EFFICACY OF ANTIOXIDANT IN THE THERAPY OF SUBCLINICAL MASTITIS IN BUFFALOES

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Received: 23 June 2019
Accepted: 26 December 2021

ABSTRACT

This study was conducted to assess the therapeutic efficiency of antioxidant in subclinical mastitis. Therapeutic trials were conducted in twelve buffaloes affected with subclinical mastitis and were randomly divided into two groups. The buffaloes of Group 1 were treated with intramuscular injection of Inj. Mofoi 5 mg/kg body weight, while Group 2 buffaloes were treated with intramuscular administration of Inj. Mofoi 5 mg/kg body weight along with antioxidant powder orally. The therapeutic efficacy was assessed based on bacteriological cure and the pre and post therapeutic values of somatic cell count, electrical resistance, pH and milk yield. The use of antioxidant along with Moxifloxacin was found to be superior than antibiotic alone by improving udder's natural defence mechanism, early restoration of milk yield and quick recovery.

Keywords: *Bubalus bubalis*, buffaloes, subclinical mastitis, oxidative stress, antioxidant

INTRODUCTION

Mastitis is an economically important disease of dairy animals all over the globe. It is caused by diversity of etiological agents and factors including oxidative stress. Mastitis is considered as a existing disease in dairy herds with the prevalence of 15 to 40 subclinical mastitis cases for every one case of clinical mastitis (Kelly, 2002). Subclinical mastitis is an inflammation of the mammary gland without conspicuous signs, with 15 to 45% drop in daily milk yield and distorted milk composition (Swinkels *et al.*, 2005; Halasa *et al.*, 2007). The approximate economic loss due to mastitis was about 526 million dollars in India, of which 70% was due to subclinical mastitis (Varshney and Naresh, 2004).

The treatment efficacy of subclinical mastitis depends on the species and strain of pathogen, therapeutic regimen, animal factors, antimicrobial resistance and the cost effectiveness of the treatment. An unconventional approach involves the use of antioxidants in the therapy

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of bovine subclinical mastitis as mastitis is related with release of free radicals due to lipid peroxidation resulting in increased total oxidant capacity and decreased total antioxidants capacity in milk (Atasever *et al*., 2010; Patnaik *et al*., 2014). Vitamins and minerals such as vitamin A and β-carotene, vitamin C, vitamin E, selenium, Zinc and copper have long been documented as antioxidants in the animal health and production. Antioxidant vitamins and minerals protect the body from free radicals either by directly scavenging free radicals or by inhibiting the action of oxidizing enzymes (Umesh *et al*., 2012).

The present study was undertaken with the objective of evaluating the efficacy of antioxidant (MnM) in combination with antibiotic to treat subclinical mastitis in buffaloes.

MATERIALS AND METHODS

Study design

The buffaloes from different dairy farms located in and around Gannavaram, Krishna district, Andhra Pradesh and lactating animals from individual holdings that were brought to Teaching veterinary clinical complex, Gannavaram, were selected for the present study. The quarters with bacteriologically positive, milk samples without any clinical abnormalities were considered positive for subclinical mastitis.

In the present study 149 quarters of 49 graded Murrah buffaloes were screened for subclinical mastitis of which, 33 quarters from 12 buffaloes were diagnosed positive for subclinical mastitis based on culture results. The buffaloes with subclinical mastitis were randomly assigned to 2 groups consisting of six buffaloes each. The antibiotic for therapy was selected based upon the *in-vitro* antibiotic sensitivity test results.

Group 1

Buffaloes of Group 1 (6 buffaloes with 18 infected quarters) were treated with a broad spectrum antibiotic Inj. Mofoi, (Moxifloxacin) once daily intramuscularly 5 mg per kg body weight.

Group 2

Group 2 buffaloes (6 buffaloes with 15 infected quarters) were subjected to treatment with intramuscular administration of Inj. Mofoi (Moxifloxacin) 5 mg per kg body weight daily along with MnM powder 60 grams per day orally (M/S Vets pharma Ltd., consisting of Vitamin A, D3, E, Niacin, Biotin, Riboflavin, Amino nitrogen, Protein hydrolysate, Trisodium citrate dehydrate, Copper sulphate, Selenium, Manganese sulphate, Zinc and Lactobacillus spores).

In both the groups, the antibiotic therapy was continued till the milk samples were culture negative up to a maximum of 7 days. The response to therapy was recorded based on bacteriological cure and the pre and post treatment somatic cell count, electrical resistance, pH and milk yield.

RESULTS AND DISCUSSIONS

The recovery was established on the basis of bacteriological cure and changes in milk characteristics (somatic cell count, electrical resistance and pH) and improvement in milk yield. The post treatment somatic cell count in the milk samples did not vary significantly with the pre treatment values in both the groups (Table 1). The mean±SE of somatic cell count (SCC) before and after treatment was 3310807.72±1579202.38 and 2829930.00±743703.90 and cells/ml respectively in

Group 1 while the same was 1480069.45±758033.30 and 1361336.95±891615.80 cells/ml) in Group 2. The elevation of somatic cell count in subclinical mastitis milk samples was in agreement with the findings of Bytyaqi *et al.* (2010); Antanaitis *et al*. (2015) who opined that estimation of SCC s a measure of intensity of inflammation. In both the groups significant difference was not observed in the mean±SE values of somatic cell count before and after therapy could be attributed to the fact that the time required for the SCC to become normal varies from few days to few months or even up to next lactation depending upon the type of microorganism involved in the infection and the amount of tissue damage (Bramley, 1992). The mean somatic cell count was insignificantly higher after therapy which could be due to persistence of infection in some of the quarters even after therapy and the type of etiological agent could also be responsible for high somatic cell count (Sharma *et al*., 2005).The differences in the SCC in the milk samples could be attributed to infection with coagulase positive Staphylococci which had a significantly higher SCC than those infected with coagulase negative Staphylococci as opined by Mamache *et al.* (2014).

After therapy, the. values of electrical resistance were (588.18±45.09 vs 570.56±100.29) in Group 1 and 694.45±125.07 vs 514.45±24.69) in Group 2. In both the groups the difference was non-significant though there was elevation of electrical resistance towards normalcy in Group 2. These favourable changes could be ascribed to the therapeutic efficacy of antioxidant in combination with antibiotic (Antanaitis *et al.*, 2015). A significant decline in pH (6.63±0.06 vs 6.95±0.02) was recorded in Group II buffaloes after therapy. This was attributed to the positive correlation between SCC and pH (Patil *et al.*, 2014). The results obtained from the present study indicated that treatment with antibiotic was not accompanied by a significant increase in milk yield (pre-treatment 5.30±1.41 vs post treatment 6.61±0.88 liters/day). Significant difference ($P<0.05$) in milk yield (4.98±0.92 to 7.43±0.72 liters/day) was noticed between pre and post treatment in Group 2. Although, a significant improvement of milk yield was recorded in Group 2, the results were in agreement with the findings of Rajala-Schultz (1999); Antanaitis *et al.* (2015) who opined that mastitis failed to return milk production to normalcy in present lactation.

Out of 18 subclinical mastitis positive milk samples In Group 1 animals, nine were found negative by 3rd day with 50% cure rate, 11 (61.11%) were negative by 5th day and 12 (66.67%) were culture negative after 7 days of therapy. In Group 1, 33.33% of the quarters (6/18) were culture positive even after therapy. The overall cure rate in Group 1, quarter-wise and buffalo wise were 66.67% and 66.67%, respectively (Table 2) In Group 2 animals, out of 15 subclinical mastitis positive milk samples, 9 were found negative by 3rd day with 60% cure rate, 10 (66.67%) were negative by 5th day and 11 (73.33%) were culture negative after 7 days of therapy. In Group 2, 26.67% of the quarters (4/15) were culture positive even after therapy. The overall cure rate in Group 2, quarter-wise and buffalo wise were 73.33 and 66.67%, respectively (Table 2). It was evident from this study that the recovery rate shown by subclinical mastitis quarters in Group 2 was highly appreciable when compared to Group 1 and could be attributed to the antioxidant action of M nM. The outcome of the treatment was due to the collective action of antibiotic and antioxidant and it may be concluded that supplementation of antioxidant could help to improve the altered milk oxidants/antioxidants balance towards normalcy and, thus, result recovery from subclinical mastitis.

Table 1. Pre and post therapeutic qualitative and quantitative changes in milk (Mean±SE) in subclinical mastitis affected buffaloes.

		Group 1		Group 2	
S No	**Parameter**	**Before treatment**	**After treatment**	**Before treatment**	**After treatment**
1	Somatic cell count (cells/ml)	3310807.72 ±1579202.38	2829930.00 ±743703.90 ^{NS}	1480069.45 ±758033.30	1361336.95 ±891615.80^{NS}
2	Electrical resistance (ER) (units)	570.56±100.29	588.18±45.09^{NS}	514.45±24.69	694.45±125.07^{NS}
3	pH	6.94±0.02	6.80±0.01^{NS}	6.95±0.02	6.63±0.07*
4	Milk yield (liters/day)	5.30±1.41	6.61±0.88^{NS}	4.98±0.92	7.43±0.72*

* Means differ significantly (P<0.05) before and after therapy, NS Means differ non-significantly (P>0.05) before and after therapy.

Table 2. (Quarter-wise and animal-wise cure rates in Group 1 and Group 2 buffaloes.

Group	**No. of buffaloes treated**	**No. of quarters treated**	**No. of quarters cured**			**Quarter wise cure rate**	**Animal wise cure rate**
			Day 3	**Day 5**	**Day 7**		
1	6	18	9 (50.00)	11 (61.11)	12 (66.67)	66.67	4 (66.67)
2	6	15	9 (60.00)	10 (66.11)	11 (73.33)	73.33	4 (66.67)

Values in parenthesis indicate percentage.

Supplementation of mastitis dairy cattle with antioxidant vitamins like vitamin A, β carotene, vitamin C, and vitamin E and antioxidant minerals such as selenium, zinc and copper helped in quicker recovery as opined by Yang and Xiao (2015).

The results of this study suggest that antioxidant therapy along with antibiotic significantly improved treatment efficacy in comparison with antibiotic alone which could be accredited to the presence of non-specific immune-modulators such as vitamins, minerals, amino nitrogens, protein hydrolysate, Trisodium citrate dehydrate and lactobacillus.

ACKNOWLEDGEMENT

The authors would like to thank the Vice Chancellor, Sri Venkateswara Veterinary University, Tirupati for providing the facilities to carry out the research.

REFERENCES

Antanaitis, R., V. Zilaitis, V. Juozaitiene, G. Palubinskas, A. Kusinskas, A. Sederevicius and D.B. Aleksiejune. 2015. Efficient diagnostics and treatment of bovine mastitis according to herd management parameters. *Vet. Zootec*., **69**(10): 3-10. Available on: https://vetzoo.lsmuni.lt/data/vols/2015/69/pdf/antanaitis.pdf

Atasever, S., H. Erdem and A. Altop. 2010. Relationships between milk somatic cell count and pH in dairy cows. *J. Anim. Vet. Adv*., **9**(11): 1575-1577. DOI: 10.3923/javaa.2010.1575.1577

Bramley, A.J. 1992. Mastitis in bovine medicine. *Diseases and Husbandry of Cattle*. Blackwell Scientific Publications, Oxford, UK. p. 289-300.

Bytyaqi, H., U. Zaugg, K. Sherifi, A. Hamidi, M. Gjonbalaj, S. Muji and H. Mehmeti. 2010. Influence of management and physiological factors on somatic cell count in raw milk in Kosova. *Vet. Arhiv*, **80**(2): 173-183. Available on: http://vetarhiv.vef.unizg.hr/papers/2010-80-2-2.pdf

Halasa, T., K. Huijps, O. Osteras and H. Hogeveen. 2007. Economic effects of bovine mastitis and mastitis management: A review. *Vet. Q*., **29**(1): 18-31. DOI: 10.1080/01652176.2007.9695224

Kelly, A.L. 2002. Test methods and standards. *In Encyclopedia of Dairy Science*. Academic Press, London, UK.

Mamache, B., S. Rabehi and T. Meziane. 2014. Bacteriological study of subclinical and setif governorates, Algeria. *Journal of Veterinary Advances*, **4**(2): 364-373.

Patil, N.A., V.R. Kasaralikar, K. Ravikanth, K. Thakur and S. Maini. 2014. "Mastidip liquid" a herbal post milking teat dip for prevention and control of subclinical mastitis in bovines. *Medical Science*, **7**(23): 23-27.

Patnaik, S., A. Prasad and S. Ganguly. 2014. Biochemical characterization and antibiogram of *Staphylococcal* microorganisms associated with subclinical mastitis in lactating crossbred cows. *Animal Science Reporter*, **8**(4): 123-129. Available on: https://www.cabdirect.org/cabdirect/FullTextPDF/2014/20143385638.pdf

Rajala-Schultz, P.J., Y.J. Grohin and C.L. Guard. 1999. Effects of clinical mastitis on milk yield in dairy cows. *J. Dairy Sci*., **82**(6):

1213-1220. DOI: 10.3168/jds.S0022-0302(99)75344-0

Sharma, N. and S.K. Maiti. 2005. Effects of dietary supplementation of vitamin E and selenium in subclinical mastitis in dairy cows. *Indian Journal of Veterinary Medicine*, **25**: 76-79.

Swinkels, J.M., J.G.A. Rooijendijk, R.N. Zadoks and H. Hogeveen. 2005. Use of partial budgeting to determine the economic benefits of antibiotic treatment of chronic subclinical mastitis caused by *Streptococcus uberis* or *Streptococcus dysgalactiae*. *J. Dairy Res*., **72**(1): 75-85. DOI: 10.1017/s0022029904000603

Umesh, D., M.C. Sharma, S.K. Singh, P. Kumar, R. Jhambh, B. Singh, S. Bandhyopadhyay and M.R. Verma. 2012. Amelioration of altered oxidant/antioxidant balance of Indian water buffaloes with subclinical mastitis by vitamins A, D3, E, and H supplementation. *Trop. Anim. Health Pro*., **45**(4): 971-978. DOI: 10.1007/s11250-012-0319-6

Varshney, J.P. and R. Naresh. 2004. Evaluation of a homeopathic complex in the clinical management of udder diseases of riverine buffaloes. *Homeopathy*, **93**(1): 17-20. DOI: 10.1016/j.homp.2003.11.007

Yang, L.F. and S.L. Xiao. 2015. Role of antioxidant vitamins and trace elements in mastitis in dairy cows. *Journal of Advances in Veterinary Animal Research*, **2**(1): 1-9. Available on: https://bdvets.org/JAVAR/V2I1/b48_pp1-9.pdf