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Ethno-veterinary Formulation for Treatment of Bovine Mastitis

Balakrishnan Nair MN[1*], Punniamurthy N[2], Mekala P[2], Ramakrishnan N[2] and Kumar SK[1]

[1]TransDisciplinary University, School of Health Sciences, Veterinary Ayurveda group, Bangalore-560106, Karnataka, India

[2]EVM Herbal Training and Research Centre, Tamil Nadu Veterinary and Animal Sciences University, Thanjavur-613403, India

Research Article

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***For Correspondence**

Balakrishnan Nair MN, TransDisciplinary University, University School of Health Sciences, Veterinary Ayurveda Group, 74/2 Jarakabandekaval, Attur Post, Yelahanka, Bangalore, Karnataka, India.

E-mail: nair.mnb@tdu.edu.in; nairunni2003@yahoo.co.uk

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ABSTRACT

Introduction: The objectives of the study were to carry out clinical trial of ethno-veterinary medicine for mastitis, to conduct an intervention impact analysis on the reduction of antibiotic residue in the milk when herbal formulation is used and to evaluate *in vitro* the pharmacological understanding of the ingredients in the formulation based on the published literature and phytochemical assessment.

Methods: The herbal formulation for mastitis was documented from a healer and the safety and efficacy were confirmed using the methodology suggested earlier [3] . Clinical mastitis was identified by California Mastitis Test (CMT) method. The pH, Somatic Cell Count (SCC) and Electric Conductivity (EC) were monitored and documented before and post treatment with Ethno-Veterinary Medicine (EVM) in the field studies involving 27 animals. Freshly prepared fine paste of herbal formulation was applied externally over the udder 10 times per day for 7 days. Before each application, the udder was washed well with water and the milk from all quarters was removed completely. The affected cows were also fed with 2 lemon fruits twice daily. Bulk milk samples were tested for the presence of antibiotic residue(s) in milk before and after intervention. The pharmacological understanding of the ingredients in the formulation was evaluated based on the literature and phytochemical assessment.

Results: The select parameters (pH, SCC, EC) in the animals with mastitis became normal within 6-7 days of treatment. The milk production returned to near normal to the pre-mastitis level. Hence, the mastitis can be efficiently managed with this formulation. The intervention impact analysis showed 18 to 49% reduction in antibiotic residues in the milk. The combination [*Aloe vera* (L.) Burm. F, *Curcuma longa* L. and calcium hydroxide] acts synergistically and provides effective cure in clinical mastitis owing to its broad spectrum antimicrobial, anti-inflammatory and immunomodulatory activities.

Conclusion: Mastitis can be efficiently managed with this formulation. The intervention impact analysis showed 18-49% reduction in antibiotic residues in the milk. The combination [*Aloe vera* (L.) Burm. F, *Curcuma longa* L. and calcium hydroxide] acts synergistically and provides an effective cure in clinical mastitis owing to its broad spectrum antimicrobial, anti-inflammatory and immunomodulatory activities.

INTRODUCTION

In the last 50 years, mastitis in India reportedly increased 115 times [1]. The incidence of resistant mastitis in dairy cows in Tamil Nadu, India is 56.01% -*Escherichia coli* 50.64%, *Staphylococcus aureus* 44.25% and Methicillin-resistant *Staphylococcus aureus* 5.11% [2]. Hence, an alternate approach was attempted.

MATERIALS AND METHODS

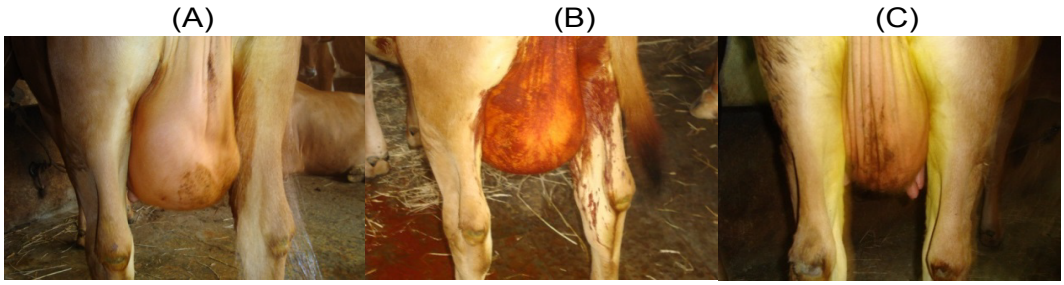
The safety and efficacy of the herbal formulation for mastitis were confirmed using the methodology suggested earlier [3]. Clinical mastitis was identified by CMT method. The pH, (SCC) and (EC) before and 6 days post treatment with EVM involving 27 animals were reported. Freshly prepared fine paste of *Aloe vera* leaves, *Curcuma longa* rhizome and calcium hydroxide was applied externally over the udder 10 times per day for 7 days. The udder was washed well with water and the milk from all quarters was removed completely before each application. The affected cows were also fed with 2 lemon fruits twice daily. Bulk milk samples collected from the farmers before and after intervention and tested for the presence of antibiotic residue(s) in milk [4,5]. The formulation was evaluated *in vitro* for the pharmacological understanding based on the literature and phytochemical assessment.

RESULTS

Figure 1. A-C shows cow treated with clinical mastitis using the herbal formulation has got cured within 6 to 7 days of treatment. The pH, SCC, and EC of milk became normal within 6 days of treatment **(Figure 2)**. The combination provides broad spectrum antimicrobial, anti-inflammatory, and immunomodulatory activities. Post-intervention impact analysis after one year showed up to 18-49% reduction in antibiotic residues **(Figures 3-7)** in the milk suggestive of the efficacy of the herbal combination in mastitis and in reducing the use of antibiotics. There was an outbreak of foot and mouth disease in certain areas in 2014 and therefore, there is an increase of tetracycline, Ceftriaxone and Ciprofloxacin in the milk certain centres **(Figure 5-7)**. The phytochemical screening of the formulation revealed the presence of alkaloids, saponins, steroids, carbohydrates, flavonoids, terpenoids, triterpenoids, sterols, emodin and diterpenes. TLC analysis revealed the presence of flavonoids, phenols and glycosides and they exhibited antioxidant activity [6,7]. The molecular docking study revealed that the active ingredients from *Aloe vera* and *Curcuma longa* interact with the proteins that play crucial role in *Staphylococcus aureus*.

DISCUSSION

As per the texts of Ayurveda, mastitis is known as *Sthanavidhradi*, a disease of *pitta* origin, the drugs used in this formulation (*Aloe vera*, *Curcuma longa* and Calcium hydroxide) is potent *pitta shamaka* (Pacifies *Pitta* humour). The formulation possesses *Krimighna* (antimicrobial), *Vranashodaka* (wound cleanser), *Vranaropaka* (wound healing), *Shothahara* (anti-inflammatory) and



Figures 1. A cross bred cow treated with clinical mastitis using the herbal formulation.

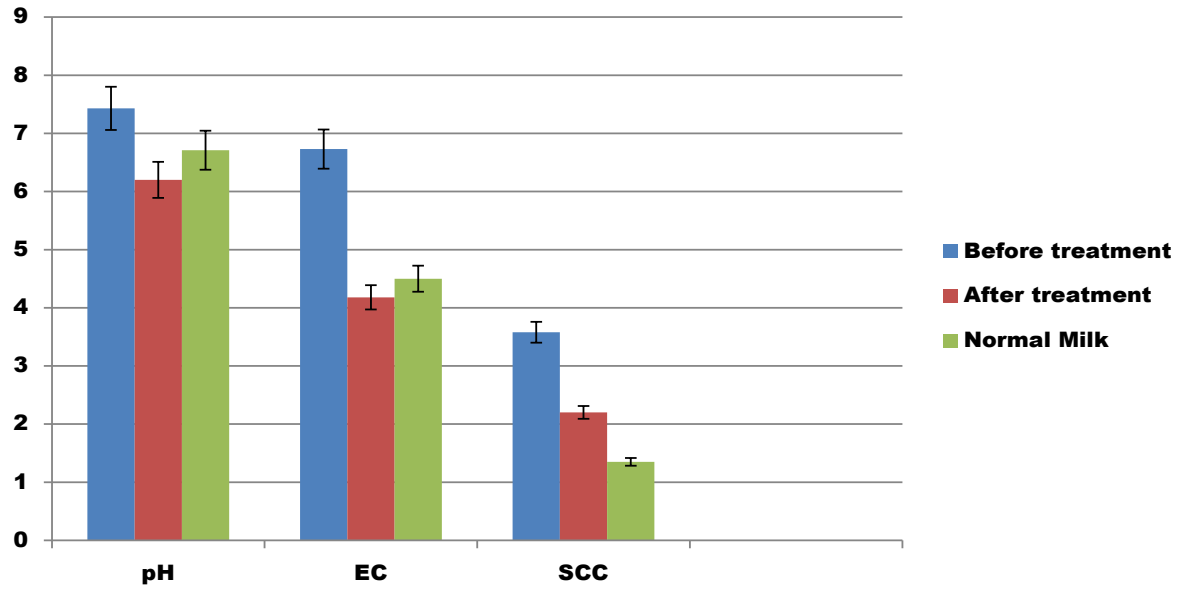


Figure 2. The figure shows reduction of pH, Electrical Conductivity (EC) and Somatic Cell Count (SCC) before and after treatment with herbal formula in comparison with normal values.

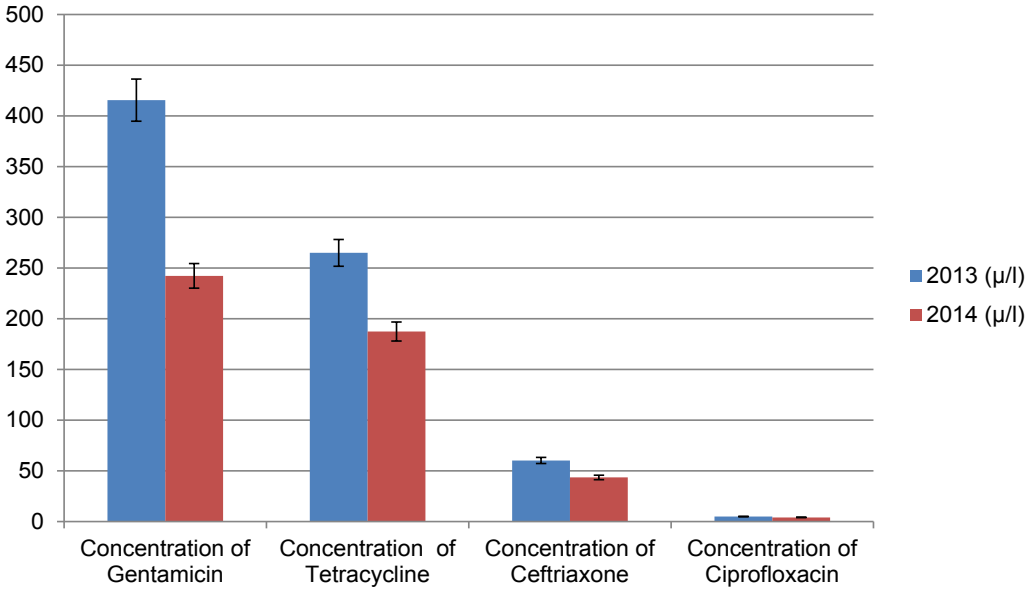


Figure 3. Mean antibiotic residue with Standard error (Gentamicin, Tetracycline, Ceftriaxone and Ciprofloxacin µg/L) in the milk before (2013) and after intervention (2014).

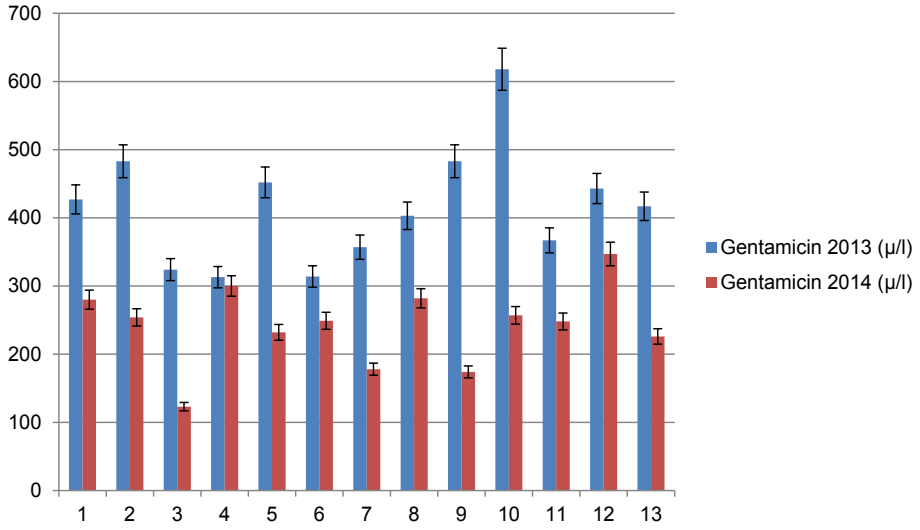


Figure 4. Gentamicin in the milk from 13 milk collection centres before (2013) and after intervention (2014).

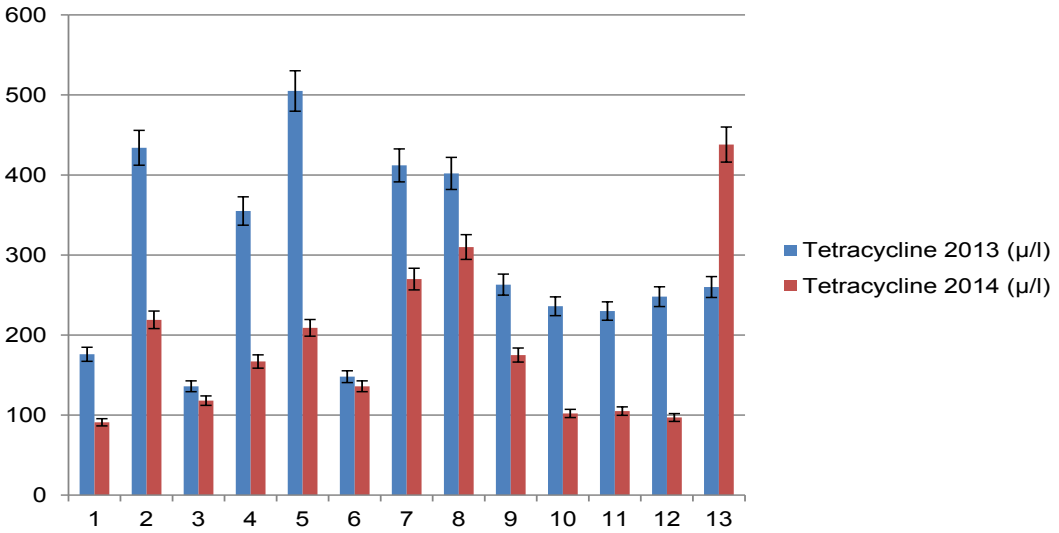


Figure 5. Tetracycline in the milk from 13 milk collection centres before (2013) and after intervention (2014).

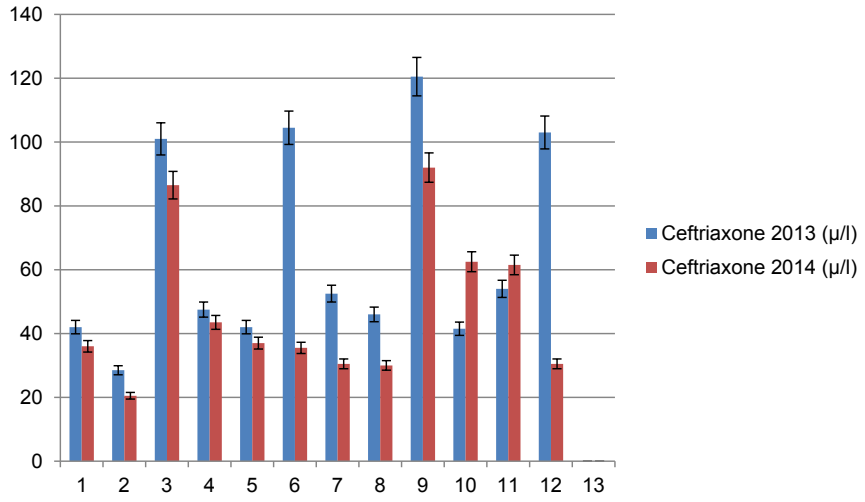


Figure 6. Ceftriaxone in the milk from 13 collection centres before (2013) and after intervention (2014).

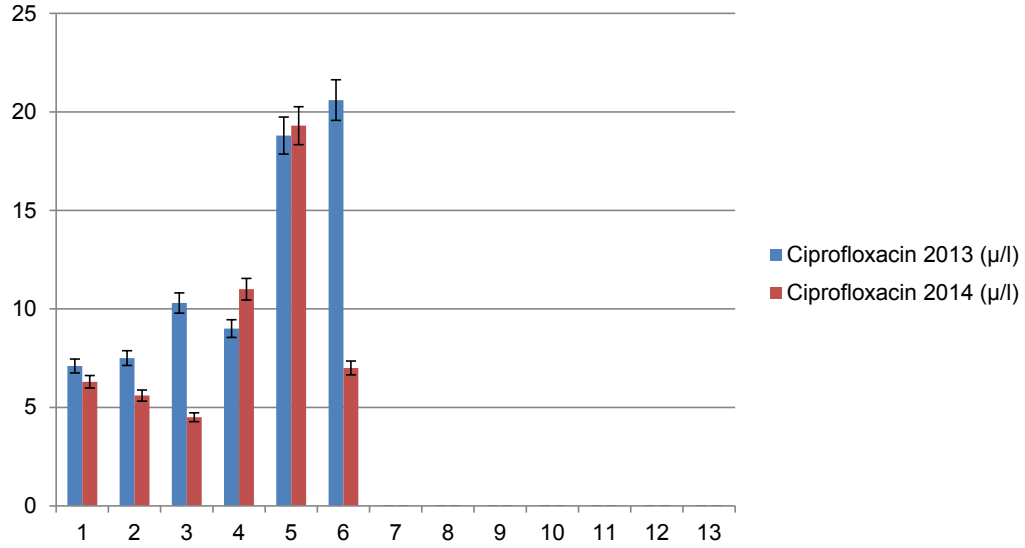


Figure 7. Ciprofloxacin in the milk from 13 milk collection centres before (2013) and after intervention (2014).

Srotoshodaka (channel cleanser) properties. Hence, mastitis can be efficiently managed with this formulation. The antimicrobial activity of *Aloe vera* is attributed to the anthraquinones (aloin and emodin), flavonoids, tannins (active against MRSA), saponins, p-coumaric acid, ascorbic acid, pyrocatechol and cinnamic acid. Alkaloids, tannins, phenolics, terpenoids, phytosterols, saponins, flavonoids, glycosides, fatty acids such as palmitoleic acid and α-turmerone in fixed oils of *Curcuma longa* also possess antimicrobial activity against wide range of bacteria. The anti-inflammatory activity of *Aloe vera* is reported to be due to Brady kinase which decreases vascular permeability, neutrophil migration, and leukocyte adhesion and reduces edema formation. It is also found to decrease the production of TNFα, inhibit PGF2α and TB4. Curcumin, the active principle of *Curcuma longa* is reported to inhibit NF-κB which in turn decreases TNF-α, superoxides, COX-2, iNOS and NO. It inhibits LOX pathway and decreases the formation of leukotriene. Calcium hydroxide is known to possess anti-inflammatory action and reduces edema formation. Thus all the three ingredients in the formulation act at various steps in the inflammatory pathway and synergistically produce anti-inflammatory effect [6-9].

Polysaccharides present in *Aloe vera* are rich in mannose and act as biological response modifier by targeting antigen presenting cell and cytokine cascade. Acemannan increases TNFα, IL-1B, IFN γ, IL-2 and IL-6; aleoride increases NFκB activation and stimulate macrophages which in turn increase nitric oxide production and other cytokines responsible for immunomodulation. Curcumin also possesses immunomodulatory and antioxidant activity[9]. The urgent need of dissemination of this traditional veterinary practice is highlighted in the light of the benefits it provides.

CONCLUSION

This traditional formulation is very effective in the cure of mastitis. The intervention impact analysis showed 18 to 49% reduction in antibiotic residues in the milk which indicates EVM based natural products is an effective alternative to synthetic chemicals in treating mastitis in cattle.

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COMPETING INTERESTS

The authors declare that they have no competing interest.

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