

Original Research Article

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Therapeutic Efficacy of Indigenous Polyherbal Formulation on Milk pH, Somatic Cell Count and Electrical Resistance Profile in Healthy and Subclinical Mastitic Dairy Cows

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ABSTRACT

Keywords

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The present study was conducted to detect subclinical mastitis from milk and also to evaluate the therapeutic efficacy of the indigenous polyherbal formulation. Twenty four lactating cow suffering from subclinical mastitis (SCM) were randomly divided into four treatment groups G-II to G-V, whereas G-I comprised of six apparently healthy cows. G-II did not receive any treatment and served as the positive control. G-III was provided with standard therapy with Amoxicillin and Cloxacillin @ 10mg/kg bwt I/M along with Meloxicam @ 0.5 mg/kg bwt I/M for 3 days. G-IV was administered with 5 gm each from *Azadirachta indica*, *Boswellia serrata*, *Vitex nigundo*, *Ocimum sanctum* and *Tinospora cordifolia* herbal powder @ 25 gm orally for 5 days. Group-V was treated with group III and IV combination therapy. The Somatic Cell Count (SCC) in G-IV to G-V was significantly reduced on 10th day of post-treatment. Similarly, electrical conductivity (ER) was significantly reduced on 7th day, which was further reduced on 10th day. The combined treatment was most effective in the reduction of ER on 7 and 10th day of post-treatments as compared to herbal treatment alone. In addition, the antibiotic treatment reduced the pH significantly on 7th day, while in combination therapy, it was reduced significantly on 3rd day onward. Hence, herbal treatment along with standard treatment was most effective in the amelioration of pH, somatic cell count and electrical resistance as compared to standard treatment or herbal treatment when given alone.

Introduction

Livestock plays a vital role in the Indian economy and in the socio-economic development of the country. These livestock sectors also play an important role in supplementing family incomes and generating gainful employment in the rural sector, particularly, among the small and marginal farmers, women and landless laborers. They are providing cheap nutritional food to millions of people. Mastitis is a common and multi-etiological complex disease affecting all milk-producing animals (Lu *et al.*, 2008).

It caused an inflammation of the parenchyma of mammary glands and characterized by physical, chemical and bacteriological changes in milk and pathological changes in mammary gland tissues (Radostits *et al.*, 2000).

The infection is transmitted by milk-contaminated fomites at milking, by the Milker's hands, or by the milking machine. Environmental mastitis transmitted by contact of the teats with contaminated soil, bedding and water with fecal materials (Barua *et al.*, 2014; Mir *et al.*, 2014).

It's considered of quite vital importance to public health due to its association with many zoonotic diseases in which the milk act as a vehicle for some infectious agents (Ahmady and Kazemi, 2013). Antibiotics have been the mainstay for mastitis prevention programs and treatment for decades.

The use of antibiotics has been accompanied by the appearance of resistant strains of common bacterial species in dairy animals (Gao *et al.*, 2012).

This rising to alarming concerned that led to the urgency of finding new and innovative treatment options for mastitis worldwide. In

the past few decades, a large quantity of research has been focused on characterizing the antibacterial effects of different herbs and aromatic plants and many other natural substances for the treatment of different animal diseases including mastitis (Baskaran *et al.*, 2009; Nurdin *et al.*, 2011).

The objectives of this study were to assess the effects of *Azadirachta indica*, *Vitex Nigundo*, *Ocimum sanctum*, *Tinospora cordifolia* and *Boswellia serrata* on milk pH, somatic cell count and electrical resistance profile in healthy and as well as mastitis-affected dairy cattle.

Materials and Methods

Experimental animals

The suspected cases of subclinical mastitis was screened for the study from those presented to Medicine Out Patient Department (OPD) of Teaching Veterinary Clinical Complex (TVCC) and Instructional Dairy Farm of college along with private dairies and Goushalas of Rewa district, Madhya Pradesh during June 2018 to July 2019.

Plant Material

The part of herbs like *Azadirachta indica* (Neem/Indian lilac/Margosa), *Vitex Nigundo* (Nirgundi/Chinese Chaste Tree), *Ocimum sanctum* (Tulsi /Holy Basil), *Tinospora cordifolia* (Giloy/Guduchi /Heart-leaved moonseed) and *Boswellia serrata* (Salai /Guggul/Shallaki/Indian Frankincense) were purchased from registered herbal shops from Rewa, Madhya Pradesh.

Diagnosis of subclinical mastitis

The cows were screened for sub clinical mastitis (SCM) by performing California Mastitis Test (CMT).

Experimental group

Twenty four lactating cows suffering from subclinical mastitis (SCM) were randomly divided into four treatment groups i.e. Group II to Group V (n=6) whereas, Group-I comprise of six apparently healthy cows was taken for comparison. Subclinical mastitis cows of Group-II were kept as an untreated control and Group-III of SCM cow was treated with Amoxicillin and Cloxacillin @ 10 mg/kg bwt I/M along with Meloxicam @ 0.5 mg/kg bwt I/M for 3 days. Group-IV of SCM cows was treated with herbal preparation which contains 5 gm leaves of *Azadirachta indica*, 5 gm leaves of *Vitex Nigundo*, 5 gm Leaves of *Ocimum sanctum*, 5 gm bark of *Tinospora cordifolia* and 5 gm Gum resin of *Boswellia serrata*. Total 25 gm of powder preparation was given orally twice daily for 5 days. Group-V was treated with both standard treatment as well as herbal preparation (Group III and IV combination treatment).

Collection of milk samples

The milk samples from affected quarters from each cow were collected after proper disinfection of teat surface. The udder and teats were cleaned and washed with potassium permanganate 0.01 % then wiped with clean cloth. First few streams of foremilk was discarded and then about 8 ml of milk from each affected quarter was collected in fresh, sterile, labeled screw cap test tubes and brought to the department in ice for further examination. Milk sample was collected on '0' day (pretreatment) and subsequently on 3rd, 5th, 7th and 10th day.

Testing of milk samples

California Mastitis Test (CMT)

California Mastitis Test (CMT) was conducted for the identification of Clinical and Sub

clinical mastitis in cattle (Ruegg and Reinemann, 2002). The milk from the four quarters was collected in the CMT paddle after discarding first stripping of the milk. The paddle was tilted so that the excess milk was drained off and all the cups in the paddle contained equal amount of milk. The CMT reagent was added to the all the cups equal to the amount of milk already contained in the cups representing the four quarters. The paddle gently swirled and the CMT score was read.

Milk pH

Milk pH was estimated by the digital pH meter. The pH reading of the normal and mastitis milk sample was recorded on 0 day (pre treatment) and on 3rd, 5th, 7th and 10th day (post treatment).

Electrical resistance (ER)

Draminski Mastitis Detector was used to measure the electrical resistance of milk to detect subclinical mastitis. A minimum of 15 ml of the first portion of milk was poured directly from the teat to measuring cup. Then the switch on button was pressed to read the result in units. After recording the milk was poured out and the steps were repeated for other quarters. The electrodes were cleaned with methylated spirit by a clean cloth or tissue. A reading below 300 units was considered as the cut-off value for subclinical mastitis and above 300 was considered as healthy (Siddiquee *et al.*, 2013).

Somatic Cell Count (SCC)

The somatic cell count (SCC) of milk samples was calculated as described by Schalm *et al.*, (1971) and the milk smear were stained with Newman- Lumpert stain. Milk sample was thoroughly mixed, so that to obtain uniform distribution of cells. Milk smears (0.01 ml

milk for each smear) was prepared on grease free glass slide with the help of a platinum loop. The slides were air dried and preserved till staining was done. Slides were immersed for 20 seconds in Newman - Lampert stain. Excess of stain was drained off and slides were air dried. Then the slides were rinsed two or three times in water and rapidly air dried after draining by gentle blotting with filter paper. The smears were stained deep blue. Calibration was done in four one square cm areas. The leukocyte count in the subclinical mastitis milk was performed to assess the degree of infection using Newman's - Lumpert stain as per the procedure described by Harmon (2001).

Statistical analysis

Data are presented in Means \pm Standard Error. The data was analyzed using statistical tools (SPSS version 20). Repeated measures ANOVA was used for pre and post treatment multiple comparisons. ANOVA followed by Duncan's Multiple Range Test was used for multiple comparisons. Statistical differences were determined at the 5% level of significance.

Results and Discussion

pH before and after treatment

Mean \pm SE value of milk pH was 6.57 ± 0.04 to 6.68 ± 0.05 in healthy control group (G-1) and 7.53 ± 0.04 to 7.60 ± 0.04 in negative control group (G-II) indicate milk pH increased due to mastitis (Table 1). Statistical analysis indicated a highly significant ($p<0.05$) difference in pH due to severity of mastitis. In treatment G-III to V indicate a significant reduction in pH during the course of treatment from day 0 to 10th day.

The results of the present study are in agreement with Horvath *et al.*, (1980) and

Haggag *et al.*, (1991) who showed that with severity of mastitis pH of the samples increased. Similarly, Charjan *et al.*, (2000) reported that milk pH increased in could serve as the best indicator to assess the condition of udder health in dairy animals.

Normal fresh milk has approximately a pH 6.6 to 6.9, which indicates that the milk is slightly acidic. The probable reason of increase pH due to severity of mastitis may be attributed to the lowered acidity as has been found in mastitis milk. The lowered acidity in mastitis milk is due to reduction in lactose contents as the lactic acid formation is minimum in this case (Haggag *et al.*, 1991; Bilal and Ahmad, 2004).

Electrical Resistance (ER) before and after treatment

Mean \pm SE value of milk ER was 356.67 ± 13.33 to 371.67 ± 13.02 in the healthy control group (G-1) and 295.00 ± 13.84 to 315.00 ± 19.45 in the negative control group (G-II) indicate milk ER decreased due to mastitis (Table 2). Statistical analysis indicated a highly significant ($p<0.05$) decreased in ER due to severity of mastitis. In treatment G-III to V indicate a significant ($p<0.05$) increased in ER during the course of treatment from day 0 to 10th day. However, the best result observed after 5th day onward in treatment group.

In this study, the electrical resistance (ER) was measured instead of electrical conductivity (EC) which gave an indirect estimate of EC.

Electrical conductivity of milk was reciprocal to electrical resistance of milk. EC is the measured by the presence of ions (Ilie *et al.*, 2010). Milk has conductive properties as it is enrich compound especially mineral salts such as sodium, chloride, potassium, calcium, magnesium and other ions (Mucchetti *et al.*, 1993; Norberg, 2005).

Table.1 pH of the healthy and SCM positive animals before and after the treatment

Day	G-1	G-II	G-III	G-IV	G-V
0	^A 6.57±0.04 ^a	^A 7.55±0.04 ^c	^{BC} 6.95±0.02 ^b	^A 7.08±0.08 ^b	^B 6.50±0.00 ^a
3	^A 6.62±0.06 ^a	^A 7.55±0.04 ^c	^C 6.98±0.05 ^b	^A 7.08±0.15 ^b	^A 6.40±0.04 ^a
5	^A 6.68±0.05 ^b	^A 7.53±0.04 ^d	^{BC} 6.90±0.03 ^c	^A 7.03±0.10 ^c	^A 6.40±0.03 ^a
7	^A 6.62±0.05 ^b	^A 7.60±0.04 ^d	^A 6.75±0.04 ^b	^A 6.90±0.07 ^c	^A 6.37±0.04 ^a
10	^A 6.65±0.06 ^b	^A 7.57±0.04 ^d	^{AB} 6.85±0.04 ^{bc}	^A 6.90±0.15 ^c	^A 6.37±0.03 ^a

Values (Mean±SE) bearing different superscript in capital and small letter differ significantly in column and row respectively (p<0.05).

Table.2 Electrical Resistance of the healthy and SCM positive animals before and after the treatment.

Day	G-1	G-II	G-III	G-IV	G-V
0	^A 356.67±13.33 ^b	^A 315.00±19.45 ^{ab}	^A 287.33±15.76 ^a	^A 300.67±17.94 ^a	^A 283.33±12.02 ^a
3	^A 363.33±15.20 ^b	^A 311.67±17.40 ^a	^{AB} 312.50±13.77 ^a	^A 288.33±13.02 ^a	^{AB} 310.33±15.75 ^a
5	^A 370.00±12.91 ^b	^A 308.33±20.23 ^a	^B 333.00±13.55 ^{ab}	^{AB} 330.00±12.91 ^{ab}	^{ABC} 325.00±12.04 ^{ab}
7	^A 371.67±13.02 ^c	^A 295.00±13.84 ^a	^B 331.67±14.47 ^{abc}	^{AB} 328.33±10.14 ^{ab}	^{BC} 341.67±14.47 ^{bc}
10	^A 370.00±18.26 ^b	^A 296.67±10.22 ^a	^B 341.67±14.47 ^{ab}	^B 346.67±16.06 ^b	^C 361.67±17.21 ^b

Values (Mean±SE) bearing different superscript in capital and small letter differ significantly in column and row respectively (p<0.05).

Table.3 Somatic cell count (nx10⁵) of the healthy and SCM positive animals before and after the treatment

Day	G-1	G-II	G-III	G-IV	G-V
0	^A 1.52±0.12 ^a	^A 17.22±3.12 ^b	^B 18.05±5.68 ^b	^B 18.73±2.62 ^b	^B 19.77±6.46 ^b
3	^A 1.49±0.11 ^a	^A 19.78±4.99 ^b	^B 17.18±3.12 ^b	^{AB} 16.35±1.94 ^b	^{AB} 13.43±1.78 ^b
5	^A 1.28±0.09 ^a	^A 29.68±5.97 ^b	^{AB} 14.30±2.43 ^a	^{AB} 11.27±6.64 ^a	^{AB} 11.13±6.51 ^a
7	^A 1.33±0.11 ^a	^A 33.23±7.06 ^b	^{AB} 8.47±2.31 ^a	^A 6.97±2.03 ^a	^{AB} 8.61±2.23 ^a
10	^A 1.44±0.15 ^a	^A 30.15±5.67 ^b	^A 4.87±0.83 ^a	^A 5.35±2.32 ^a	^A 4.48±1.02 ^a

Values (Mean±SE) bearing different superscript in capital and small letter differ significantly in column and row respectively (p<0.05).

The results of the present study are in agreement with Hussain *et al.*, (2012) and Gaspard *et al.*, (2012) who showed that higher values of electrical conductivity in mastitis animals might be due to the presence of clinical and subclinical infection and could be used as an adjunct test for diagnosis of mastitis in animals along with other available tests.

Norberg *et al.*, (2004) indicate that cows with mastitis may not always show an increased

electrical conductivity of milk from the infected quarter, but the variation in EC of milk from infected quarters may be larger than variation in EC of milk from healthy quarters.

The ionic changes that occur during inflammation as a result of increased sodium and chloride concentrations in mastitis milk might be responsible for the alterations in electrical conductivity/resistance (Popovic, 2004).

The concentration of Na⁺ and Cl⁻ ions is increased and concentration of K⁺ and lactose is decreased when the cows and buffalos were suffering with mastitis due to inflammation of udder, hence increase the EC. Although EC is also affected by some other factors such as breed, lactation stage and milking interval (Kamal *et al.*, 2014).

Somatic Cell Count (SCC) before and after treatment

Mean±SE value (nx10⁵ somatic cells/ml) of milk SCC was 1.28±0.09 to 1.52±0.12 in the healthy control group (G-1) and 17.22±3.12 to 33.23±7.06 in the negative control group (G-II) indicate milk SCC increased due to mastitis (Table 3).

Statistical analysis indicated a highly significant difference in SCC due to severity of mastitis. In treatment G-III to V indicate a significant increase in SCC during the course of treatment from day 0 to 10th day. The combination therapy (G-V) give the best result a significant (p<0.05) reduction was found only on 10th day.

The major factor affecting the SCC at the herd and individual level is the presence of intramammary infections (Radostits, 2007).

Normal milk from uninfected quarters generally contains < 2x10⁵ somatic cells/ml. The rise in the leukocyte number in milk and in the mammary gland might be due to the pathogens or to their metabolites which lead to an increase in somatic cell count (Harmon, 1994; Atasever, 2012).

The high SCC content in infected quarters as compared with uninfected quarters was similar to that reported by previous studies (Bruckmaier *et al.*, 2004; Bonfoh *et al.*, 2005).

It could be concluded that the feeding of polyherbal formulation to SCM infected dairy

cows showed improvement in the pH, Somatic Cell Count and Electrical resistance profile of milk. It indicated that polyherbal therapy potentiates the udder immunity, not only eliminates udder infection in subclinical mastitis but also control the mastitis without any side effects.

It also augments repair of mammary gland, firmness and normalizes udder functioning with improved milk quality. Thus polyherbal formulation may be recommended for the prevention and treatment of subclinical Mastitis in bovines.

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