip in preventing intramammary infection. It is known that teat dipping effectively reduces intramammary infections by the bacteria transmitted to the teat end during milking (Pankey, 1986). Our findings corroborate with authors (Biradar *et al.*, 2022), who evaluated the efficacy of three herbal formulations and found all three of them to be effective in reducing subclinical mastitis as compared to untreated groups. Similarly, studies have found that teat dipping with herbal essential oils proved to be effective in lowering the incidence of subclinical mastitis in dairy cows (Rasool *et al.*, 2021). Another researcher (Patil *et al.*, 2014) conducted 30 days of herbal teat dip trial with herbal Mastidip liquid and observed that during 30 days of trial, no new intramammary infection was found; therefore, the study concluded that post-milking teat dipping with herbal Mastidip liquid was effective in reducing the incidence of intramammary infection in dairy cows. However, in our study, results were recorded for a longer duration and the incidence of IMI did not increase until July when environmental conditions favour mastitis outbreak. At the beginning of the experiment, the teat ends were dry and cracked with teat end score 3 to 4 in all animals (Fig. 4a & 5a). After 1 month of application of herbal teat dip, the teat end score reduced to 1 to 2 in herbal/group III animals (Fig. 4b & 5b), but remained same in chemical teat dip/group II animals. The teat ends of the herbal group showed marked healing owing to the anti-inflammatory, antiseptic, immunomodulatory, moisturising and skin-conditioning effects of Aloe vera, Tulsi and Turmeric (Avancini *et al.*, 2008). This could be the reason for the better efficacy of herbal teat dip than chemical teat dip as animals having teat end lesions become more predisposed to mastitis.

As mentioned earlier, latent infection was not considered while dividing animals into groups; hence, animals with latent infection were present in all 3 groups at the beginning of the experiment. The pathogens did not get eliminated from the milk samples and were frequently isolated throughout the study period from all groups. In the dairy farms where the present study was conducted, the culling of chronic cases of mastitis was not practised. These animals act as reservoirs for pathogenic bacteria and further maintain infection at dairy farm. From the above findings, it is clear that the reduction in intramammary infections in a dairy herd requires more than just the use of teat dips, and other aspects of the mastitis control programme should be implemented strictly.

Animals receiving herbal teat dip formulation had low SCC, EC and pH than animals receiving chemical teat dip or no teat dip, suggesting that herbal teat dip performed better in preventing subclinical mastitis. Also, herbal teat dip preparation was found to have better efficacy than conventional teat dips in reducing the incidence of intramammary infections. Further, herbal teat dip had no corrosive effect on teat skin, had healing properties and no side effects which were present with chemical teat dipping (teat end score >2, irritation and dry cracked teat end). However, one drawback was found that herbal teat dip could not eliminate mastitis pathogens, and latent infections persisted in the animal.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: SK: Involved in herbal teat dip preparation, sample collection and processing; DKG: Engaged in conceptualization, data curation, supervision and final editing; RSS: Involved in statistical analyses, methodology and editing; DN: Involved in microbiological work of isolation and identification of bacteria.

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