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Therapeutic efficacy of topical polyherbal preparation of turmeric, aloe vera, sesame oil and calcium hydroxide in subclinical mastitis in crossbred dairy cattle

Mohneet Kaur, Sikh Tejinder Singh, Dhiraj Kumar Gupta, Deepti Narang#*

Department of Veterinary Medicine, # Department of Veterinary Microbiology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

Abstract

Udder health maintenance is an important factor in dairy farming economics and subclinical mastitis is the most prevalent disease. The present study aims at controlling subclinical mastitis in dairy cattle by ethnoveterinary medicine. The study was carried out in 30 HF × Sahiwal crossbred cattle at University dairy form. The animals having SCC more than 2 lakh cells/ ml were selected and randomly divided into two Treatment (n = 20) and Control (n = 10) groups. The Treatment group was treated with topical polyherbal preparation of turmeric powder (50 gms), aloe vera gel (250 gms), sesame oil (10 ml) and calcium hydroxide (5 gms) twice a day for five days. It was applied to the whole udder post milking. The milk culture, SCC and MCMT of quarter milk and SCC, MCMT, phagocytic activity, phagocytic index, milk pH and electrical conductivity, biochemical composition of milk was evaluated at day 0, 7, 14 and 21. The culture of the milk revealed presence of E. coli, *Staphylococcus aureus, Staphylococcus hemolyticus, Staphylococcus chromogens, Streptococcus agalactiae and Streptococcus uberis*. The composite milk SCC and MCMT declined significantly ($p<0.05$) whereas phagocytic activity and phagocytic index increased significantly ($p>0.05$) during different days of treatment in the Treatment group. No such effect was seen in the control group. The quarter milk SCC and MCMT score also decreased in Group I i.e., Infected, treatment. There was no effect of the therapy on the composite milk pH, electrical conductivity and milk composition such as fat, SNF, protein and lactose concentration. Hence, the findings of the present study indicate the therapeutic potential of the polyherbal preparation.

Keywords: cattle, subclinical, mastitis, polyherbal, ethnoveterinary, medicine.

Dairy industry is one of the major contributors to the gross domestic products (GDP) of India that contributes to about 5 percent of the economy. India ranks first in the world in total milk production, and the dairy sector provides direct employment to about 8 crore farmers. Udder health maintenance is an important factor in dairy farming economics. Disorders of the udder results in lower profitability, unplanned culling, poor quality of milk and poor milk hygiene. Mastitis is an endemic disease affecting dairy herds all over the world (Halasa *et al*., 2007). The most common type is the subclinical mastitis that has no visible symptoms and causes insufficient milk production, changes in milk consistency (density), a reduced potential of appropriate milk processing, low protein and a significant risk of milk hygiene because it may include pathogenic organisms. Milk somatic cell count (SCC) values are frequently used to determine quality standards and detect subclinical mastitis. It has been demonstrated that somatic cell count (SCC) in milk is a good indicator of subclinical mastitis (Paape *et al*., 2002). The composite SCC above a threshold value of 200,000 cells/mL has been considered as occurrence of udder infection (Bradley and Green, 2005). The mainstay treatment for mastitis is antibiotic therapy. The cost of antibiotic treatment, antibiotic residues in milk and antimicrobial resistance are important concerns. One strategy to lessen the burden of mastitis is to improve the cow's natural capacity to endure infections. Alternative therapy options for treatment of mastitis includes use of medicinal herbs that function as antibacterial, anti-inflammatory or immune-modulatory substances (Mushtaq *et al*., 2018). Herbal plants are important component of ethno-veterinary medicine (Van der Merwe *et al*., 2001). The EVM is concerned with people's knowledge, skills, techniques, practices, and ideas about managing and keeping their animals healthy, which are learned during practical experience and passed down from generation to generation (McCorkle, 1986). Aloe vera (*Aloe barbadensis miller*) is known to possess anti-inflammatory, astringent, emollient, anti-fungal and anti-viral properties (Bashir *et al*., 2011). It has been reported that aloe vera has antibacterial activity against *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa*,

*Corresponding author: sikhtejindersingh@yahoo.co.in

E. coli, Staphylococcus aureus and *Salmonella typhi* (Djeraba and Quere, 2000). It is known to have healing, soothing and moisturising properties also. Turmeric (*Curcuma longa*) is rich in bioactive chemicals that have anti-inflammatory and antioxidant activities. Curcumin is an anti-inflammatory chemical found in turmeric (Nelson *et al*. 2017). The sesame (*Sesamum indicum*) is a flowering plant belonging to the Sesamum genus and family Pedaliaceae. Sesame oil has been found to help in variety of conditions, including oxidative stress. Sesame oil is used as a solvent in pharmaceutical industry due to its resistance to oxidation (Chang *et al*., 2002). It is a popular ingredient of massage in the ayurvedic medicine. It has antioxidant, anti-inflammatory, and antibacterial properties (Chen *et al*., 2005) (Heidari-Soureshjani *et al*., 2016). Calcium hydroxide is added to the polyherbal preparation to improve their penetration through the skin. It has two significant properties: it inhibits bacterial enzymes, which has an antibacterial impact, and it activates tissue enzymes, such as alkaline phosphatase. When some plants are used together, there is synergistic action of certain pharmacological actions of the active constituents which are not seen when they are used individually (Parasuraman *et al*., 2014). Ethno-veterinary medicine may provide an effective, affordable and farmer and animal friendly prevention and control solution for mastitis.

Materials and Methods

The study was conducted on the crossbred dairy cattle (Holstein Frisian × Sahiwal) maintained at the dairy farm of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. The animals were kept in a loose barn with head-to-head housing system. They were provided free access to ad-libitum drinking water, seasonal green and concentrate mixture as per the standard recommendations. The animals were milked by using automatic machine milking in the morning and evening. Post milking teat dip with a mixture of povidone iodine and glycerine (4:1) was practiced. Thirty crossbred cattle suffering from subclinical mastitis i.e., having milk somatic cell count more than 2 lakh cells/ml) were selected for the study. The selected cattle were randomly divided into two groups i.e., Treatment (n=20) and Control (n=10) groups. Treatment group was treated with a topical polyherbal preparation and other group was taken as control. The control group was not given any treatment. The polyherbal preparation comprised of turmeric powder (50 gms), aloe vera gel (250 gms), sesame oil (10 ml) and calcium hydroxide (5 gms). All the ingredients were weighed and mixed uniformly to make a paste. The polyherbal preparation was applied to whole udder post milking twice a day for five days in the Treatment group. It was prepared fresh every time before application.

Composite and quarter milk samples were collected for each animal before treatment (day 0) and on day 7, 14 and 21. Proper cleanliness and dryness of the udder was ensured during sample collection. Composite milk samples (50 ml) were collected in plastic disposable vials and analysed for somatic cell count, MCMT score point, phagocytic activity, phagocytic index, milk pH, milk electrical conductivity and biochemical composition of milk (fat, SNF, protein and lactose concentration). Quarter milk samples (10 ml) were collected in sterilized test tubes and assessed for milk culture, MCMT score point and somatic cell count.

The quarter milk samples were streaked on blood agar plates and incubated aerobically at 37ºC for 18 to 24 hours and examined for presence of any bacterial growth. The individual bacterial colonies were streaked on BHI agar and the organisms were identified using MALDI-TOF MS. Modified California Mastitis Test (MCMT) was conducted and interpreted as per standard method described by Pandit and Mehta (1969). The results were interpreted as no mastitis (0), doubtful (1), positive (2) and strong positive (3) depending upon the degree of gel formation. The analysis of milk samples for SCC was done by using SomaScope Smart, an automatic milk somatic cell counter from DELTA Instruments, BV Kelvinlaan 3, 9207 JB Drachten and results were expressed in × 10^3 cells/ ml of milk. The pH of milk was recorded with the help of digital pH meter Mettler Toledo, Five Easy Plus. The electrical conductivity of milk samples was recorded with the help of Digital Conductivity Meter, CON 700, Eutech instruments and the results were expressed in milli Siemens per cm (mS/cm). The biochemical composition of the milk i.e., fat, SNF, protein and lactose were analyzed by using Milk analyzer Lactoscan LA from Milkotronic Ltd., Bulgaria and the results were expressed in % (w/v). Phagocytic activity of milk neutrophils was done according to the method described by Shafi *et al*. (2013). Mean concentrations of various parameters were calculated and compared between different days in treatment and control groups by using one way analysis of variance (ANOVA).

Fig. 1. Application of topical polyherbal preparation on the udder of cattle.

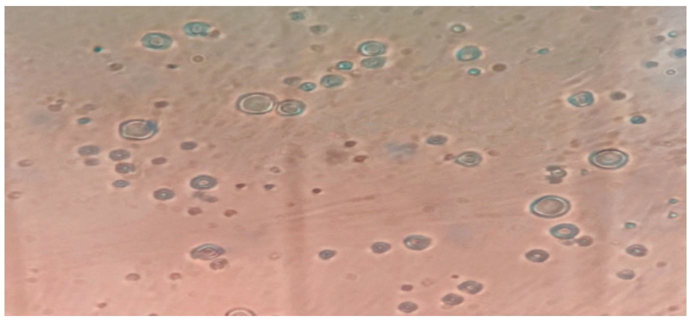

Fig 2. Phagocytic activity of milk leucocytes against C. albicans (40X)

Results and Discussion

Culture and Somatic cell count

The organisms isolated on day 0 in dairy cattle were E. coli, *Staphylococcus aureus, Staphylococcus hemolyticus, Staphylococcus chromogens, Strepreptococcus agalactiae and Streptococcus uberis.* The effects of topical polyherbal preparation application on composite milk somatic cell count in Treatment and Control groups during different days is presented in Table 1. There was significant effect of the treatment on the composite milk somatic cell count ($p<0.05$). No such effect was seen in the control group ($p>0.05$). In Treatment group, the mean somatic cell count of composite milk on day 0 was 576.10±62.30 $\times 10^3$ cells/ml that ranged from 250.00 to 1274.00 $\times 10^3$ cells/ml. It reduced significantly to 268.70±31.02 $\times 10^3$ cells/ml on day 7, 215.10±35.56 $\times 10^3$ cells/ml on day 14, and 251.50±33.88 $\times 10^3$ cells/ml on day 21.

The effects of the topical polyherbal preparation application on the quarter milk somatic cell count are presented in Table 2. Treatment group was further sub-divided into two groups viz. Group 1 (Infected, treatment) and Group II (Non-infected, treatment) on the basis of milk culture. Control group was further sub-divided into two groups viz. Group III (Infected, control) and Group IV (Non-infected, control) on the basis of milk culture. There was significant effect of the therapy in Group I i.e., infected, treatment group ($p<0.05$). The mean quarter milk somatic cell count on day 0 was 587.34±42.65 $\times 10^3$ cells/ml. It decreased to 314.78±54.68 $\times 10^3$ cells/ml on day 7, 302.95±51.64 $\times 10^3$ cells/ml on day 14 and 257.73±46.13 $\times 10^3$ cells/ml on day 21. No significant effect of the therapy ($p>0.05$) was seen in Group II, Group III and Group IV. Similarly, Maramulla *et al*. (2019) observed decrease in milk SCC in 16 affected quarter from 10 animals treated with topical application of paste prepared from *Moringa oleifera* leaves, turmeric powder and common salt. Panigrahi *et al*. (2022) found significant reduction in the SCC in 6 quarters suffering from subclinical mastitis and fed with the polyherbal formulation of *Moringa oleifera, Ocinum sanctum* and *Azadirachta indica* leaves and *Curcuma longa* rhizome @10 mg/kg.

Modified California mastitis test point score

The effects of topical polyherbal preparation application on composite milk modified California mastitis test point score in the Treatment and Control groups is presented in Table 1 There was significant effect of therapy on the composite milk modified MCMT in the Treatment group $p<0.05$); whereas, in the Control

Table 1. Effects of topical polyherbal preparation on composite milk parameters in dairy cattle suffering from subclinical mastitis (Mean±SE).

Parameters	Group	Day 0	Day 7	Day 14	Day 21	F value
SCC ($\times 10^3$/ ml)	Treatment	576.10±62.30^{a}	268.70±31.02^{b}	268.70±31.02^{b}	251.50±33.88^{bd}	F = 15.371, df = 3 (p<0.05)
	Control	866.70±222.46^{a}	894.00±146.10^{a}	901.80±304.35^{a}	1030.30±250.66^{a}	F = 0.094, df = 3 (p>0.05)
MCMT score point	Treatment	1.60±0.15^{a}	0.70±0.10^{b}	0.70±0.12^{bc}	0.55±0.11^{bd}	F = 14.516, df = 3 (p<0.05)
	Control	1.70±0.26^{a}	1.80±0.20^{a}	1.90±0.27^{a}	1.70±0.21^{a}	F = 0.159, df = 3 (p>0.05)
Phagocytic Activity (%)	Treatment	14.67±0.59^{a}	27.33±1.22^{b}	24.50±0.50^{bc}	22.83±0.79^{cd}	F = 41.976, df = 3 (p<0.05)
	Control	15.67±0.88^{a}	15.00±0.63^{a}	15.17±0.83^{a}	12.33±1.05^{a}	F=3.014, df = 3 (p>0.05)
Phagocytic Index	Treatment	1.10±0.01^{a}	1.30±0.01^{b}	1.27±0.01^{bc}	1.24±0.01^{d}	F = 65.470, df = 3 (p<0.05)
	Control	1.13±0.01^{a}	1.13±0.01^{a}	1.12±0.01^{a}	1.09±0.01^{a}	F=1.542, df = 3 (p>0.05)
Milk pH	Treatment	6.53±0.02^{a}	6.55±0.02^{a}	6.54±0.02^{a}	6.56±0.02^{a}	F = 0.267, df = 3 (p>0.05)
	Control	6.57±0.06^{a}	6.64±0.03^{a}	6.60±0.03^{a}	6.63±0.04^{a}	F = 0.512, df = 3 (p>0.05)
Milk electrical conductivity (mS/cm)	Treatment	4.53±0.06^{a}	4.43±0.06^{a}	4.44±0.05^{a}	4.47±0.05^{a}	F = 0.507, df = 3 (p>0.05)
	Control	4.78±0.16^{a}	4.86±0.16^{a}	4.86±0.20^{a}	4.53±0.17^{a}	F = 0.771, df = 3 (p>0.05)
Milk fat (%)	Treatment	2.13±0.14^{a}	2.18±0.18^{a}	2.20±0.13^{a}	2.29±0.14^{a}	F = 0.201, df = 3 (p>0.05)
	Control	2.43±0.30^{a}	2.36±0.25^{a}	3.04±0.97^{a}	2.82±0.31^{a}	F = 1.183, df = 3 (p>0.05)
Milk SNF (%)	Treatment	9.94±0.08^{a}	9.79±0.08^{a}	9.87±0.06^{a}	9.74±0.11^{a}	F = 1.005, df = 3 (p>0.05)
	Control	9.95±0.15^{a}	9.91±0.17^{a}	9.90±0.21^{a}	9.96±0.21^{a}	F = 0.023, df = 3 (p>0.05)
Milk protein (%)	Treatment	3.63±0.03^{a}	3.58±0.03^{a}	3.61±0.02^{a}	3.56±0.03^{a}	F = 0.813, df = 3 (p>0.05)
	Control	3.62±0.05^{a}	3.64±0.07^{a}	3.61±0.07^{a}	3.63±0.07^{a}	F = 0.035, df = 3 (p>0.05)
Milk lactose (%)	Treatment	5.45±0.04^{a}	5.36±0.04^{a}	5.42±0.04^{a}	5.34±0.06^{a}	F = 1.174, df = 3 (p>0.05)
	Control	5.41±0.08^{a}	5.39±0.08^{a}	5.37±0.12^{a}	5.40±0.12^{a}	F = 0.025, df = 3 (p>0.05)

Values with common superscript (a, b, c) in a row do not differ significantly (p<0.0

Table 2. Effects of topical polyherbal preparation on quarter milk somatic cell count 10³ cells/ml) in dairy cattle suffering from subclinical mastitis (Mean±SE).

Day	Quarter wise groups			
	Group I (Infected, treatment) (n=41)	Group II (Non-infected, treatment) (n=39)	Group III (Infected, control) (n=24)	Group IV (Non-infected, control) (n=16)
0	587.34±142.65[a]	177.79±52.50[a]	629.50±158.50[a]	565.12±273.25[a]
7	314.78±54.68[b]	84.17±17.65[a]	1042.40±250.43[a]	519.38±128.60[a]
14	302.95±51.64[b]	85.15±20.19[a]	1260.90±401.00[a]	1114.80±482.47[a]
21	257.73±46.13[bd]	117.72±25.44[a]	839.00±203.30[a]	373.06±67.83[a]
F value	F = 3.190, df = 3 (p<0.05)	F = 1.871, df = 3 (p>0.05)	F = 1.012, df = 3 (p>0.05)	F = 1.286, df=3 (p>0.05)

Values with common superscript (a, b, c) in a column do not differ significantly (p<0.05)

group, no significant effect was observed (p>0.05). The mean MCMT score in the Treatment group on day 0 was 1.60±0.15, with the range of 1 to 3 and decreased significantly on day 7 to mean of 0.70±0.10, with the range of 0 to 1. The mean MCMT on day 14 and 21 was 0.70±0.12 and 0.55±0.1, respectively. The effects of the topical polyherbal preparation application on quarter milk modified California mastitis test point score is presented in Table 7. There was significant effect of the treatment in Group I (p<0.05). No significant effect (p>0.05) was observed in any of the other groups viz. Group II, Group III and Group IV. The mean CMT on day 0 was 1.24±0.15 in Group I and reduced to 0.73±0.12 on day 7, 0.68±0.10 on day 14 and 0.65±0.10 on day 21. Similarly, Shafi et al. (2016) showed a significant decline in the CMT score from 1.65±0.17 on day 0 to 0.80±0.28 on day 14, and 0.40±0.23 on day 28 after oral supplementation of O.sanctum leaf powder in twenty crossbred cattle. Tawheed et al. (2018) found significant decline in CMT after treatment with non-antibiotic preparation Masticure. The present findings are also in agreement with Gupta (2010) who reported a significant reduction in CMT and SCC after treatment with oral administration of the herbal powder mix containing O. sanctum and W. somnifera.

Phagocytic activity and Phagocytic index

The effects of topical polyherbal preparation application in Treatment and Control groups on composite milk phagocytic activity and phagocytic index is presented in Table 1. The mean phagocytic activity differed significantly in the Teatment group (p<0.05). However, no such effect was observed in the Control group (p>0.05). The mean phagocytic activity on day 0 was 14.67±0.59% and ranged between 10 to 17%. The highest phagocytic activity with the mean of 27.33±1.22% and range of 22 to 34% was seen on day 7 after therapy in the Treatment group. The phagocytic activity remained elevated with the mean of 24.50±0.50% on day 14 and 22.83±0.79% on

Table 3. Effects of topical polyherbal preparation on quarter milk modified california mastitis test point score (CMT) in dairy cattle suffering from subclinical mastitis (Mean±SE).

Day	Quarter wise groups			
	Group I (Infected, treatment) (n=41)	Group II (Non-infected, treatment) (n=39)	Group III (Infected, control) (n=24)	Group IV (Non-imfected, control) (n=16)
0	1.24±0.15[a]	0.43±0.12[a]	1.29±0.20[a]	1.25±0.19[a]
7	0.73±0.12[b]	0.17±0.07[a]	1.70±0.20[a]	1.18±0.20[a]
14	0.68±0.10[b]	0.20±0.07[a]	1.66±0.19[a]	1.50±0.25[a]
21	0.65±0.10[bd]	0.62±0.10[a]	1.62±0.17[a]	1.25±0.21[a]
F value	F = 4.892, df = 3 (p<0.05)	F = 1.702, df = 3 (p>0.05)	F = 0.945, df = 3 (p>0.05)	F = 0.397, df = 3 (p>0.05)

Values with common superscript (a, b, c) in a column do not differ significantly (p<0.05).

day 21 in Treatment group. There was significant effect of the treatment on composite milk phagocytic index in the Treatment group ($p<0.05$). No such effect was seen in the Control group ($p>0.05$). The mean phagocytic index of composite milk on day 0 was 1.10±0.01 and increased to 1.30±0.01 on day 7. It decreased to 1.27±0.01 on day 14, and 1.24±0.01 on day 21, but still it was a significant change as compared to day 0. Similarly, Shafi *et al*. (2016) found a significant increase in the phagocytic activity at day 7 of oral treatment with O.sanctum leaf powder in twenty crossbred cattle. It increased from 17.10 ± 0.64% on day 0 to 29.10 ± 1.62% on day 7. Phagocytic index was also significantly increased in the animals. Similar to this, Gupta *et al*. 2016 found that oral treatment with Tinospora cordifolia dried stem powder for subclinical mastitis (100 mg/ kg BW) demonstrated a significant increase in the mean phagocytic index and total serum immunoglobulin levels.

pH and electrical conductivity

The effects of topical polyherbal preparation application on composite milk pH and electrical conductivity in Treatment and Control groups is presented in Table 1. There was no significant effect of the treatment on the composite milk pH and electrical conductivity ($p>0.05$) in Treatment and Control groups. No literature was found in this support. On the contrary, Tawheed et al. (2018) reported decrease in milk pH and electrical conductivity in the animals treated with combination therapy of Masticure granules and spray. Gupta (2010) also reported decrease in the pH and electrical conductivity in animals after treatment with oral herbal powder mix of O. sanctum and W. somnifera. Waghmare *et al*. (2013) studied that after application of herbal teat dip post-milking the pH and SCC of milk was significantly ($P < 0.01$) improved in the treated groups and was normalized on 30th day post application. Kolte *et al*. (2008) used a paste of W. somnifera, O. sanctum, *Curcuma amada* and *Asparagus reacemosus* topically in subclinical mastitis in cows and recorded a significant decrease in pH, sodium ions and potassium ions after 10 days of therapy.

Milk composition

The effects of topical polyherbal preparation application on the composite milk composition i.e., milk fat, SNF, lactose and protein concentration in Treatment and Control is presented in Table 1. There was no significant difference in both the groups during different days of treatment on the milk composition. Similarly, Hase *et al*. (2013) found no change in the milk fat concentration after topical treatment with mastilep gel. Panigrahi *et al*. (2022) also reported non-significant changes in milk lactose and milk protein concentration during different observation days of study in the animals fed with polyherbal formulation of *Azadirachta indica*, *Moringa oleifera, Ocimum sanctum* and *Curcuma longa*. On the contrary Tawheed *et al*. (2018) reported significant improvement in milk fat, SNF, protein and lactose concentration in animals treated with combination therapy (Masticure granules and spray) with or without antibiotic.

Conclusions

The findings of the present study indicate the therapeutic potential of topical polyherbal preparation of turmeric, aloe vera, sesame oil and calcium hydroxide in subclinical mastitis in dairy cattle. The effects of the therapy are attributed to the antimicrobial, anti-inflammatory, antioxidant and immunomodulatory properties of the polyherbal ingredients. It is substantiated by decrease in the milk SCC and MCMT score; and increase in the phagocytic activity and phagocytic index of the milk. The alternative ethnoveterinary medicines are cheaper, farmer friendly and easily available. The concerns for antibiotic resistance can also be addressed.

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