The Pharma Innovation Journal 2022; SP-11(4): 1638-1642

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(4): 1638-1642

www.thepharmajournal.com
Received: 10-02-2022
Accepted: 12-03-2022

Ravishankar Biradar
PG Scholar, Department of LPM, Veterinary College Bengaluru, KVAFSU, Karnataka, India

Vivek M Patil
Associate Professor & Head, Department of LPM, Veterinary College Bengaluru, KVAFSU, Karnataka, India

Mahadevappa D Gouri
Assistant Professor, Department of LPM, Veterinary College Bengaluru, KVAFSU, Karnataka, India

Shivaraj BM
Assistant Professor (Senior Scale), SRDDL, Institute of Animal Health & Veterinary Biologicals, Bengaluru, KVAFSU, Karnataka, India

SB Prasanna
Associate Professor & Head, Department of LFC, Veterinary College Gadag, KVAFSU, Karnataka, India

Kalmath GP
Associate Professor, Department of VPB, Veterinary College Bengaluru, KVAFSU, Karnataka, India

Corresponding Author
Vivek M Patil
Associate Professor & Head, Department of LPM, Veterinary College Bengaluru, KVAFSU, Karnataka, India

Efficacy of herbal teat disinfectants on microbiological quality of milk in cattle with subclinical mastitis

Ravishankar Biradar, Vivek M Patil, Mahadevappa D Gouri, Shivaraj BM, SB Prasanna and Kalmath GP

Abstract
Sub-clinical mastitis is one of the major reasons for low yield and poor-quality milk and ranks first among the diseases that cause substantial loss to owners. The existing practice of treatment of udder infections, whether sub-clinical level or clinical mastitis involves the use of higher antibiotics causing anti-microbial resistance and residues in milk leading to public health concerns. Thus, the current study was conducted to study the efficacy of alternative, low-cost, post-milking herbal teat disinfectants in different combinations against sub-clinical mastitis. A total of 16 lactating dairy HF crossbred cows in second and subsequent lactations were divided into four groups each having four cows; T1 (Control fresh clean water), T2 (*Aloe vera* extract 5 grams, ozonated oil 2.5 grams, wood vinegar 7.5 grams, turmeric extract 0.5 grams, excipient q.s), T3 (*Aloe vera* extract 5 grams, ozonated oil 2.5 grams, wood vinegar 5.0 grams. turmeric extract 0.5 grams, excipient q.s.), and T4 (*Aloe vera* extract 5 grams, ozonated oil 2.5 grams, wood vinegar 2.5 grams. turmeric extract 0.5 grams, excipient q.s.). The study for period of 60 days showed that treatment groups had significantly better SCC, pH and bacterial load as compared to untreated animals. All three herbal combinations were effective in combating SCM, with T4 showing slightly better bacterial load values.

Keywords: Sub-clinical mastitis, herbal agents, SCC, pH, bacterial load

Introduction
Dairy farming in India is one of the important community businesses that makes a vital contribution to people's welfare. It is also an important source of subsidiary income to small/marginal farmers and agricultural labourers. Milk production in India has been growing at a Compounded Annual Growth Rate (CAGR) of 4.5% over the last 20 years compared to 2% worldwide.

Sub-clinical mastitis is defined as inflammation of parenchyma of mammary glands and is characterized by physical, chemical and usually bacteriological changes in milk and pathological changes in glandular tissues. It is a global problem as it adversely effects animal health, quality of milk and economics of milk production and every country, including developed ones, suffer huge financial losses related to culling, decreased production, decreased fecundity, and treatment costs. In comparison with clinical mastitis, sub-clinical mastitis is considered as a major and also a silent problem that leads to huge economic losses [7]. Apart from causing huge losses in case of milk production, the sub-clinically affected animals remain a continuous infection source to the other animals [13]. It is one of the major reasons for low yield and poor-quality milk and ranks first among the diseases that cause substantial loss to owners.

Conventional antibiotic treatment is a proven method for prevention and control of mastitis. However, it is of public health importance on account of the indiscriminate usage of antibiotics, non-compliance with milk withdrawal period post-treatment leading to antibiotic residues in milk and related health issues, and incomplete duration of treatment and associated antimicrobial resistance. Herbal teat disinfectants can greatly reduce the usage of antibiotics by the farmers, thus reducing occurrence of antimicrobial resistance in pathogenic bacteria and antibiotic residues in milk.

With the increasing awareness among public regarding the quality of milk and milk products, it is inevitable to improve our efforts to control sub-clinical mastitis through effective, sustainable and alternate control measures. The present study was conducted to test the efficacy of commonly used herbal agents that could be applied as a post-milking teat disinfectant for the control of sub-clinical mastitis.

Materials and Methods

The present study was conducted Nandagokula dairy farm at village Antarahalli, taluka Doddaballapura, district Bengaluru Rural, Karnataka, India. A total of sixteen Holstein-Friesian crossbred dairy cows in second and subsequent lactations were selected for the study. Each animal was identified with numbered plastic ear tags. The selected cows were in the mid-lactation and had a milk production of 7-10 lit/day. The presence of sub-clinical mastitis was ascertained by preliminary screening with California Mastitis Test and confirmation using somatic cell counter; only cows with somatic cell count in the range 2.0-3.5 lakhs/ml were considered for the study.

The cows were maintained under conventional housing system and standard management conditions. Each cow was given adequate silage and dry fodder twice a day. Clean, safe drinking water was provided *ad libitum*. Cows were milked twice a day in a milking parlour using machine milking. During milking, the cows were offered required quantity of concentrates. Cows were washed once daily. The shed floor was made of cement concrete with adequate slope and drainage without rubber mat. Cleaning of the shed including manger was done every day. Dung and fodder residues were cleaned regularly. Milking machine and utensils used for milk collection were washed after every milking session and kept for drying in a clean area. The milking parlour was also washed and dried. Shed floor was washed twice daily with pressurized water sprays.

The cows were divided into four groups of four each based on parity, body weight, days in milk and daily milk yield, and subjected to different post-milking herbal teat disinfectants as per the details given in Table 1. The herbal teat disinfectants were taken in separate teat dip applicators and labelled accordingly. At the completion of the milking process, the teat dip applicator was raised onto each teat and squeezed gently to ensure that a coating of the herbal teat disinfectant was applied onto the teat (Plate 1). In T1 group (Control), teats were cleaned and dried with single service paper towel and no post-milking teat disinfectant was used. The study was conducted for a period of 60 days.

Somatic Cell Count (SCC): Milk samples collected from all the cows (Plate 2) were subjected to the estimation of SCC on day 0, 15, 30, 45 and 60 using a DeLaval cell counter (DCC). After milking of each cow, the milk samples were collected and stored at 3–8ºC for no more than 12 hours until assay. No preservatives were added as per the instruction book of the DCC [2]. Required sample was loaded in the DCC cassette provided with the device. The cassette was then inserted in the provided slot and readings (SCC per microlitre) were taken as per the manufacturer's instructions (Plate 3).

Milk pH: Milk pH was determined using a digital pH meter (Konvio pH meter) as per the manufacturer's instruction manual. The device was calibrated using standard buffer solutions of pH 4.0, 6.86 and 9.18. To record the milk pH, the protective cap was removed. The electrodes of the pH meter were rinsed with distilled water and blotted dry with the help of filter paper. The device was switched on and the electrode was immersed in the milk sample to be tested (not over the immersion line) and gently stirred. Within 30 seconds, the reading was displayed and recorded.

Bacterial load: autoclaving at 121 ºC for 15 minutes at 15 psi. The raw milk samples collected from experimental animals were subjected to microbial analysis for determination of Standard Plate Count by serial dilution and pour plate technique on SPC Agar Medium. After fore-stripping, the teats were washed with clean water followed by drying with single service paper towels. Samples of milk were collected aseptically from all quarters on day 0, 15, 30, 45 and 60 in Tarsons sample bottles from each cow separately. These bottles were properly labelled and kept in an ice box maintained at 4-5 ºC. The raw milk samples were analysed within 4 hours of their collection. Plate count agar medium of M/s Himedia was used in the present study. The plate count agar was prepared as per the manufacturer's instructions by autoclaving at 121 ºC for 15 minutes at 15 psi. The raw milk samples collected from experimental animals were subjected to microbial analysis for determination of Standard Plate Count by serial dilution and pour plate technique on SPC Agar Medium. The petri plates were incubated at 37 ºC for 24 hrs after which the numbers of colonies on the plates were counted and results expressed as CFU/µl (Plate 4).

CFU/µl = (no. of colonies x dilution factor) / volume of culture plate

Each animal was considered as one experimental unit for the purpose of statistical analysis. The data pertaining to various parameters was analyzed using standard statistical packages in Microsoft Excel 2019 software. All the means were compared at 5 per cent level of significance.

Results and Discussion

Somatic Cell Count (SCC): Increase in somatic cell counts could be considered as early sign of emergence of inflammatory changes in mammary glands [5] and is directly related the seasonal occurrence and presence of bovine sub-clinical mastitis agents [1]. The overall somatic cell count (thousands/ml) of T1, T2, T3 and T4 was 315.35±6.686, 233.05±6.070, 243.25±6.049 and 249.15±5.891, respectively (Table 2). From day 15 onwards, there was significant (P<0.05) decrease in somatic cell counts of herbal disinfectant groups as compared to untreated group. The mean SCC (thousands/ml) of T1 group was significantly (P<0.05) higher at day 45 and 60 as compared to day 0. The SCC of T2 group was significantly (P<0.05) lower on day 30-60 as compared to day zero. The SCC of T3 and T4 groups at day 30-60 was significantly (P<0.05) lower than day 0-15.

The results are in agreement with [14] wherein significant reduction in somatic cell counts were found in the groups treated with herbal combinations of *Aloe vera*, turmeric and lime as teat dip was done. Similarly, this was also in agreement with [11] where *Aloe vera* gel was prepared by squeezing it from the leaf and 95% *Aloe vera* gel was mixed with 2.5% olive oil and 2.5% glycerine. Significant reduction in somatic cell counts in the herbal combinations could be attributed to its antibacterial and anti-inflammatory properties [3, 8, 10, 12]. The study was also in agreement with [16] in which turmeric powder and *Aloe vera* were components of the herbal paste applied and led to reduced somatic cell counts. Similar findings were reported by [9] using herbal paste, where there was significant reduction in somatic cell counts; this might be due to presence of other herbal components like *Murraya koenigii* in teat dip liquid.

Milk pH: Milk pH serves as the best indicator to assess the

condition of udder health in dairy animals. Normal pH range of cow milk is 6.5-6.7. The milk from the mastitis affected animals is alkaline in nature. Increased pH could be due to reduction of lactose content which, in turn, reduces lactic acid in those treatment groups and vice-versa [15]. The overall milk pH of T1, T2, T3 and T4 was 7.18±0.035, 6.95±0.036, 7.09±0.053 and 6.97±0.042, respectively (Table 3). Form day 45 onwards, there was significantly (P<0.05) lower pH in herbal treatment group as compared to untreated group. There was a gradual decrease but no significant difference in milk pH during different stages of trial in herbal treatment groups; however, there was a significant (P<0.05) increase in the milk pH of untreated group as the trial progressed.

The results with respect to the group with herbal disinfectant treatment are in accordance with findings of [16] where they used Mastidip herbal liquid for teat dip and found reduction in milk pH. Similar results were reported by [9, 14] wherein *Aloe vera*, turmeric were the components in the treatments.

Bacterial load: The overall bacterial load of T1, T2, T3 and T4 was 331.08±8.510, 327.67±9.416, 299.00±6.178 and 276.58±10.393 CFU/ml, respectively (Table 4). Herbal treatment groups had significantly (P<0.05) lower bacterial count than untreated group by day 30. T1-3 groups did not show any significant differences in bacterial load (CFU/ml) during the course of the trial. There was a significant (P<0.05) reduction in bacterial load in T4 on day 60 as compared to day 0.

The results of present study with respect to counts of bacterial load are in agreement with findings of [6]. Similar findings were reported by [4, 9] where combinations of herbal dips along with antibiotics and herbal agents were used, respectively. This indicates the antibacterial property of the herbal agents. The results were also in accordance with [8] where treatment using ozonized sunflower oil was studied. Similar results were found on effect of ozonated oils used as antimicrobials and anti-inflammatory agents [3]. Results of wood vinegar as effective antimicrobial agent are also in accordance with [12] where wood vinegar was tested for its antibacterial effects in goats. Significantly lower bacterial load and associated SCC in the milk of herbal treated groups may be due to the antibacterial, antifungal, antiseptic and anti-inflammatory properties of wood vinegar, turmeric, *Aloe vera* which are the constituent ingredients of all the herbal combinations of study.

Table 1: Details of experimental groups and composition of the herbal teat disinfectants

Group	No. of animals	Description of the treatment
T1 (Control)	4	Teats were cleaned and dried with single service paper towel.
T2	4	Application of herbal teat disinfectant comprising aloevera extract 5 grams, ozonated oil 5 grams, wood vinegar 7.5 grams, turmeric extract 0.5 grams, excipient q.s. to make 100 ml
T3	4	Application of herbal teat disinfectant comprising aloevera extract 5 grams, ozonated oil 2.5 grams, wood vinegar 5.0 grams. turmeric extract 0.5 grams, excipient q.s. to make 100 ml
T4	4	Application of herbal teat disinfectant comprising aloevera extract 5 grams, ozonated oil 2.5 grams, wood vinegar 2.5 grams. turmeric extract 0.5 grams, excipient q.s. to make 100 ml

Table 2: Effect of herbal teat disinfectants on somatic cell counts (thousands/ml) recorded at different intervals in dairy cattle

Group		Day 0	Day 15	Day 30	Day 45	Day 60	Overall	P value
T1	Mean	281.00^{Y}	305.25^{aXY}	312.25^{aWY}	342.75^{aW}	335.50^{aWX}	315.35	0.001
	SE	4.378	11.947	10.873	15.708	8.510	6.686	
T2	Mean	265.25^{W}	247.00^{bWX}	233.50^{bXY}	218.75^{bYZ}	200.75^{bYZ}	233.05	0.001
	SE	10.234	11.218	8.411	3.750	5.006	6.070	
T3	Mean	278.25^{W}	262.50^{bW}	237.50^{bX}	223.00^{bXY}	215.00^{bY}	243.25	0.000
	SE	2.287	11.751	5.923	5.212	2.739	6.049	
T4	Mean	280.75^{W}	272.75^{bW}	241.75^{bX}	229.25^{bXY}	221.25^{bY}	249.15	0.000
	SE	6.183	5.072	7.620	5.543	4.250	5.891	
Overall	Mean	276.31	271.88	256.25	253.44	243.13		
	SE	3.341	7.209	9.185	13.933	14.123		
P value		0.309	0.010	0.000	0.000	0.000		

Note: Means within a column (^{abc}) or row (^{WXYZ}) having different superscripts differ significantly (P<0.05)

Table 3: Effect of herbal teat disinfectants on milk pH recorded at different intervals in dairy cattle

Group		Day 0	Day 15	Day 30	Day 45	Day 60	Overall	P-value
T1	Mean	7.01^{Z}	7.08^{YZ}	7.19^{XY}	7.27^{aX}	7.35^{aX}	7.18	0.004
	SE	0.040	0.061	0.056	0.043	0.068	0.035	
T2	Mean	7.10	6.99	6.92	6.91^{b}	6.82^{b}	6.95	0.131
	SE	0.065	0.063	0.092	0.069	0.072	0.036	
T3	Mean	7.28	7.18	7.09	7.03^{ab}	6.88^{b}	7.09	0.155
	SE	0.089	0.123	0.122	0.109	0.092	0.053	
T4	Mean	7.14	7.00	6.97	6.86^{b}	6.91^{b}	6.97	0.296
	SE	0.108	0.092	0.101	0.077	0.071	0.042	
Overall	Mean	7.13	7.06	7.04	7.02	6.99		
	SE	0.043	0.044	0.051	0.054	0.064		
P-value		0.186	0.412	0.238	0.014	0.001		

Note: Means within a column (^{abc}) or row (^{WXYZ}) having different superscripts differ significantly (P<0.05)

Table 4: Effect of herbal teat disinfectants on bacterial load (CFU/ml) recorded at different intervals in dairy cattle

Group		Day 0	Day 30	Day 60	Overall	P-value
T1	Mean	329.50	325.25^{a}	338.50^{a}	331.08	0.839
	SE	15.548	14.773	17.495	8.510	
T2	Mean	351.50	319.50^{a}	312.00^{a}	327.67	0.200
	SE	19.324	15.080	9.000	9.416	
T3	Mean	318.00	291.25^{ab}	287.75^{ab}	299.00	0.077
	SE	12.396	6.575	6.356	6.178	
T4	Mean	310.25^{X}	265.75^{bXY}	253.75^{bY}	276.58	0.045
	SE	12.072	16.605	13.269	10.393	
Overall		327.31	300.44	298.00		
		7.865	8.718	9.755		
P-value		0.288	0.036	0.003		

Note: Means within a column (^{abc}) or row (^{WXYZ}) having different superscripts differ significantly (P<0.05)

Plate 1: Herbal teat disinfectant application

Plate 2: Collection of milk sample

Plate 3: SCC evaluation using DeLaval Cell Counter

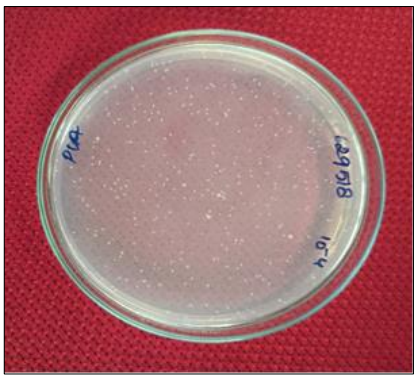

Plate 4: Assessment of bacterial load using Standard Plate Count technique

Conclusion

Sub-clinical mastitis can be defined as an infection that is accompanied by increase in leukocyte count of milk. The existing practice of treatment of udder infections, whether sub-clinical level or clinical mastitis involves the use of higher antibiotics causing anti-microbial resistance and residues in milk leading to public health concerns. Herbal teat disinfectants comprising of various levels of *Aloe vera* extract, ozonated oil, wood vinegar and turmeric extract were found to be effective in controlling SCM in crossbred cattle. The treatment groups had significantly better SCC, pH and bacterial load as compared to untreated animals. All three herbal combinations were effective in combating SCM, with T4 showing slightly better bacterial load values. Apart from slight reduction in SCC, there was no significant improvement in other parameters beyond 30 days of treatment.

References

1. Antanaitis R, Juozaitienė V, Jonike V, Baumgartner W, Paulauskas A. Milk lactose as a biomarker of sub-clinical mastitis in dairy Cows. Animals. 2021;11(6):1736.
2. DeLaval, 2005. http://www.delavalus.com/ Products/MilkingEquipment/Milk_Quality/DCC/default. htm Accessed on Jan 21, 2022. 2:12 pm
3. Enginler SÖ, Sabuncu A, Kahraman BB, Koçak Ö, Yildar E, Güzel Ö. Comparison of intramammary ozone administration doses in dairy cows with clinical mastitis. Acta Sci. Vet. 2015;43:1-7.
4. Hadiya K, Yadav V, Borthakur A, Ravikanath K, Maini S. Efficacy evaluation of Mastilep gel in sub-clinical mastitis in cattle. Int. J Agri. Innovations Res. 2017;5(5):2319-1473.
5. Kaswan S, Mukherjee J, Prasad S, Dang AK. Phagocytic activity of blood neutrophils and its relationship with plasma concentration of TNF-α, IL-6 and milk SCC in crossbred cows during early lactation. Indian J Anim. Sci. 2012;82(7):737-740.
6. Kučević D, Plavšić M, Trivunović S, Radinović M, Kučević DS. Effect of post-milking teat dipping on hygienic quality of cow's milk. Biotechnol. Anim. Husb. 2013;29(4):665-673.
7. Kumari T, Bhakat C, Choudhary RK. A review on sub-clinical mastitis in dairy cattle. Int. J Pure Appl. Biosci. 2018;6(2):1291-1299.
8. Quintana MCF, Isadora MD, Andrea RR. Use of ozonized oil in the treatment of sub-clinical mastitis in jersey cows: Case Report. Pubvet. 2019;13:1-4.
9. Raut NP, Shafi TA, Sakhare MP, Siddiqui MF, Gaikward SS, Ranvir GD. Therapeutic evaluation of *Murraya koenigii* in bovine subclinical mastitis. J Pharmacol. Phytochem. 2019;10(4):40-45.
10. Sahoo AK, Das D, Panda SK, Jena AK. Economic impact of sub-clinical and clinical mastitis in Odisha, India. Int. J Curr. Microbiol. Appl. Sci. 2018;7(3):3651-3654.
11. Sharma N, Mukherjee R, Mishra A. Comparative evaluation of *Aloe vera* and glutaraldeyde teat antisepsis on somatic cell count in organized farm. Indian Vet. J. 2014;91(4):40-41.
12. Soares WNC, Lira GPO, Santos CS, Dias GN, Pimenta AS, Pereira AF, *et al*. Pyroligneous acid from *Mimosa tenuiflora* and *Eucalyptus urograndis* as an antimicrobial in dairy goats. J Appl. Microbiol. 2021;131(2):604-614.
13. Swami SV, Patil RA, Gadekar SD. Studies on prevalence of sub-clinical mastitis in dairy animals. J Entomol. Zool. Stud. 2017;5(4):1297-1300.
14. Thangadurai R, Venilla MA, Shanmugam PS. Management of mastitis in dairy cattle using herbal combination. J Krishi Vigyan. 2017;5(2):164-167.
15. Tomar A, Shukla PC, Singh B, Sheikh AA. Comparative

therapeutic efficacy of various teat dip solutions in caprine mastitis. Int. J Chem. Stud. 2018;6(4):123-12.
16. Waghmare SP, Kolte AY, Ravikanth K, Thakur A. Applications of herbal teat dip mastidip liquid in sub-clinically mastitic animals and its role in further prevention of mastitis. Int. J Agri. Sci. Vet. Med. 2013;1:43-49.