The Pharma Innovation Journal 2023; 12(3): 4651-4654

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(3): 4651-4654

www.thepharmajournal.com
Received: 01-12-2022
Accepted: 05-01-2023

Meera Sakhare
Department of Veterinary Medicine, College of Veterinary and Animal Sciences, Parbhani, MAFSU, Maharashtra, India

Md. Ferozoddin Siddiqui
Department of Veterinary Medicine, College of Veterinary and Animal Sciences, Parbhani, MAFSU, Maharashtra, India

Tawheed Ahmed Shafi
Department of Veterinary Medicine, College of Veterinary and Animal Sciences, Parbhani, MAFSU, Maharashtra, India

Bharti Negi
Research and Development Unit, Ayurvet Limited, Baddi, Himachal Pradesh, India

Bhaskar Ganguly
Research and Development Unit, Ayurvet Limited, Baddi, Himachal Pradesh, India

Corresponding Author:
Bhaskar Ganguly
Research and Development Unit, Ayurvet Limited, Baddi, Himachal Pradesh, India

Efficacy evaluation of some polyherbal formulations in the management of subclinical mastitis in buffaloes

Meera Sakhare, Md. Ferozoddin Siddiqui, Tawheed Ahmed Shafi, Bharti Negi and Bhaskar Ganguly

Abstract
Subclinical mastitis is an important problem for the dairy industry, responsible for reduced milk production and economic losses. The present study was conducted to evaluate the efficacy of some polyherbal formulations in the management of subclinical mastitis in buffaloes. 30 buffaloes with subclinical mastitis were assigned to one of three groups. In group G1, Mastilep® spray was applied on teats and udder twice a day after milking for five days; in group G2, Mastilep® gel was applied on teats and udder twice a day after milking for five days, and in group G3, Brand A spray was applied on teats and udder twice a day after milking for five days. Mastilep® (M/s Ayurvet Limited, India) is a scientifically prepared polyherbal formulation for control of mastitis available in gel and spray forms. Its key ingredients include oils of *Cedrus deodara*, *Curcuma longa*, *Eucalyptus globulus*, *etc*. The efficacy of various treatments was compared based on improvements in milk fat, milk yield, and somatic cell count (SCC). Based on the findings of the study, Mastilep® spray was found to be more efficacious in management of subclinical mastitis in buffaloes followed by Mastilep® gel.

Keywords: Buffaloes, herbal, mastilep, subclinical mastitis**,** somatic cell count

Introduction
Mastitis is considered to be most prevalent and costly disease of the dairy animals as it causes huge financial losses to the dairy industry in terms of compromised health of animals, and poor quality and quantity of milk [1]. Mastitis is mainly characterized by pathological changes such as inflammation of the udder, and physico-chemical and microbiological changes in the milk [2]. However, macroscopic changes in udder and milk are absent in cases of subclinical mastitis and, thus, have to be usually screened by laboratory tests. Mastitis is predominantly caused by bacterial pathogens and may also involve fungi and yeasts [3]. Antimicrobial intramammary infusions are the mainstay of treatment against mastitis but due to increase in prevalence of multi-drug resistance bacteria and antibiotic residues in milk, there is a need for herbal alternatives for the control of mastitis. The present study was designed to evaluate the efficacy of some polyherbal formulations in the management of subclinical mastitis in buffaloes.

Materials and Methods
The clinical trial was undertaken at the Instructional Livestock Farm Complex, COVAS, Parbhani (Latitude: 19.25˚N and longitude: 76.78˚E) and in and around Parbhani from November, 2020 to April, 2021. 30 buffaloes diagnosed positive for subclinical mastitis by modified California Mastitis Test (mCMT), Mastrip® test, cultural examination, and somatic cell count (SCC) were assigned to one of three equal groups (G1-G3) as shown in Table 1.

Table 1: Experimental design

Group (n=10)	Treatment
G1	Mastilep® spray twice daily for 5 days (applied on teats and udder after each milking)
G2	Mastilep® gel twice daily for 5 days (applied on teats and udder after each milking)
G3	Brand A spray twice daily for 5 days (applied on teats and udder after each milking)

Milk samples were collected under aseptic conditions from all the groups and subjected to SCC, milk yield, and milk fat on days 0 (pre-treatment) and 5, 14, 21, and 28 (post-treatment). The efficacy of various treatments was compared based on improvements in milk fat, milk

yield, and SCC. Data were subjected to statistical analysis by using SPSS *v*.20.0.0 and $p \leq 0.05$ was considered statistically significant [4].

Results and Discussion

The present study aimed to evaluate the efficacy of some polyherbal formulations in the management of subclinical mastitis in buffaloes. Results of SCC, milk yield, and milk fat on days 0 (pre-treatment) and 5, 14, 21, and 28 after treatment with Mastilep® spray, Mastilep® gel, and Brand A spray have been summarized in Table 2; SCC and 4% fat-corrected milk yields have also been compared in Figures 1a and b.

Table 2: SCC, milk yield, and milk fat before and after treatment with Mastilep spray, Mastilep gel, and Brand a spray.

Parameter		G1	G2	G3
Somatic cell count (SCC; $\times 10^3$/mL)	day 0	$538.108^{xa} \pm 23.80$	$608.108^{xa} \pm 44.34$	$444.408^{ya} \pm 35.46$
	day 5	$485.659^{xb} \pm 22.98$	$511.159^{ya} \pm 39.50$	$412.258^{yb} \pm 35.41$
	day 14	$401.557^{zb} \pm 26.82$	$412.457^{zb} \pm 30.57$	$322.457^{yb} \pm 31.64$
	day 21	$306.124^{zc} \pm 14.39$	$290.224^{zb} \pm 27.77$	$230.224^{xb} \pm 32.67$
	day 28	$167.752^{xd} \pm 9.58$	$174.372^{xc} \pm 19.16$	$185.67^{xb} \pm 20.92$
Milk yield (L)	day 0	$3.795^{xa} \pm 0.21$	$4.495^{xa} \pm 0.22$	$4.395^{xa} \pm 0.13$
	day 5	$4.055^{yb} \pm 0.22$	$4.555^{xa} \pm 0.22$	$4.430^{xa} \pm 0.13$
	day 14	$4.280^{zc} \pm 0.204$	$4.828^{yb} \pm 0.21$	$4.590^{xa} \pm 0.123$
	day 21	$4.479^{zd} \pm 0.213$	$5.205^{xb} \pm 0.212$	$4.942^{yb} \pm 0.137$
	day 28	$4.860^{ye} \pm 0.184$	$5.524^{xb} \pm 0.213$	$5.249^{yc} \pm 0.135$
Milk Fat (%)	day 0	$5.328^{xa} \pm 0.247$	$5.028^{xa} \pm 0.352$	$5.528^{xa} \pm 0.167$
	day 5	$5.417^{xa} \pm 0.159$	$5.117^{xa} \pm 0.358$	$5.617^{xa} \pm 0.250$
	day 14	$5.721^{xb} \pm 0.264$	$5.347^{ya} \pm 0.353$	$5.820^{xb} \pm 0.148$
	day 21	$6.026^{zc} \pm 0.272$	$5.627^{yb} \pm 0.356$	$6.125^{yc} \pm 0.146$
	day 28	$6.339^{zd} \pm 0.270$	$5.797^{yb} \pm 0.355$	$6.347^{yd} \pm 0.153$

(Means bearing superscript a, b, c, d, e differ significantly within the group and means bearing superscript x, y, z differ significantly between the groups; $p \leq 0.05$)

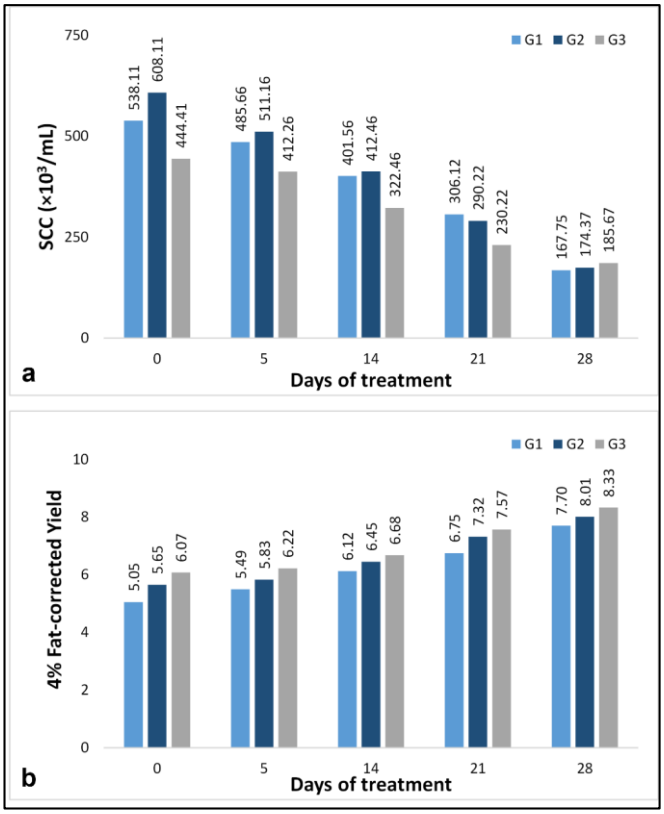

Fig 1: SCC and 4% fat-corrected milk yield before and after treatment with Mastilep spray, Mastilep gel, and Brand A spray.

Animal with SCC below 2 lac cells/mL are considered to be healthy [5]. Increase in SCC indicates inflammatory reaction of udder tissue and this increase might be due to shift of leucocyte to the udder as a defense mechanism [6]. In group G1 and G3, SCC reduced significantly on days 5, 14, 21, and 28 of treatment whereas non-significant decrease in mean SCC was observed in group G2 on day 5 and significant decrease was found on days 14, 21 and 28 of treatment as compared to day 0 before initiation of treatment. On day 28 of treatment, SCC returned to normal in all the groups; overall reduction in SCC being 68.82%, 71.32%, and 58.22%, respectively. Higher decrease in SCC in Mastilep® gel and Mastilep® spray treated animals might be attributed to antibacterial and anti-inflammatory properties of their constituent herbs [7, 8]. Similar findings on the ability of topical herbal preparations to reduce SCC has also been reported [9, 10, 11]. Previous studies on Mastilep have also reported its usefulness in reducing SCC to normal in subclinical mastitis in cows [12, 13].

Mastitis is one of the costliest diseases in terms of production losses especially milk yield, which accounts for approximately 49% of the total loss per lactation [14]. In group G1, milk yield increased significantly (6.85%) on day 5 of treatment whereas in other two groups non-significant increase was found on day 5 of treatment as compared to day 0. On days 14, 21, and 28 of treatment, milk yield increased significantly in groups G1 and G2 whereas in group G3, significant increase in milk yield was seen on days 21 and 28 of treatment as compared to day 0. Overall, 28.06%, 22.89%, and 19.43% increase in milk yield was observed on day 28 in groups G1, G2, and G3, respectively. Similarly, Balakrishnan *et al*. reported improvement in milk production in animals treated for mastitis with certain herbal preparation because of combined action of herbs owing to antimicrobial, anti-inflammatory, and immunomodulatory activities [15]. Similar to our findings, Hadiya *et al*. also reported significant increase in milk yield on application of Mastilep for treatment of subclinical mastitis in cattle [16]. This observed response of Mastilep may be due to antibacterial and anti-inflammatory property of the key constituents present in Mastilep such as *Cedrus deodara*, *Curcuma longa* and *Eucalyptus globulus*. Further, better permeation of bioactive compounds due to the presence of solvents may, possibly, be a factor contributing to the slightly better results obtained with the spray form over

the gel form.

Subclinical mastitis in buffaloes results in decreased fat content in milk due to impaired synthetic and secretary activity of epithelial cells [17]. In groups G1 and G3, milk fat increased significantly on days 14, 21, and 28 of treatment whereas in group G2, significant increase in milk fat was found on days 21 and 28 of treatment as compared to day 0. Highest increase in milk fat content, *i.e.* 18.97%, was recorded in group G1 followed by group G2 (15.29%) and groups G3 (14.81%) on day 28 of treatment. This increase might be due to synergistic action of the bioactive compounds of the constituent herbs that reduces bacterial load and inflammation of the udder [18, 19]. In contrast to our study, Nurdin *et al*. reported no significant improvement in fat content of milk of mastitic dairy cow [20]. However, in line with the findings of our study, Maramulla *et al*. reported increase in fat content of milk of cows suffering from subclinical mastitis after treatment with herbal preparations [21]. In agreement with our findings, Bansal and co-workers reported improvement in milk fat % upon application of Mastilep spray for treatment of mastitis in cows [22].

Mastilep is a non-antibiotic approach for control of mastitis that improves udder immunity *via* strengthening and maintaining integrity of teat canal. Bioactive compounds present in the constituents of Mastilep eliminate udder microbial loads. Both Mastilep and Brand A contain oils of *Eucalyptus globulus*, *Ocimum sanctum*, etc. Besides these ingredients, Mastilep also contains oils of *Cedrus deodara*, *Curcuma longa*, and *Azadirachta indica* that are reputed for their antimicrobial and anti-inflammatory properties [23-26]. In previous studies, Mastilep has shown efficacy not only in cows but also in other species such as goats. Digraskar *et al*. reported remarkable efficacy *i.e.* 83.33% of Mastilep gel along with antioxidant mineral supplement for treatment of caprine mastitis [27].

Conclusion

In conclusion, subclinical mastitis in buffaloes resulted in decreased milk yield, milk fat, and increased SCC, which were improved by topical application of spray and gel. Among treatments, Mastilep® spray was found to be most efficacious at reducing SCC and increasing milk yield and milk fat percentage followed by Mastilep® gel and popular competitor spray of Brand A.

Acknowledgements

The authors are grateful to the Associate Dean, CoVAS, Parbhani, India for facilitating the study.

Funding

This study was financially supported by M/s Ayurvet Limited; grant no. 035866, dated 11.02.2019.

Conflict of Interest

Mastilep and Mastrip are commercial brands of M/s Ayurvet Limited, India. This study was financially supported by M/s Ayurvet Limited and authors BN and BG are full-time employees of M/s Ayurvet Limited. The financial support or affiliation of the co-authors did not influence the study outcomes or interpretation of results in any manner.

References

1. De Vliegher S, Fox LK, Piepers S, McDougall S, Barkema HW. Invited review: Mastitis in dairy heifers: Nature of the disease, potential impact, prevention, and control. Journal of Dairy Science. 2012;95(3):1025-1040.
2. Shittu A, Abdullahi J, Jibril A, Mohammed AA, Fasina FO. Sub-clinical mastitis and associated risk factors on lactating cows in the Savannah Region of Nigeria. BMC Veterinary Research. 2012;8(1):1-8.
3. Baloch H, Rind R, Kalhoro DH, Kalhoro AB. Study on the incidence of clinical mastitis in buffaloes caused by bacterial species. Pakistan Journal of Agriculture, Agricultural Engineering and Veterinary Sciences. 2011;27(1):83-93.
4. Snedecor GW, Cochran WG. Statistical methods 8th edn., Oxford and IBH Publishing company, New Delhi; c1994.
5. Dhakal IP. Normal somatic cell count and subclinical mastitis in Murrah buffaloes. Journal of Veterinary Medicine, Series B. 2006;53(2):81-86.
6. Kehrli Jr ME, Shuster DE. Factors affecting milk somatic cells and their role in health of the bovine mammary gland. Journal of Dairy Science. 1994;77(2):619-627.
7. Devmurari VP. Antibacterial evaluation of ethanolic extract of Cedrus deodara wood. Archives of Applied Science Research. 2010;2(2):179-183.
8. Ranjith D, Nisha AR, Nair SN, Litty M, Rahman M, Juliet S. Evaluation of analgesic and anti-inflammatory activity of herbal formulation used for mastitis in animals. International Journal of Applied Science and Engineering. 2018;6(1):37-48.
9. Kolte AY, Waghmare SP, Mode SG, Handa A. Efficacy of indigenous herbal preparation on altered milk pH, somatic cell count and electrolyte profile in subclinical mastitis in cows. Veterinary World. 2008;1(8):239-240.
10. Hase P, Digraskar S, Ravikanth K, Dandale M, Maini S. Management of subclinical mastitis with mastilep gel and herbal spray (AV/AMS/15). International Journal of Pharmacy and Pharmacology. 2013;4:64-67.
11. Shafi TA, Bansal BK, Gupta DK, Nayyar S. Evaluation of immunotherapeutic potential of *Ocimum sanctum* in bovine subclinical mastitis. Turkish Journal of Veterinary & Animal Sciences. 2016;40(3):352-358.
12. Verma AK, Nauriyal DS. Therapeutic potential of a topical herbal gel against bovine subclinical mastitis. Indian Journal of Animal Sciences. 2009;79(3):275-277.
13. Krishnakumar S, Yadav V, Borthakur A, Ravikanth K. Efficacy evaluation of topical herbal spray in sub clinical mastitis in bovines. World Journal of Pharmaceutical and Medical Research. 2017;3(8):172-175.
14. Sinha MK, Thombare NN, Mondal B. Subclinical mastitis in dairy animals: incidence, economics, and predisposing factors. The Scientific World Journal; c2014. p. 1-4.
15. Balakrishnan MN, Punniamurthy N, Mekala P, Ramakrishnan N, Kumar SK. Ethno-veterinary formulation for treatment of bovine mastitis. Journal of Veterinary Sciences. 2017;18(S1):377-382.
16. Hadiya K, Yadav V, Borthakur A, Ravikanath K, Maini S. Efficacy evaluation of Mastilep gel in sub-clinical mastitis in cattle. International Journal of Agriculture Innovations and Research. 2017;5(5):2319-1473.
17. Schultz LH. Somatic cell counting of milk in production testing programs as a mastitis control technique. Journal of the American Veterinary Medical Association. 1977;170(10.2):1244-1246.

18. Bhatt VD, Shah TM, Nauriyal DS, Kunjadia AP, Joshi CG. Evaluation of a topical herbal drug for its *in-vivo* immunomodulatory effect on cytokines production and antibacterial activity in bovine subclinical mastitis. Ayu. 2014;35(2):198-205.
19. Harjanti DW, Wahyono F, Afifah DN. Milk production and milk quality of sub-clinical mastitis cows feed with different supplementation of herbal in the diet. In: IOP Conference Series: Earth and Environmental Science, IOP Publishing. 2019;250(1):012062.
20. Nurdin E, Amelia T, Makin M. The effects of herbs on milk yield and milk quality of mastitis dairy cow. Journal of the Indonesian Tropical Animal Agriculture. 2011;36(2):104-108.
21. Maramulla A, Gadige A, Kosqapati L, Bommu S, Katta P. Efficacy of herbal preparations in the therapy of sub clinical mastitis in cows of periurban areas of Hyderabad. The Pharma Innovation Journal. 2019;8(11):186-188.
22. Bansal B, Gupta D, Randhawa S, Ravikanth K, Adarsh MS. Mastitis treatment and prevention of new intramammary infections with topical herbal spray international. Journal of Medical Science and Clinical Inventions. 2015;2(7):1105-1113.
23. Grover M. An Overview on the ornamental coniferous tree *Cedrus deodara* (Roxburgh) G. Don (Himalayan Cedar). Journal of Ayurveda and Integrated Medical Sciences. 2021;6(4):291-302.
24. Solanki P, Choudhary S, Soni R. Evaluation of antimicrobial properties of ethanolic extracts of stem of *Azadirachta indica* (Neem) and *Tinospora cordifolia* (Giloy) in subclinical mastitis affected cattle. The Pharma Innovation Journal. 2022;SP-11(7):587-591.
25. Baby AR, Freire TB, Marques GDA, Rijo P, Lima FV, Carvalho JCMD, *et al*. *Azadirachta indica* (Neem) as a Potential Natural Active for Dermocosmetic and Topical Products: A Narrative Review. Cosmetics. 2022;9(3):58.
26. Sabalingam S, Siriwardhene MA. A systematic review of the effects of Curcuma longa topical formulations on wound healing. Asian Journal of Pharmaceutical Research and Development. 2022;10(6):71-76.
27. Digraskar SU, Muley VD, Maini S, Ravikanth K. Therapeutic efficacy of polyherbal gel (Mastilep gel®) and antioxidant-mineral formulation (Uniselit®) against clinical mastitis in goats. Veterinary Practitioner. 2011;12(1):128-30.