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Efficacy of a composite formulation (masticure®) as an adjunct therapy in the treatment of mastitis in bovines

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Abstract
Mastitis is a complex disease with multifaceted etio-pathogenesis and causes huge economic losses worldwide including India. The present study was aimed to evaluate commercially available non-antibiotic preparation of minerals, probiotics, amino acids and enzymes with phyto extracts, "Masticure®" in the treatment of bovine mastitis. The present therapeutic trial involved 24 lactating cows with at least one specific subclinical mastitic quarter. The cows were divided randomly into 4 groups of 6 animals each. The therapeutic potential of current therapy in bovine mastitis was evaluated in terms of elimination of intramammary infections, subsiding of udder inflammation, improvement of milk quality and quantity up to d 28 post-initiation of treatment. The therapy in combination with and without parental antibiotic was found highly efficacious in eliminating intramammary infections and subsiding udder inflammation as evidenced from a significant decline in CMT score, SCC score, pH and EC. Also therapy enhanced the milk yield and milk biochemical parameters like protein, SNF and lactose. The overall effect of therapy was more seen in the group III treated with combination therapy i.e, Masticure granules orally and Masticure spray locally in combination with parental antibiotic ceftizoxime followed by group IV treated with combination therapy i.e, Masticure granules orally and Masticure spray locally without parental antibiotic.

Keywords: mastitis, masticure, therapeutic trial, intramammary infections, inflammation

1. Introduction

Mastitis, the inflammation of mammary glands results in huge economic losses to the dairy industry. It is a multi-factorial disease with cow, micro-organisms, management and environment playing key roles in its risk and these risk factors must be properly addressed for its prevention and control. The disease generally occurs in two forms; subclinical and clinical. On an average subclinical mastitis is prevalent in 47% of cows and 33% buffaloes in India. The clinical mastitis affects 6-7 percent of dairy animals. Subclinical mastitis, though in-apparent, causes up to 25% loss in milk production where as in clinical mastitis there may be total loss of milk production. The disease besides causing direct economic losses by decreased milk production has great public health significance. The presence of mastitis causative organisms and antibiotic residues in milk following therapy of mastitis poses a major threat to the consumer health. The use of antimicrobials has, overtime, increased the number of antimicrobial-resistant microbes globally [14]. Another concern is their effect on the manufacture of dairy products and the development of hypersensitivity syndromes in human beings. Further, the antibiotics used for the treatment of mastitis depress the activity of the polymorphonuclear cells (PMNs) that are considered primary cellular defences of the mammary gland [5]. Development of resistance after antimicrobial treatment of mastitis is an important consideration especially under the aspect of potential build-up of antibiotic-resistant organisms. Most developed countries tried to guarantee the food safety by the installation of drug residue detection systems for antibiotic residues in milk and milk products. In addition to the application of milk quality regulation to reduce the potential risk of milk contamination to the consumer, there is a need to reduce the frequency of antibiotic treatment of mastitis cases.

Another important and inevitable fact is the adverse effects of mastitis on the compositional and keeping quality of milk and milk products. Mastitis results in increase in the somatic cell count (SCC) and bacterial load of milk. The high SCC in mastitic milk has a lipolytic effect on fat and so there is increased tendency for rancidity of milk and milk products.

In mastitis milk desirable components such as lactose, casein, butterfat, solids not fat, calcium and phosphorus decreases while undesirable milk components such as lipase, whey proteins, immunoglobulins, sodium and chloride increase. Thus, to meet the major challenge posed by mastitis to the fastest growing dairy industry, timely and cost effective management of disease is very important. Till date antibiotics are the only primary agents used in the treatment and control of mastitis, but their use in mastitis therapy at large scale is not free from its hazardous effects. For this reason, antibiotic alternative methods of mastitis treatment and control are receiving increased attention now-a-days. The present study was therefore, designed to evaluate commercially available non-antibiotic preparation of minerals, probiotics, amino acids and enzymes with phyto extracts "Masticure®" as an adjunct therapy in the treatment of bovine mastitis and to evaluate its economics.

2. Materials and Methods

2.1. Selection of animals and experimental protocol

Twenty four adult HF cross dairy cows weighing around 500 Kg, found positive for specific subclinical mastitis were assigned randomly into four different groups taking into possible consideration of animal specific physiological data such as calving, lactation stage and milk yield and treated (Figures1 and 2) as per the protocol given below;

Group 1: Animals treated with appropriate antibiotic (Inj. Ceftizoxime @ 5 mg / Kg body weight) (n= 6, Control).

Group 2: Animals treated with appropriate antibiotic (Inj. Ceftizoxime@ 5 mg / Kg body weight) along with Masticure® granules @ 30 g BID, P.O. daily × 7 days (n= 6, Treatment I)

Group 3: Animals treated with appropriate antibiotic (Inj. Ceftizoxime @ 5 mg / Kg body weight) and a combination of Masticure® granules (@ 30 g BID, P.O. daily × 7 days) and topical Masticure® spray twice daily for 7 days (n= 6, Treatment II)

Group 4: Animals treated with a combination of Masticure® granules (@ 30 g BID, P.O. daily × 7 days) and topical Masticure® spray twice daily for 7 days (n= 6, Treatment III)

The antibiotic Ceftizoxime was found highly sensitive (80%) against the isolated organisms from the study area and was therefore selected in the present study as standard antibiotic. Injectable Ceftizoxime preparation was given parentally at the dose rate of 5 mg per kg of body weight. The milk samples were collected in sterile vials after proper teat preparation for assessment of California mastitis test (CMT), somatic cell count (SCC), pH, electrical conductivity (EC) and biochemical composition (fat, protein, lactose and SNF). Therapy was evaluated in terms of elimination of intramammary infection and improvement of the milk quality/quantity at quarter and animal level. The milk samples were collected on day zero before treatment and on days 4, 7, 14 and 28 post initiation of treatment to assess the long term effects of the therapy in dairy animals having specific mastitis (positive for culture and having CMT score ≥ 1) in at least one out of four quarters.

2.2. Milk sampling and analysis of milk samples

The milk samples were collected ensuring proper cleanliness and dryness of udder and teat. The individual quarter fore milk samples (about 10 ml) were collected in sterilized test tubes after cleaning the teat orifice with 70% ethyl alcohol and after discarding the first few streams of milk (Figure 3). Also about 40 ml of bucket milk (cow composite) samples were collected in clean disposable 50 ml plastic vials. The milk samples were transferred to the laboratory for undertaking various analytical parameters.

2.2.1. Isolation and identification of bacteria

The milk samples brought to the laboratory were mixed thoroughly and streaked on Blood agar plates. Each plate was divided into four equal parts for inoculation of samples from the four quarters of each cow. The sample was streaked in the respective parts of the agar plates with sterilized platinum loop and was incubated aerobically at 37ºC for 24 to 36 hours. The individual bacterial colonies were noted and the organisms were identified on the basis of colony morphology, Gram's staining and growth on selective media [11].

2.2.2. California Mastitis Test

The test was conducted as per standard method described by Pandit and Mehta [10]. The results were read as negative (-), trace, one plus (+), two plus (++) and three plus (+++) depending upon the degree of gel formation and graded as 0, 1, 2, 3 & 4 respectively (Figure 4).

2.2.3. pH

The milk pH was determined using a digital pH meter. It was standardized by the known buffer solutions.

2.2.4. Electrical conductivity

The electrical conductivity was recorded using Digital Conductivity Meter (Systronics, Digital Conductivity Meter). The results were expressed in milli Siemens per cm (mS/cm).

2.2.5. Somatic cell count

Milk Somatic Cell Count (SCC) was determined as per the standard method described by Schalm *et al.* [12]. The milk smears were prepared from the test samples on a clean, grease-free glass slide and were dried in air. Thereafter, the slides were stained by Newman-Lampart stain. Cells under 25 random fields were counted under the oil immersion objective lens. Total number of cells/ml of milk was estimated by multiplying total number of cells in 25 fields with the working factor of the microscope (WF = 20,000) used.

2.2.6. Milk yield and biochemical composition of the milk

Daily record of milk yield was made to observe the effect of therapy on milk production. The biochemical composition parameters of the milk i.e. fat, SNF, protein and lactose were analyzed by Milk analyzer. The samples were brought to room temperature before doing analysis and results were expressed in % (w/v).

Fig 1: Mixing and feeding of Masticure granules

Fig 2: Application of Masticure spray on mastitic udder

Fig 3: Aseptic collection of milk sample

Fig 4: California Mastitis Test (CMT)

3. Results and Discussion

3.1. Effect of therapy on level of intramammary infections (IMI)

The effect of therapy on the quarter infection level was compared to a control group treated with standard antibiotic (Ceftizoxime) as detailed in Table 1. The therapy with appropriate antibiotic (Inj. Ceftizoxime) along with Masticure® granules could eliminate, 6/18 (33.33%) of intramammary infections at d 14 post-treatment. The combination of Masticure® granules and topical Masticure® spray along with Inj. Ceftizoxime eliminated 13/22 (59.09%) of intramammary infections and combination of Masticure® granules and topical Masticure® spray eliminated 9/18 (50.0%) intramammary infections on d 14 post-treatment against the control group (Group I) in which 5/18 (27.78%) of intramammary infections were eliminated.

Table 1: Elimination of intramammary infections (within group comparison)

	Number of quarters infected (out of 24 quarters) at the start of the study and at day 14							
Group Time	G1 (n=24)		G2 (n=24)		G3 (n=24)		G4 (n=24)	
	NIQ	NHQ	NIQ	NHQ	NIQ	NHQ	NIQ	NHQ
Day 0	18 (75)	6 (25)	18 (75)	6 (25)	22 (91.6)	2 (8.4)	18 (75)	6 (25)
Day 14	13 (54.2)	11 (45.8)	12 (50)	12 (50)	9 (37.5)	15 (62.5)	9 (37.5)	15 (62.5)
Chi square statistics	2.3		3.2		15.4**		6.8**	

NIQ= No. of infected quarters, NHQ= No. of healthy quarters, Figures in parentheses indicate percentages, out of 24 quarters
** indicates significant difference at p<0.01, comparison by chi square test between no. of healthy quarters at day 0 and day 14

The differences in elimination of intramammary infections were observed to be statistically significant in group III and group IV ($p < 0.01$) with χ^2 values being 15.4 and 6.8 respectively (Table 1). However elimination of intramammary infections in group I and group II was statistically nonsignificant. The elimination of intramammary infections could be attributed to the use of antibiotic in group III, however the statistically significant difference in elimination of infection in group IV treated with Masticure granules and Masticure spray could be attributed to the presence of antimicrobial components and such effects have been reported earlier by other workers also [2, 13]. Mir [8] used a non-antibiotic therapy comprising of vitamin A 01 million IU, Amino-nitrogen 300mg and Lactobacillus 60 million spores in 35 dairy cows having 67 mastitis quarters and found an overall elimination of infection/cure of 38.81% quarters in the treated group as compared to 14.81% quarters in the control group. Nayak [9] evaluated the efficacy of Dermanol, a herbal anti-inflammatory cream, in cases of udder inflammation and oedema (with or without mastitis) in cattle and buffaloes and reported that Dermanol cream treatment led to a full recovery along with disappearance of inflammation and other associated symptoms within 3-5 days.

3.2. Effect of therapy on inflammatory reaction of udder and milk quality/quantity

The combination therapy of Masticure® granules and topical Masticure® spray with (Group III) and without antibiotic Ceftizoxime (Group IV) was found highly efficacious in subsiding udder inflammation. There was a significant ($p<0.05$) decline in CMT and SCC score in the treatment groups undergoing combination therapy (Table 2) and the subsiding effect was more in Group III in which CMT and SCC score show a significant decline from day 4 onwards, however comparable with Group IV which also showed significant decline in inflammatory markers. Similarly, the pH value decreased significantly ($p<0.05$) on day 7 in Group III and on day 14 in Group IV respectively and the EC showed a significant ($p<0.05$) decline on day 4onwards (Table 3) in Group III and Group IV respectively.

The therapy resulted in a gradual increase in the milk yield. The increase in the milk yield was observed from the beginning of the therapy and was significant on day 28 (Table 4) in groups treated with combination therapy of Masticure granules and spray with or without antibiotic (Group III and Group IV).

Table 2: Effect of therapy on inflammatory reaction of udder in comparison to control

Parameters	Group	Days after initiation of treatment (AT)				
		Day 0	Day 4	Day7	Day14	Day28
CMT point score	G1	1.5 ± 0.55^{a1}	1.41± 0.46^{a1}	1.33 ± 0.52^{a1}	1.17 ± 0.41^{a1}	0.83 ± 0.41^{a2}
	G2	1.67 ± 0.82^{a1}	1.27 ± 0.38^{a2}	1.17 ± 0.41^{a12}	1.00 ± 0.00^{b2}	0.67 ± 0.52^{b3}
	G3	1.67 ± 0.52^{a1}	1.26± 0.41 ^{b2}	1.00 ± 0.00^{b2}	0.50 ± 0.15^{c3}	0.17 ± 0.11^{c3}
	G4	1.83 ± 0.75^{a1}	1.44± 0.45^{b1}	1.17± 0.41^{a1}	0.50 ± 0.25^{c2}	0.33 ± 0.12^{c3}
SCC (×10^3 /ml)	G1	844.33± 134.03^{a1}	815.34± 123.34^{a1}	790.17± 147.69^{a1}	749.33± 124.23^{a1}	644.17 ± 126.36^{a2}
	G2	893.67± 227.06^{a1}	774.67± 232.36^{ab1}	746.17± 87.63^{b2}	682.50 ± 32.93^{b3}	610.17 ± 180.88^{b3}
	G3	882.67± 143.10^{a1}	715.36± 164.12^{b2}	674.83 ± 33.64^{c2}	542.33± 110.0^{c3}	444.67 ± 114.27^{c4}
	G4	924.0± 168.69^{a1}	812.30± 168.69^{b2}	726.50 ± 98.91^{b2}	586.17± 89.51^{bc3}	519.67 ± 77.14^{bc3}

Superscripts in each row (a, b) and each column (1, 2) differ significantly (p<0.05).

Table 3: Effect of therapy on milk EC and pH in control and treatment groups

Parameters	Group	Days after initiation of treatment (AT)				
		Day 0	Day 4	Day7	Day14	Day28
EC (mS/cm)	G1	5.99 ±0.42^{a1}	5.78± 0.34^{a1}	5.60 ± 0.49^{a1}	5.43 ± 0.16^{a1}	5.20 ± 0.37^{a1}
	G2	6.03 ±0.52^{a1}	5.87 ± 0.36^{ab1}	5.58 ± 0.38^{a1}	5.25 ± 0.11^{a2}	5.07 ± 0.16^{a2}
	G3	6.16 ±0.49^{a1}	5.56 ± 0.28^{b2}	5.25 ± 0.10^{a2}	4.93 ± 0.47^{a2}	4.73 ± 0.35^{b2}
	G4	6.09 ±0.60^{a1}	5.78 ± 0.38^{b2}	5.35 ± 0.38^{a2}	5.07 ± 0.53^{a2}	4.83± 0.45^{ab3}
pH	G1	7.21 ±0.19^{a1}	7.14± 0.18^{a1}	7.10 ± 0.17^{a1}	6.99 ± 0.13^{a1}	6.90 ± 0.17^{a1}
	G2	7.34 ±0.20^{a1}	7.25± 0.20^{a1}	7.11 ± 0.21^{a1}	6.95 ± 0.07^{a2}	6.84 ± 0.20^{a2}
	G3	7.29 ±0.18^{a1}	7.06± 0.11^{b1}	6.95 ± 0.08^{b1}	6.82 ± 0.13^{b2}	6.72 ±0.19^{b2}
	G4	7.25 ±0.30^{a1}	7.14± 0.21^{a1}	7.01 ± 0.12^{a1}	6.86 ± 0.11^{b2}	6.77 ± 0.14^{b2}

Superscripts in each row (a, b) and each column (1, 2) differ significantly (p<0.05).

The therapy resulted nonsignificant improvement in fat, protein, lactose and SNF in animals under group I and II, however significant statistical improvement in fat, protein, lactose and SNF was observed in animals in group III and IV treated with combination therapy (Masticure granules and spray) with or without antibiotic (Table 4).

Table 4: Effect of therapy on milk composition in G1 (control) and treatment groups

Parameters	Group	Days after initiation of treatment				
		D 0	D 4	D 7	D 14	D 28
Milk Yield (Kg)	G1	17.46 ± 1.65^{a1}	17.52 ± 1.68^{a1}	17.50 ± 1.84^{a1}	17.46 ± 1.65^{a1}	17.46 ± 1.65^{a1}
	G2	18.14 ± 1.81^{a1}	18.89 ± 1.82^{a1}	18.04 ± 1.71^{a1}	18.68 ± 1.81^{ab2}	18.88 ± 1.81^{ab2}
	G3	16.17 ± 0.98^{a1}	16.52 ± 1.05^{a1}	16.83 ± 1.17^{a1}	17.63 ± 1.20^{a2}	17.63 ± 1.20^{a2}
	G4	18.33 ± 1.51^{a1}	18.68a ± 1.64^{b2}	19.00 ± 1.73^{b2}	19.96 ± 1.81^{b2}	19.96 ± 1.81^{b2}
Fat (%)	G1	4.16 ± 0.13 ^{a1}	4.16 ± 0.13^{a1}	4.18 ± 0.17^{a1}	4.17 ± 0.23 ^{a1}	4.20 ± 0.19 ^{a1}
	G2	4.13 ± 0.30 ^{a1}	4.15 ± 0.33^{a1}	4.12 ± 0.30 ^{a1}	4.25 ± 0.25 ^{a1}	4.32 ± 0.15 ^{a1}
	G3	4.22 ± 0.27 ^{a1}	4.27 ± 028^{a1}	4.35 ± 0.10 ^{a1}	4.41 ± 0.21 ^{a1}	4.46 ± 0.14^{b2}
	G4	4.20 ± 0.27 ^{a1}	4.20 ± 0.27^{a1}	4.28 ± 0.19^{a1}	4.39 ± 0.16 ^{a1}	4.40 ± 0.13 ^{a1}
Protein (%)	G1	3.30 ± 0.15 ^{a1}	3.31 ± 0.16^{a1}	3.33 ± 0.08^{a1}	3.30 ± 0.15 ^{a1}	3.32 ± 0.14 ^{a1}
	G2	3.36 ± 0.19 ^{a1}	3.38 ± 0.11^{a1}	3.41 ± 0.12^{a1}	3.36 ± 0.19^{a1}	3.42 ± 0.05 ^{a1}
	G3	3.33 ± 0.13 ^{a1}	3.36 a ± 0.11^{a1}	3.44 ± 0.08 ^{a1}	3.41 ± 0.06 ^{a1}	3.46 ± 0.06 ^{a2}
	G4	3.35 ± 0.15 ^{a1}	3.39 ± 0.17^{a1}	3.43 ± 0.10^{a1}	3.40 ± 0.08 ^{a1}	3.42 ± 0.11 ^{a1}
SNF (%)	G1	8.59 ± 0.14 ^{a1}	8.62 ± 0.15^{a1}	8.68 ± 0.16 ^{a1}	8.73 ± 0.18 ^{a1}	8.58 ± 0.24 ^{a1}
	G2	8.53 ± .20 ^{a1}	8.55 ± 0.20^{a1}	8.58 ± 0.16 ^{a1}	8.67 ± 0.18 ^{a2}	8.64 ± 0.17 ^{a2}
	G3	8.53 ± 0.17 ^{a1}	8.58a ± 0.19^{a1}	8.68 ± 0.11 ^{a1}	8.73 ± 0.16 ^{a2}	8.79 ± 0.14 ^{a2}
	G4	8.55 ± 0.17 ^{a1}	8.56a ± 0.17^{a1}	8.62 ± 0.17 ^{a1}	8.69 ± 0.19 ^{a2}	8.76 ± 0.06 ^{a2}
Lactose (%)	G1	4.70 ± 0.23 ^{a1}	4.72 ± 0.16^{a1}	4.68 ± 0.09 ^{a1}	4.67 ± 0.19 ^{a1}	4.61 ± 0.22 ^{a1}
	G2	4.68 ± 0.18 ^{a1}	4.56 ± 0.21^{a1}	4.44 ± 0.22 ^{a1}	4.59 ± 0.19^{a1}	4.47 ± 0.27 ^{a1}
	G3	4.73 ± 0.23 ^{a1}	4.48b ± 0.21^{a1}	4.32 ± 0.15^{b1}	4.27 ± 0.17^{b2}	4.30 ± 0.14 ^{b2}
	G4	4.75 ± .020 ^{a1}	4.66 a ± 0.18^{a1}	4.35 ± 0.16 ^{a1}	4.29 ± 0.14^{b2}	4.38 ± 0.13 ^{b12}

Superscripts in each row (a, b) and each column (1, 2) differ significantly (p<0.05).

There was a significant (p<0.05) improvement in CMT score, SCC, pH, EC and milk yield in all the treatment groups from day 14 onwards as compared to the control (Group I).

The present findings are in agreement with Gupta [3] who reported a significant reduction in CMT, SCC, TBC, EC and pH post treatment in subclinical mastitis affected animals treated with oral administration of the herbal powder mix. He also observed a significant rise in lactose and SNF values following treatment. Kolte *et al.* [7] used a herbal paste topically in subclinical mastitis in cows and recorded a significant decrease in CMT, pH, sodium ions and potassium ions after 10 days of therapy. Acharya *et al.* [1] conducted a study to assess the immunomodulatory effect of Immu-21, a herbal product and its synergistic effect along with the antibiotic therapy in the treatment of subclinical mastitis in cows and observed 60% recovery with Immu-21 alone in subclinical mastitis cases and 100% when used with antibiotics. Gupta and Pachauri [4] while using Bonmilk (an herbal oral therapy) in mastitis observed a significant decrease in CMT and mean SCC values, and disappearance of clinical abnormalities in milk and mastitic quarters with a 75% cure in bovine mastitis. Kamal *et al.* [6] used Bonmilk in four mastitic cows involving 6 quarters, and observed recovery with significant improvement in clinical signs, SCC and pH within

10 days of drug administration.

3.3. Evaluation of economics of composite formulation as an adjunct therapy

The present study revealed that the therapy of Group III was the costliest out of the four selected groups; however, its therapeutic efficacy was comparable to Group IV.

Table 5: Evaluation of economics of composite formulation as an adjunct therapy

Group	N	Treatment	Frequency	Unit Cost (Rs.)	Total Cost (Rs.)
1	6	Inj. Ceftizoxime	2.5 gm IM Once	629.00	629.00
2	6	Inj. Ceftizoxime Masticure® granules	2.5 gm IM Once 30 g BID × 7 days	629.00 95 × 7 = 665.00	1295.00
3	6	Inj. Ceftizoxime Masticure® granules Masticure® spray	2.5 gm IM Once 30 g BID × 7 days BID × 7 days	629.00 95 × 7 = 665 140.00	1434.00
4	6	Masticure® granules Masticure® spray	30 g BID × 7 days BID × 7 days	95 × 7 = 665.00 140.00	805.00

The therapy of Group IV was observed to be economical out of the four selected groups as it is effective in reducing the percentage of infected quarters at par with the costliest group (Table 5). From the study it can be inferred that the therapy with Masticure® granules and Masticure® spray is efficient in terms of subsiding udder inflammation, improvement in milk quality/ quantity and because of its cost effectiveness. This therapy can be tried alone in subclinical mastitis reserving antibiotics for severe acute mastitis cases which require a prompt response and also decreases the unnecessary antibiotic exposure and risk of developing resistant microbes. It is worth mention that the combination of therapy with Masticure® granules and Masticure® spray has yielded results at day 4 which will further reduce the cost of treatment if followed for 4 days only.

4. Conclusion

Results obtained from the study indicate the beneficial effects of Masticure therapy against subclinical mastitis of lactating dairy cows. The presence of minerals, probiotics, amino acids and enzymes with phyto extracts in the "Masticure®" possess anti-bacterial, anti-inflammatory and milk yield enhancing potential as substantiated by elimination of intramammary infections, decrease of milk CMT, SCC, ph, EC and increase in milk yield.

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