

## TRI-SODIUM CITRATE: A SUPPORTIVE THERAPY FOR MASTITIS IN DAIRY COWS

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### ABSTRACT

*Mastitis remains one of the most significant health issues in dairy herds, reducing both productivity and profitability. In this study, 86 cows with different forms of mastitis were clinically evaluated at the Teaching Veterinary Clinical Complex, Namakkal. The affected quarters showed an increased milk pH ranging from 7.6 to 8.0 and somatic cell counts between  $1.5-5 \times 10^5$  cells/ml. Collected samples were subjected to bacteriological culture and antibiotic sensitivity testing. A treatment regimen combining local udder sanitation with 3% potassium permanganate, intramammary flushing with saline and metronidazole, systemic administration of 15mg per kg.bw tri-sodium citrate intravenously, 2mg per kg.bw tolfenamic acid intravenously, antihistamines, oxytocin, and supportive vitamin supplementation was adopted. Post-treatment observations revealed normalization of milk pH (6.5–6.7) and somatic cell counts ( $0.5-1 \times 10^5$ /ml). Clinical recovery was achieved in 96% of cases, while 4% progressed to fibrosis or gangrenous changes. The results highlight tri-sodium citrate as a cost-effective, residue-free adjunct in mastitis therapy, reducing reliance on antibiotics and safeguarding milk quality.*

**Key words:** Mastitis, Tri-sodium citrate, pH, somatic cell count, udder hygiene antibiotic residue

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### INTRODUCTION

Mastitis, the inflammation of the bovine mammary gland, is a multifactorial disease with infectious, metabolic, and environmental etiologies. It remains a critical challenge to the global dairy

industry and has adverse effects on milk yield, quality, and animal welfare (Radostits *et al.*, 2000). Beyond reduced productivity, mastitis causes significant economic losses from discarded milk, treatment expenses, increased veterinary costs and culling of affected animals. The disease is considered as one of the most economically important disorder of cattle after foot-and-mouth disease in India (Singh *et al.*, 1982).

The condition manifests in two forms: clinical mastitis, easily identified by

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udder inflammation and abnormal milk, and subclinical mastitis, which is more prevalent yet silent, diagnosed mainly through elevated somatic cell count and altered milk pH. Subclinical cases are especially troubling since they jeopardize milk quality and serve as reservoirs for infection within herds (Watts and Owens, 1989).

*Staphylococcus aureus*, *Streptococcus agalactiae*, *Escherichia coli*, *Klebsiella* spp., and *Enterococcus* spp. are the main pathogens linked to mastitis, and their frequency varies by region (Shpigel *et al.*, 1998). Management strategies typically rely on intra-mammary or systemic antibiotic therapy, but their effectiveness is often limited. Rising antimicrobial resistance, consumer demand for residue-free milk, and regulatory restrictions on antibiotic use necessitate the development of alternative therapeutic approaches (Ram Bahal Rai *et al.*, 2013).

Tri-sodium citrate, a safe food-grade salt and natural component of milk, has emerged as a promising adjunct therapy. It functions as a pH modulator and calcium chelator, reducing udder edema, improving milk flow, and creating an environment unfavourable to bacterial survival. Studies have shown that when combined with traditional therapy, it can decrease SCC and restore mammary gland function. (Palanivel *et al.*, 2008, 2010).

Despite its potential, field-level evaluation of tri-sodium citrate therapy in Indian dairy herds is limited. Thus, the purpose of this study was to evaluate its efficacy as a supportive therapy in cases of

mastitis, with a focus on improving milk quality, reducing antibiotic reliance, and promoting clinical recovery.

The condition is primarily caused by bacterial pathogens, with *Streptococcus agalactiae*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterococcus* spp. being the most common (Shpigel *et al.*, 1998). While contagious pathogens such as *S. aureus* persist within the herd, environmental pathogens like *E. coli* are acquired from contaminated surroundings (Watts and Owens, 1989). The prevalence of mastitis in Indian dairy herds is high, with reports suggesting that 50% of cows and up to 25% of quarters may be affected at some stage of lactation (Singh *et al.*, 1982).

The mainstay of mastitis treatment has been intra-mammary or systemic antibiotic administration, coupled with supportive therapy such as udder massage and frequent milking (Ram Bahal Rai *et al.*, 2013). However, antibiotic therapy has variable efficacy, particularly against chronic *Staphylococcus aureus* infections, and often leads to recurrence. Furthermore, the existence of antibiotic residues in milk limits the milk's appropriateness for human consumption and contributes to antimicrobial resistance, which is a serious public health concern (Watts and Owens, 1989).

Milk pH and somatic cell count (SCC) are regarded as trustworthy markers of udder health. Normal milk pH ranges from 6.5–6.7, but it rises during mastitis due to leakage of blood constituents and

accumulation of alkaline substances in milk. Similarly, SCC increases in response to infection and inflammation, with levels above  $2 \times 10^5$  cells/ml considered indicative of subclinical or clinical mastitis (Singh and Baxi, 1980). Monitoring these parameters is essential for diagnosis and treatment evaluation.

Tri-sodium citrate, a naturally occurring salt and GRAS (generally recognized as safe) food additive, has gained attention as a mastitis therapy. It functions as a pH modulator and calcium chelator, reducing udder edema and restoring mammary gland homeostasis. Kerstin Uvnaes-Moberg *et al.* (2001) reported that citrate administration improved milk ejection by modulating oxytocin release and mammary gland function.

In Indian studies, Palanivel *et al.* (2008, 2010) highlighted the effectiveness of tri-sodium citrate in lowering SCC and enhancing clinical recovery rates when used as an adjunct therapy. Its major advantage lies in its residue-free nature, making it safe for consumers and beneficial for maintaining milk quality standards.

## MATERIALS AND METHODS

### Animals and Case Selection

Total of eighty-six of Jersey cross, Holstein Friesian cross, and nondescript kinds of lactating cows presented to the Teaching Veterinary Clinical Complex, Namakkal, with clinical signs of mastitis were included. Diagnosis was based on history, clinical examination of the udder,

and milk abnormalities.

### Laboratory Examination

Milk samples collected from affected quarters were analyzed for:

- California-Mastitis Test (CMT): Performed cow-side using commercial CMT reagent. The degree of gel formation was scored as negative, trace, +, ++, or +++, indicating increasing severity of subclinical mastitis (Schalm *et al.*, 1971).
- pH: measured using digital pH meter (range 7.6–8.0 in affected cases).
- Somatic Cell Count (SCC): using microscopic counting method ( $1.5\text{--}5 \times 10^5$  cells/ml) (Ruegg, 2017).
- Bacteriology: Grow on Eosin Methylene Blue agar, Mannitol salt agar, and MacConkey agar and incubated for 24 hours at 37 °C (Kromker and Leimbach, 2017).
- Antibiotic Sensitivity Test (ABST): performed using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar (Kromker and Leimbach, 2017).

### Treatment Protocol

- Udder cleansing with 3% potassium permanganate solution.
- Oxytocin 10IU in peripheral ear vein followed by stripping out of milk from all affected quarters by full hand method (Kerstin Uvnaes-Moberg *et al.*, 2001)
- Intramammary infusion of 25 ml saline, followed by 500mg metronidazole, followed by massaging and re-flushing after 10 min.
- Finally affected quarters were infused

with 25ml of normal saline and 2ml of chlorpheniramine maleate. Then administration of tri sodium citrate injection @ of 15mg per kg.bw intravenously, tolfenamic acid injection @ of 2mg per kg.bt and chlorepheniramine maleate @ of 10mg, vitamin AD3E 10ml as a single injection intra muscularly.

### Post-treatment Monitoring

Animals were observed for clinical signs, udder consistency, milk pH, and SCC on days third and fourth post-treatment as most of mastitis cases recovered on these days. Success was defined as normalization of milk quality and return to normal udder function.

## RESULTS AND DISCUSSION

### Clinical Outcome

Out of 86 mastitis cows treated, 83 animals (96%) showed clinical improvement within between three and four days of treatment. Clinical signs such as udder swelling, heat, and pain subsided, while milk returned to near-normal consistency and appearance. The remaining 3 animals (4%) progressed to chronic mastitis with fibrosis and gangrenous changes, making recovery unachievable. These findings are consistent with other research showing how difficult it is to reverse chronic mastitis after irreversible tissue damage has occurred (Shpigel *et al.*, 1998).

### Effect on Milk Parameters

The CMT scores of affected quarters ranged from + to +++, reflecting moderate to severe mastitis (Figure 1). After treatment with tri-sodium citrate-based therapy, CMT scores reduced markedly, with most quarters showing negative or trace reactions by day 4, indicating successful reduction in inflammatory changes. These findings were correlated well with SCC reduction and pH normalization in line with the interpretation standards described by Schalm *et al.* (1971).

The CMT scores of affected quarters ranged from + to +++, reflecting moderate to severe mastitis. Right and left fore-quarter wells show moderate gel formation (+ to ++), while the right and left hind quarter wells indicate strong gelation (+++), consistent with severe mastitis (Fig.1)

The milk's pH ranged from 7.6 to 8.0 prior to therapy, showing alkalinity brought on by persistent infection and cellular damage (Fig 2). A higher SCC ( $1.5-5 \times 10^5$  cells/ml) indicated an inflammatory response. Following tri-sodium citrate therapy, milk pH reduced to 6.5–6.7, within the physiological range, while SCC dropped to  $0.5-1 \times 10^5$  cells/ml. This significant normalization suggests that tri-sodium citrate helped restore the mammary gland's biochemical balance. Similar observations of improved milk quality following supportive mastitis therapy have been reported by Palanivel *et al.* (2010).

## Bacteriological Findings

Culture results identified *Staphylococcus* spp. as the predominant causative agent, followed by *Klebsiella*, *Enterococcus*, and *Streptococcus* species (Fig 3). Hind quarters were commonly affected than forequarters, consistent with his findings (Singh and Baxi, 1980). The predominance of *Staphylococcus aureus* is of particular concern due to its chronic nature, ability to form biofilms, and reduced response to antibiotic therapy.

## Therapeutic Implications

A high recovery rate was observed in this study. This highlights the potential of tri-sodium citrate as an effective adjunct to conventional therapy. It has ability to modulate pH and improve udder physiology and reduce the need of prolonged antibiotic administration. This is especially relevant when considering food safety, as antibiotic residues in milk are a persistent concern for both consumers and regulators. The cost-effectiveness of the protocol further enhances its applicability in field conditions, particularly in resource-limited rural dairy farms.

The results are in line with the past studies that highlighted the benefits of supportive therapy for improving mastitis healing and lowering medication use. (Watts and Owens, 1989; Kerstin Uvnaas-Moberg et al., 2001). Integrating tri-sodium citrate into mastitis treatment protocols could contribute

to sustainable dairy production by effective disease control measures with consumer safety.

## CONCLUSION

The current study shows that dairy cow mastitis is predominantly caused by *Staphylococcus* spp., followed by *Klebsiella*, *Enterococcus*, and *Streptococcus* species, with hindquarters more frequently affected. The tri-sodium citrate-based supportive therapy, combined with udder hygiene and selective antibiotics, achieved a high clinical recovery rate of 96%. Treated animals showed normalization of milk pH (6.5–6.7) and somatic cell counts ( $0.5-1 \times 10^5/\text{ml}$ ), reflecting restoration of mammary gland health. Only 4% of cases progressed to chronic fibrosis or gangrene. These findings highlight tri-sodium citrate as a safe, cost-effective, and residue-free adjunct therapy that enhances recovery, maintains milk quality, and reduces reliance on prolonged antibiotic use. Adoption of this protocol, along with proper udder sanitation and hygiene, can improve herd health, ensure milk safety, and support sustainable dairy production.

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Fig .1. California Mastitis Test (CMT) results from milk samples of affected quarters.

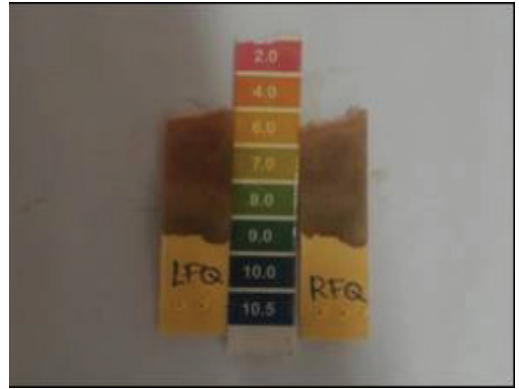


Fig .2. Milk pH evaluation using indicator strips before treatment.

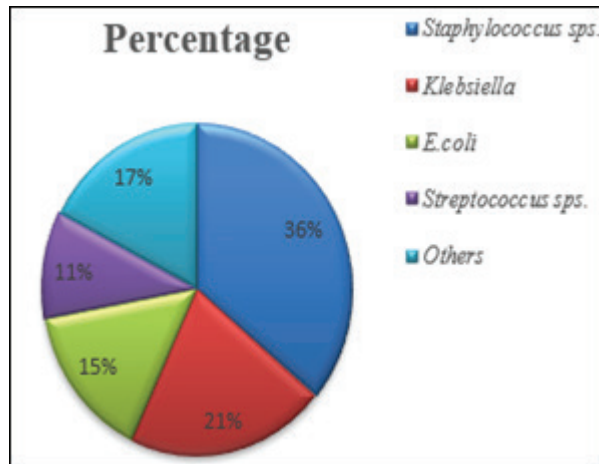


Fig .3. Bacterial isolates distribution from milk samples during mastitis.

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