

ASSESSMENT OF TRISODIUM CITRATE – VITAMIN C BASED ORAL PREPARATION IN THE TREATMENT OF SUBCLINICAL MASTITIS IN INDIGENOUS ANIMAL (CATTLE, BUFFALO)

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ABSTRACT: The major disease that affects dairy industry in Pakistan is mastitis. Mastitis therapy mostly fails due to pathological changes in udder, pharmacokinetics of drugs used and poor husbandry practices. The present study is conducted to evaluate non-antibiotic therapy including Trisodium citrate and Vitamin C for the treatment of subclinical mastitis of cows and buffaloes. The product to be tested is composed of extemporaneous by mixing the Trisodium citrate 30gm and vitamin C 6gm. A field trial on therapeutic efficacy of above product has been conducted on buffaloes and cows affected with subclinical mastitis at Livestock Production Research Institute Bahadurnagar, Okara. Fifteen buffaloes with 10 animals in treatment group (A1), 5 animals in control group (A2); fifteen cows with 10 animals in treatment group (A3), 5 animals in control group (A4) are use in trial. Surf Field Mastitis Test (SFMT) is used for grading of animals and treatment is given in both the groups of cattle and buffalo. Buffalo group A1 and Cattle group A3 shows significantly higher ($p<0.05$) cure rates. The milk pH in both the groups decreased up to 6.6. The efficacy of therapeutic protocol was calculated by Analysis of Variance (ANOVA).

Key words: Cow, Buffalo, Mastitis, SFMT, Trisodium citrate, Vitamin C.

(Received 29.11.2022

Accepted 28.01.2023)

INTRODUCTION

Pakistan is fourth largest milk producing country in the world with annual milk production of 36.2 million tons (Anonymous, 2020-21). Milk is considered as most perfect and balanced diet for human beings and produced by domestic mammalian animals. It provides all constituents for body growth (Prakash *et al.*, 2009). Average milk yield in Pakistan is 3.17 liters per animal per day. Per capita consumption of milk is 158.3 liters/annum (Shaheen, 2010) and is far less than desired level of 0.5 liter per person per day (Sarwar *et al.*, 2002). Pakistan is at least 4 billion liters short of local demands and the only option to overcome the demand of milk seems to develop dairy industry on scientific lines (Express Tribune, //). The major disease that effects milk production is mastitis. Mastitis has always been economically drastic disease impeding desired progress and huge economic losses in dairy industry of Pakistan (Latif *et al.*, 2014). Mastitis lowers the milk production upto 30% (Prakash *et al.*, 2009) and is very costly disease of dairy animals (DeGraves and Fetrow, 1993; Kossabati *et al.*, 1998). Cost mainly arises from therapeutic intervention, loss due to antibiotic residues in milk, decrease milk production and quality and extra labour (Prakash *et al.*, 2009). Antibiotics are the standard therapy for mastitis (Rai *et al.*, 2013). However persistent

use of antibiotics lead to development of antibiotic resistance in bacteria and also milk and meat of treated animals may contain undesirable residues (Deluyker *et al.*, 2005). Mastitis therapy mostly fails due to pathological changes in udder, pharmacokinetics of drugs used and poor husbandry conditions. Antibiotics therapy also fails due to bacterial factors, increased antimicrobial drugs resistance and decreased endogenous lactoperoxidase activity in milk (du Preez, 2000). Various studies show that mastitis causes changes in milk composition (Prakash *et al.*, 2009). The changes in composition and antibiotic residues hinders the process of fermentation i.e., cheese and yogurt formation. This emphasizes the use of non-antibiotic formulation in dairy industries desperately.

Citrate is important for milk synthesis, so any decrease in citrate contents will lead to faulty milk synthesis and also related with mastitis in dairy animals. Citrate contents are also much lower in mastitic milk. Citrate is main constituent of buffer system in udder (Dhillon and Singh, 2013). Citrate reduces the pH of mastitic udder and chloride contents, increases the other components of mastitic milk (Prakash *et al.*, 2009). Trisodium Citrate also reduces the incidence of mastitis (Rai *et al.*, 2013). It also cures the subclinical mastitis effectively (Yousaf *et al.*, 2009). Citrate is main constituent of buffer system that maintains the pH of

udder (6.5); it also regulates the equilibrium of Ca⁺⁺ and H⁺ ions and fluidity of milk through its effect on casein micelles (Faulkner and Peaker, 1982; Shennan and Peaker, 2000). Citrate ensures isolation of soluble Ca⁺⁺ in milk and there is significant synchronization between citrate and Ca⁺⁺ (Holt and Muir, 1979). Hence deficiency leads to clumping of Ca⁺⁺ which in turn causes flakes in milk. These flakes can injure the parenchymatous tissue in udder alveoli and this injury stimulates inflammatory response and permeability of blood milk barrier is disrupted (Dhillon and Singh, 2013).

Vitamin C (ascorbic acid) acts as potent antioxidant against oxidative stress. It is water soluble vitamin and essential nutrient for animal species (Nweze *et al.*, 2015; Sauberlich, 1994). It is co-factor for many enzymatic reactions. In animals, vitamin C is especially important in wound-healing and in preventing bleeding from capillaries (Nweze *et al.*, 2015). Dairy cows suffering from acute or subclinical mastitis have lower level of vitamin C in their serum (Kleczkowski *et al.*, 2005; Ranjan *et al.*, 2005). This decrease level of vitamin

C leads to an increase level of lipid hydroperoxide in RBCs of dairy cows affected with acute mastitis. The severity of clinical signs is directly proportional to decrease in vitamin C level in serum (Weiss *et al.*, 2004)

The aim of this study was study to determine the effectiveness of this non-antibiotic therapy consists of Trisodium citrate- Vitamin C.

MATERIALS AND METHODS

The trial was conducted in 30 subclinical mastitic animals. A total of 10 animals having subclinical mastitis were left untreated as control, 20 animals received Trisodium citrate – Vitamin C based oral treatment (Table-1) composed of extemporaneous by mixing the following:

- Trisodium citrate 35gm/day/cow, buffalo
- Vitamin C 6gm/day/cow, buffalo

The selection of subclinical mastitic animals were based on the SFMT grading.

Table-1: Animals with subclinical mastitis and treatment provided.

Animal group	Animal specie	No. of animals	Treatment	Duration of treatment
A1	Buffalo	10	Oral administration of extemporaneously prepared product containing Trisodium citrate and Vitamin C	5 days
A2	Buffalo	5	No treatment (untreated control)	NA
A3	Cow	10	Oral administration of extemporaneously prepared product containing Trisodium citrate and Vitamin C	5 days
A4	Cow	5	No treatment (untreated control)	NA

The efficacy of therapeutic protocol, Df, SS, MS & F-value were calculated by Analysis of Variance (ANOVA).

RESULTS

In group A1, 7 out of 10 animals showed significant decrease in SFMT grading, hence indicated moderate cure rates in buffalo while in cow group A3, 9 out of 10 animals showed significantly higher ($p < 0.05$) cure rates (Table-4 and Table-5).

Grading

- No flakes in milk (full recovery)
+ Mild flakes in milk
++ Moderate flakes in milk along with jell formation

Table-2 Effect of Trisodium Citrate and Vitamin C Based Oral Formulation on the pH Value in Sub Clinical Mastitis after Trial.

Groups	Range	Average pH
A1, A3	6.98-6.46	6.66

Table-3. Effect of Trisodium Citrate and Vitamin C Based Oral Formulation in Sub Clinical Mastitis after Trial

Groups	Recovery	Remarks
Control Groups A2, A3	++	Moderate flakes with jell formation
Treatment group, A, A2	-	No flakes

Table -4 Analysis of Variance for Buffalo Group After Treatment

Source	DF	SS	MS	F	P
Treatment	1	9.64	.634	30.5	0.0001
Error	13	4.10	0.32		
Total	14	13.73			

Table -5. Analysis of Variance for Cattle Group After Treatment

Source	DF	SS	MS	F	P
Treatment	1	13.34	13.34	39.40	0.000
Error	13	4.40	0.34		
Total	14	17.733			

DISCUSSION

Mastitis is basically inflammation of parenchyma of mammary glands. According to Awale *et al.* (2012) and Schroeder (2012), it accounts for 38% of total direct cost of the disease and results in heavy economic losses due to reduced milk yield (upto 70%). It's the major hindrance in expansion of dairy as a source of livelihood and poverty alleviation (Pyorala, 2003; Viguier *et al.*, 2009). Sharma *et al.* (2007) stated that the therapy of mastitis aims to reduce the microbial load in the mammary glands not to sterilize it. Since for one liter of milk synthesis, it is estimated that 400 liter of blood passes through udder, thus practically it is difficult to sterilize for microbes. Furthermore, in various studies it has been established that in above 60% cases, pathogens enter the udder through teat canal (Reyther *et al.*, 2012). The present study is a novel approach to develop a chemical based formulation which when administered orally can reduce the load of mastitis causing pathogens. The present formulation has been developed on a concept to provide substrate for continuous milk synthesis, reducing the microbial load and inflammation. Citrate deficiency adversely influences the secretory alveoli and optimum level of citrate is required for Krebs cycle.

Conclusion: It can be concluded that the formulation of Trisodium citrate 30gm and vitamin C 6gm is much efficacious and cost effective as far as prevention and recovery from subclinical mastitis in both cattle and buffalo controlling sub clinic mastitis.

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